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ROBERTH FAGUNDES DE SOUZA

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Tese apresentada à Universidade Federal de Uberlândia, como parte das exigências para obtenção do título de Doutor em Ecologia e Conservação de Recursos Naturais

Orientador:
Prof. Kleber Del Claro

Uberlândia, MG
Fevereiro de 2015

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"*True glory consists in doing what deserves to be written, in writing what deserves to be read, and in so living as to make the word happier and better for our living in it*". Pliny the Elder

TESE DE DOUTORADO

COMPETIÇÃO POR MUTUALISMOS:

HIERARQUIA DE DOMINÂNCIA INTERESPECÍFICA MEDIADA PELA QUALIDADE DO RECURSO REGULANDO INTERAÇÕES FORMIGA-PLANTA

ROBERTH FAGUNDES, KLEBER DEL-CLARO

DOCTORAL THESIS

COMPETITION FOR MUTUALISMS:

INTERSPECIFIC DOMINANCE HIERARCHY MEDIATED BY RESOURCE QUALITY REGULATING ANT-PLANT INTERACTIONS

ROBERTH FAGUNDES, KLEBER DEL-CLARO

Resumo

As formigas que recolhem o néctar das plantas com nectários extraflorais (EFN) podem defendê-las contra ataques de herbívoros. Muitos casos desse tipo de mutualismo foram descritos mas ainda é preciso entender os mecanismos que estruturam essas interações. Neste estudo, esse problema foi abordado através de observações de campo e experimentos sobre o comportamento das formigas compilados em cinco capítulos. Especificamente, foi avaliado o investimento da planta em atrair formigas (qualidade do néctar) e a relação competitiva entre formigas pelo néctar (hierarquias de dominância) como mecanismos estruturadores das interações formiga-planta (partição das interações). Todo o estudo ocorreu em áreas do ecossistema Campo Rupestre, localizadas em Ouro Preto (Brasil), de junho de 2011 a março de 2014. No Capítulo 1, observamos que redes de interação formiga-planta em Campo rupestre é aninhada e generalizada como esperado para mutualismos, mas difere da rede formiga-hemíptero, seu equivalente ecológico, apesar de compartilharem as mesmas espécies de formigas interagentes. No Capítulo 2, nós aprofundamos no efeito dos recursos estruturando interações, e mostramos que a qualidade mais do que a quantidade de néctar determina o padrão de interação entre formigas (32 espécies) e plantas (10 espécies), bem como os benefícios desta interação (benefícios condicionais). No Capítulo 3, nós demonstramos através de encontros interespecíficos pareados que as formigas são organizadas em hierarquias de dominância comportamental baseadas em agressividade. Essa hierarquia afeta a dominância numérica por territorialidade e, mais importante, o domínio das interações e do néctar. Mantendo esta linha de pensamento, demonstramos no Capítulo 4 que nem todas as formigas que coletam néctar realmente protegem a planta contra herbívoros mas somente as espécies dominantes e altamente interagidas. Finalmente, fechamos a tese demonstrando no Capítulo 5 que as espécies de formiga que interagem com as plantas durante o dia não são o mesmo à noite, mas esta rotatividade diurno-noturno não afeta a estrutura das interações devido a troca específica da formiga dominante. Este estudo traz evidências comportamentais e empíricas sobre a importância da identidade de espécies de formiga e sua dominância ecológica, assim como da qualidade do recurso, para a estrutura de rede da formiga-planta e eficácia do mutualismo.

Palavras chave: mutualismo, rede mutualista, rede formiga-planta, herbivoria, dominância ecológica, partição de recurso, competição interespecífica, ciclos diurnos, Cerrado.

Abstract

Ants that collect nectar from plants bearing extrafloral nectaries (EFN) can defend them against the attacks of herbivores. Many cases of this food-for-protection mutualism have been described and the next step is to understand the mechanisms that structure these interactions. In this study, this issue was addressed through field observations and experiments on ant behaviour in studies compiled into five chapters. Specifically, we evaluated the plant's investment in attracting ants (nectar quality) and the competitive relationship between ants (resource dominance hierarchies) as structuring mechanisms of ant-plant interactions. The entire study took place in areas of the ecosystem known as Rupestrian Fields, located in Ouro Preto (Brazil), between June 2011 and March 2014. In Chapter 1, we observe that ant-plant interaction network in Rupestrian Fields is nested and generalized as expected for mutualisms, but differs from ant-hemipteran network, its ecological equivalent, despite both share ant species interactions. In Chapter 2, we delve into the study of the effect of resources in interaction frequency, and we showed that the quality more than quantity of nectar determines the pattern of interaction between plants (10 species) and ants (32 species), as well as the benefits of this interaction (conditional outcomes). In the Chapter 3, we focus on the ants to demonstrate, using paired encounters, that ants species are organized in dominance hierarchies based on aggressiveness. This hierarchies affects the numerical dominance based on territoriality and, more importantly, in the dominance of interactions and nectar. Keeping this line of thought, we demonstrated in Chapter 4 that not all ants that collect nectar really protect the plant against herbivores but only the ecological dominant and highly interacted species. Finally, we close the thesis demonstrating in Chapter 5 that the ant species interacted with plants during the day are not the same at night, but this diurnal-nocturnal turnover does not affect the structure of the interactions by only changing the dominant species. This study brings behavioural and empirical evidences on the importance of the identity of the ant species and its ecological dominance, as well the effect of the nectar quality, for the structure of ant-plant interactions and effectiveness of mutualisms.

Key words: mutualism, mutualistic network, ant-plant network, herbivory, resource partitioning, ecological dominance, interspecific competition, co-occurrence, daily turnover, Cerrado.

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Introdução geral

Introdução Geral

Comunidades de formigas arborícolas são comumente organizadas em hierarquias de dominância e distribuição (Leston 1973; Blüthgen et al. 2000). Nessa estrutura, poucas espécies consideradas dominantes agressivas se evitam, ocupam grandes territórios e monopolizam recursos tróficos), enquanto outras espécies, submissas, co-ocorrem com cada dominante (Leston 1973). Esse padrão, conhecido como Mosaico de Formigas, é mantido por níveis de tolerância entre as espécies através de comunicação química, estratégias de defesa e baixa frequência de encontro espaço-temporal (Majer et al. 1994; Davidson 1997; Hölldobler e Wilson 2000). Portanto, existe uma constante competição entre formigas arborícolas por recursos, como o alimento fornecido pelas plantas na forma de néctar extrafloral e pelos hemípteros herbívoros sugadores na forma de honeydew. Essa competição por um ou outro recurso pode a afetar a frequência na qual as espécies de formigas interagem com plantas e hemípteros, e por fim, alterar os benefícios recebidos pelos parceiros. Enquanto alguns trabalhos discutem a competição entre diferentes espécies de formigas pela utilização exsudatos de herbívoros sugadores de seiva (Del-Claro e Oliveira 1999; Blüthgen et al. 2000; Del-Claro e Oliveira 2000) nenhum estudo avaliou essas mesmas hipóteses para o mutualismo entre formigas e plantas via nectários extraflorais (NEFs). No Cerrado esses mutualismos planta-formiga é muito comum (Rico Gray e Oliveira 2007), principalmente através de NEFs (Schoereder et al. 2010), e portanto essa interação pode ser mecanismo chave para compreender a biodiversidade de formigas nesse ecossistema.

A territorialidade (alto recrutamento e permanência espacial) é a principal característica das formigas dominantes (Leston 1973). A manutenção de territórios é uma estratégia que envolve perda de operárias em confrontos, investimento em guarda e sistemas de recrutamento em massa (Hölldobler e Wilson 1990). Assim, fontes de recursos constantes e energéticos são importantes para a manutenção desses comportamentos (Jackson 1984). Um exemplo desse tipo de recursos são os exsudatos de hemípteros e lepidópteros e secreções de nectários extraflorais que são amplamente utilizados pelas formigas arborícolas (Blüthgen et al. 2000) e contribuem significativamente para o fitness das mesmas (Byk e Del-Claro 2011). Entretanto, as formigas são insetos onívoros e também necessitam de fontes de proteína, principalmente para suas larvas (Hölldobler e Wilson 1990). Assim, elas também forrageiam ativamente nas plantas em busca de presas, como os insetos herbívoros (Hölldobler e Wilson 1990; Davidson 1997). Essa alta predação associada a formação de territórios de defesa em torno das fontes de recurso (nectários extraflorais), gera importantes benefícios para a planta (Del-Claro 2004; Rosumek et al. 2009).

As formigas podem fornecer diversos benefícios para a planta quando em interação, como redução do número de herbívoros visitantes e consequentemente da taxa de herbivoria (Nascimento e Del-Claro 2010). Essa proteção se reflete na planta em forma de maior produção de folhas (Freitas et al. 2000), flores, frutos e sementes (Del-Claro et al. 2006; Moreira e Del-Claro 2005). Rosumek et al. (2009) revisam o papel protetor das formigas e mostram que plantas não visitadas por formigas apresentam maior quantidade de herbívoros e herbivoria, além de menor produção de biomassa e potencial reprodutivo (Rico-Gray e Oliveira 2007; Schoereder et al. 2010). Em contrapartida as formigas recebem um limitado e energético alimento, logo vantajoso, que favorece o crescimento da colônia (Byk e Del-Claro 2011). Porém, o caráter protetor das formigas é variável principalmente em dois sentidos: o tipo e quantidade de recurso (qualidade) e a espécie e quantidade de formigas protetoras (dominância). Rosumek et al. (2009) discutem que plantas que possuem uma espécie de formiga muito abundante e frequente como protetora (espécie dominante) são mais bem protegidas. Essas espécies de formigas dominantes são mais comumente encontradas em associações com hemípteros, nectários extraflorais e/ou estruturas de nidificação (Blüthgen et al. 2000). Logo, plantas que apresentam recursos mais vantajosos, e consequentemente formigas agressivas, apresentariam maior fitness do que plantas que não apresentam esse tipo de recurso (Rosumek et al. 2009).

Recentemente, a teoria de redes complexas de interação se mostrou uma importante ferramenta no estudo de interações mutualísticas (Bascompte e Jordano 2007; Guimarães et al. 2011), principalmente entre formigas e plantas com nectários extraflorais (Guimarães et al. 2006; Dáttilo et al. 2013). De acordo com a teoria de redes, os pares de espécies interagentes são componentes de uma complexa rede de interações múltiplas que envolvem diferentes espécies com diferentes graus de especialização (Guimarães et al. 2006; Blüthgen et al. 2007; Guimarães et al. 2007). Nessas redes, as espécies são apresentadas como nós (ou vértices) e as interações como linhas (ou arestas). Várias propriedades emergem dessa estrutura tridimensional, como conectância, assimetria grau médio, aninhamento, modularidade, entre outras (veja revisão em Vázquez et al. 2009). Essas propriedades respondem a mudanças na comunidade e no ambiente e permitem comparação entre redes em diferentes contextos e o entendimento de mecanismos ecológicos que influenciam as interações em escala de comunidade (Vázquez et al. 2009; Jordano 2010).

De acordo com a teoria ecológica, competição é a interação entre duas espécies caracterizada pela disputa de recurso limitado cujo consumo beneficia o fitness da espécie dominante. Nesse caso, o néctar fornecido por plantas portadoras de NEFs seria um recurso gerador de competição por ser vantajoso, limitado e seu consumo impulsionaria o crescimento das colônias de formigas. Nesse contexto, a presente tese de doutorado avaliou hipótese de competição por mutualismos, ou seja, interações entre duas espécies em disputa pela possibilidade de interagir com uma planta e coletar o néctar (adquirir o benefício). Nós avaliamos a estrutura das interações entre formigas e plantas portadoras de NEFs em Campos rupestres, assim como os mecanismos competitivos reguladores dessas interações. Testamos a hipótese central de que as espécies de formigas coletam NEF em uma hierarquia de dominância do recurso e essa hierarquia regula a frequência das interações e os benefícios do mutualismo. Essa ampla hipótese foi trabalhada em cinco capítulos. No Capítulo 1 avaliamos a estrutura de duas redes de mutualismos ecologicamente equivalentes: formiga-planta e formiga-hemíptero, e avaliamos a disponibilidade de néctar e honeydew como mecanismo regulador dessas interações. No Capítulo 2 focamos no nível trófico das plantas, e avaliamos a qualidade e quantidade de néctar como fatores determinantes das interações com formigas realizadas pelas plantas. Além disso, avaliamos a importância da quantidade de interações com formigas e da agressividade dessas formigas em resposta a qualidade do néctar (contexto-dependente), para a proteção recebida pela planta contra herbivoria (benefícios condicionais). No Capítulo 3, partimos pro ponto de vista do nível trófica das formigas, e avaliamos a competição interespecífica pelo recurso de melhor qualidade como mecanismo regulador das interações com plantas (competição por mutualismos) através de hierarquias de dominância do recurso (partição de interações). No capítulo 4, aprofundamos na efetividade de defesa de plantas entre as diferentes espécies de formigas e a condicionalidade dessa defesa a qualidade do recurso ofertado (relação de dependência recuso-consumidor). Por fim, no Capítulo 5, nós avaliamos a partição temporal das interações entre as espécies dominantes para explicar a alta sobreposição espacial das interações.

Métodos Gerais

Área de estudo

Esse estudo foi realizado no Parque Estadual do Itacolomi (PEIT), Unidade de Conservação (UC) criado em 14 de Junho de 1967 pela lei estadual 4.495 (IEF/MG). O PEIT situa-se entre os meridianos 43°32'30" e 43°22'30" de longitude oeste e 20°22'30" de latitude sul (Figura 1). O PEIT cerca 7,200Km^2 de área situado entre as cidades de Ouro e Mariana (MG/Brasil), na porção sul da Cadeia do Espinhaço que se estende entre Minas Gerais e Bahia (Figura 1). Especificamente, o parque corresponde à região sudeste do Quadrilátero Ferrífero, com solo formado por rochas matassedimentares clásticas das quais predominam quartzitos, filitos e xistos, rochas intrusivas, depósitos de ferro representado pelas cangas e depósitos aluvionares dos Rios Prazeres e Maynart (Fujaco 2007). Sua altitude varia entre 700m, em vale íngremes e profundos, e 1,772m acima do nível do mar, nas encostas rochosas do Pico do Itacolomi (Fujaco 2007). O clima do parque é subtropical úmido (Cwb, Koepen), com verões quentes e chuvosos entre Outubro e Março e invernos secos e frios entre Abril e Setembro. A precipitação anual média varia entre 1,000mm e 1,500mm e a temperatura anual média flutua entre 19°C e 22°C (Fujaco 2007).

O PEIT protege principalmente os ecossistemas de Cerrado e Floresta Atlântica (Messias 2006; Figura 2). O Cerrado é composto basicamente por Campos Rupestres Quartzíticos e Ferruginosos (56% da área), enquanto que o sistema florestal é composto por Florestas Estacionais Semideciduais e Florestas Paludosas (44%) (Fujaco et al. 2007). As florestas restringem-se a encostas baixas, escarpas e vales e caracterizam-se principalmente pelas famílias Myrtaceae, Melastomataceae, Annonaceae, Fabaceae e Rubiaceae (Pedreira e Souza 2011). Os Campos Rupestres localizam-se nos platôs elevados e encostas rochosas (acima de 900m) e são formados pro gramíneas, ervas e arbustos baixos em sua maioria das famílias Poaceae e Asteraceae (Lemes 2009). No PEIT são encontrados seis tipos de Campo Rupestre de acordo com Dutra (2009) e Alves et al. (2014), que se diferenciam pelas constituições de solo e microclima, logo distinguem-se pela fitossociologia mas assemelham-se quanto a florística: (1) Campos Graminos Secos, com amplas extensões de grama e poucos arbustos esparsos nos platôs elevados. (2) Campos Graminosos Úmidos em platôs de menor altitude e constantemente alagados onde predominam gramíneas. (3) Campos Graminosos Úmidos de Altitude, semelhante ao anterior mais em áreas muito elevadas. (4) Campos quartzíticos de Afloramentos Rochosos, formado por vegetação de gramíneas, arbustos e epífitas sobre ou entremeados às rochas. (5) Campos Ferruginosos, semelhante ao anterior mas sobre rocha e concreções ferruginosa conhecida como Canga. E, (6) Escrubes, vegetação de transição entre Campo Rupestres e Floresta Estacional dominada por alta densidade de ervas, arbustos e arvoretas em solo de quartzito e filito no topo de morros e elevações entremeados por esparsos afloramentos rochosos (Figura 3).

Modelos de estudo

Apesar de ser parte do Cerrado, o Campo Rupestre é um ecossistema bastante específico em flora e fauna com grande número de espécies endêmicas (Messias 2006; Alves et al. 2014). No Cerrado, o estudo das interações mutualísticas entre formigas e plantas ou formigas e hemípteros é bastante amplo e diversos estudos descrevem as interações, mecanismos estruturantes, dinâmica espacial e temporal, comportamento e evolução (Rico-Gray e Oliveira 2007 e referências, veja também artigos publicados de Kleber Del-Claro e Paulo S. Oliveira). Por outro lado, o estudo de interações mirmecófilas em ecossistemas de Campos Rupestres é bastante limitado. Existe um estudo sobre formigas coletoras de néctar floral (Romero 2002), três estudos sobre interação entre formigas e hemípteros produtores de honeydew (Fagundes et al. 2011 e 2013; Guerra 2011). Sobre interações entre formigas e plantas portadores de nectários extraflorais em Campos Rupestres, até o janeiro de 2015, encontramos apenas três estudos publicados ou em publicação (Dáttilo et al. 2014, Fagundes et al. 2015 e Barbosa et al. 2015).

Na presente tese de doutorado, nós avaliamos principalmente interações entre formigas e plantas portadoras de nectários extranupciais, ou extraflorais (NEFs). Os NEFs são órgãos produtores de néctar semelhantes aos nectários florais mas que não se envolvem em polinização, mesmo quando presentes em estruturas florais (Shmid 1988). São normalmente encontrados no pedúnculo, estípula e base abaxial ou adaxial das folhas, mas também podem ser encontrados nos pedúnculos e cálices das flores, na haste das inflorescências e também nos frutos (Shmid 1988). Geralmente, estudos relatam a função do néctar extrafloral como de atração de animais, principalmente formigas, que agem como defensores das plantas contra o ataque de insetos herbívoros (Rico-Gray e Oliveira 2007). Nós buscamos as plantas em áreas de Escrube, Campos quatizítcos e Campos Ferruginosos em uma área de aproximadamente 15ha, nas encostas do Morro do Cachorro e Calais, na porção noroeste do PEIT (MG). A coleta das interações entre formigas e plantas ocorreu entre Junho de 2011 e Março de 2014. Diferentes porções dessa área foram usadas para os experimentos em diferentes períodos de tempo, portanto, consulte a seção “Métodos” de cada capítulo para informações específicas.

No total, 12 espécies de plantas foram usadas nos diferentes estudos que compõem essa tese de doutorado (Tabela 1; Figura 4): (sigla: BAC) *Banisteriopsis campestris* (A. Juss.) Little (Malpighiaceae), (BAM) *Banisteriopsis malifolia* (Nees e Mart.) B. Gates (Malpighiaceae), (CHD) *Chamaecrista desvauxii* (Collad.) Killip. (Caesalpinioideae), (CHM) *Chamaecrista mucronata* (Spreng.) H.S. Irwin e Barneby (Caesalpinoideae), (CRM) *Crotalaria micans* Link (Fabaceae), (HEU) *Heteropterys umbellata* A. Juss. (Malpighiaceae), (INS) *Inga sessilis* (Vell.) Mart., (INV) *Inga vulpina* Mart. ex Benth., (PET) *Peixotoa tomentosa* a. Juss. (Mapighiaceae), (SEP) *Senna pendula* (Willd.) Irwin & Barneby (Fabaceae), (SER) *Senna reniformis* (g. Don) H.S.Irwin & Barneby (Fabaceae) and (STG) *Stachytarpheta glabra* Cham. (Verbenaceae). Além disso, nós estudamos 32 espécies de formigas, das quais 10 espécies foram observadas em mais 95% das amostras (Tabela 2): (sigla: BRH) Brachymyrmex heeri Forel 1974 (Formicinae), (CAC) *Camponotus crassus* Mayr, 1862 (Formicinae), (CAN) *Camponotus novogranadensis* Mayr, 1870 (Formicinae), (CAR) *Camponotus rufipes* Mayr 1775 (Formicinae), (CAS) *Camponotus senex* Smith 1858 (Formicinae), (DOB) *Dorymyrmex brunneus* Mayr 1908 (Dolichoderinae), (PSG) *Pseudomyrmex gracilis* Fabricius 1804 (Pseudomyrmicinae), (PSP) *Pseudomyrmex pallidus* Smith 1855 (Pseudomyrmicinae), (PST) *Pseudomyrmex termitarius* Smith 1855 (Pseudomyrmicinae).

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Tabelas

Tabela 1. Parâmetros descritivos das 12 plantas portadoras de nectários extraflroais (NEFs) estudadas entre 2011 e 2014 em ecossitema de Campo Rupestre, Ouro Preto (MG, Brasil). Essas espécies foram utilizadas nos estudos que compões a presente tese de doutorado. Médias e desvios padrão correpondem à amostras de 20 arbustos por espécie.

Plant species	**Code**	**Height (m)**	**EFN per plant**	**EFN per leaf**	**Sugar concentration**	**Volume de nectar (µL/24h)**
Banisteriopsis campestris (Malpighiaceae)	BAC	0.5 ± 0.13	21.8 ± 11.3	2	36.6 ± 8.9	0.2 ± 0.1
Banisteriopsis malifolia (Malpighiaceae)	BAM	0.94 ± 0.32	278.8 ± 184.0	2	8.3 ± 3.9	1.0 ± 0.6
Chamaecrista desvauxii (Caesalpinoideae)	CHD	0.58 ± 0.14	94.6 ± 43.2	1	11.8 ± 5.7	1.5 ± 0.9
Chamaecrista mucronata (*Caesalpinoideae*)	CHM	1.12 ± 0.34	515.7 ± 519.4	1	33.3 ± 15.1	0.8 ± 0.7
Crotalaria micans (Faboideae)	CRM	1.69 ± 0.42	742.9 ± 598.1	10	33.4 ± 7.4	7.7 ± 6.7
Heteropterys umbellata (Malpighiaceae)	HEU	1.17 ± 0.37	660.6 ± 488.2	2	49.9 ± 15.5	4.0 ± 4.1
Inga marginata (Mimosoideae)	INM	0.48 ± 0.08	36.1 ± 14.6	2	-	-
Inga sessilis (Mimosoideae)	INS	3.67 ± 1.14	3350.4 ± 3115.4	6	-	-
Peixotoa tomentosa (Malpighiaceae)	PET	2.53 ± 0.29	56.2 ± 34.8	2	11.2 ± 4.8	0.6 ± 0.2
Senna macranthera (Caesalpinoideae)	SEM	1.11 ± 0.17	16.0 ± 6.2	3	13.0 ± 6.7	0.6 ± 0.3
Senna renniformis (Caesalpinoideae)	SER	1.10 ± 0.22	278.8 ± 139.0	6	22.02 ± 7.3	0.8 ± 0.2
Stachytarpheta glabra (Verbenaceae)	STG	1.34 ± 0.39	91006.5 ± 106731.3	15	74.3 ± 2.5	2.9 ± 1.8

Tabela 2. Espécies de formigas estudadas na presente tese de doutorado entre 2011 e 2013. Todas as espécies foram observadas em Campo Rupestre, Ouro Preto (MG, Brasil).

- Family Formicidae
  - Subfamily Dolichoderinae
    - *Dorymyrmex brunneus* Mayr 1908
    - *Linepithema micans* Forel 1908
    - *Linepithema* sp. 1
    - *Linepithema* sp. 2
  - Subfamily Formicinae
    - *Brachymyrmex heeri* Forel 1974
    - *Brachymyrmex pictus* Mayr 1887
    - *Camponotus atriceps* Smith 1858
    - *Camponotus crassus* Mayr 1862
    - *Camponotus melanoticus* Emery 1894
    - *Camponotus novogranadensis* Mayr 1870
    - *Camponotus rufipes* Mayr 1775
    - *Camponotus senex* Smith 1858
    - *Myrmelachista catharinae* Mayr 1887
    - *Tapinoma* sp1
  - Subfamily Ectatomminae
    - *Gnamptogenys sulcata* Smith 1858
  - Subfamily Myrmicinae
    - *Cephalotes pusillus* Klug 1824
    - *Crematogaster* sp. 1
    - *Crematogaster* sp. 2
    - *Crematogaster* sp. 3
    - *Pheidole* sp. 1
    - *Pheidole* sp. 2
    - *Pheidole* sp. 3
    - *Pheidole* sp. 4
    - *Wasmannia* sp. 1
    - *Wasmannia* sp. 2
    - *Wasmannia* sp. 3
    - *Wasmannia* sp. 4
  - Subfamily Ponerinae
    - *Pachycondyla* sp. 1
    - *Pachycondyla* sp. 2
  - Subfamily Pseudomyrmicinae
    - *Pseudomyrmex gracilis* Fabricius 1804
    - *Pseudomyrmex pallidus* Smith 1855
    - *Pseudomyrmex termitarius* Smith 1855

Figuras

Figura 1. Mapa do Parque Estadual do Itacolomi (PEIT), localizado entre Ouro Preto e Mariana (MG, Brasil). Mapas na lateral esquerda localizam o PEIT em diferentes escalas geográficas. Pontos 1, 2, 3 e 10 representam as áreas de estudo contempladas nessa tese de doutorado. (Fonte: IBGE).

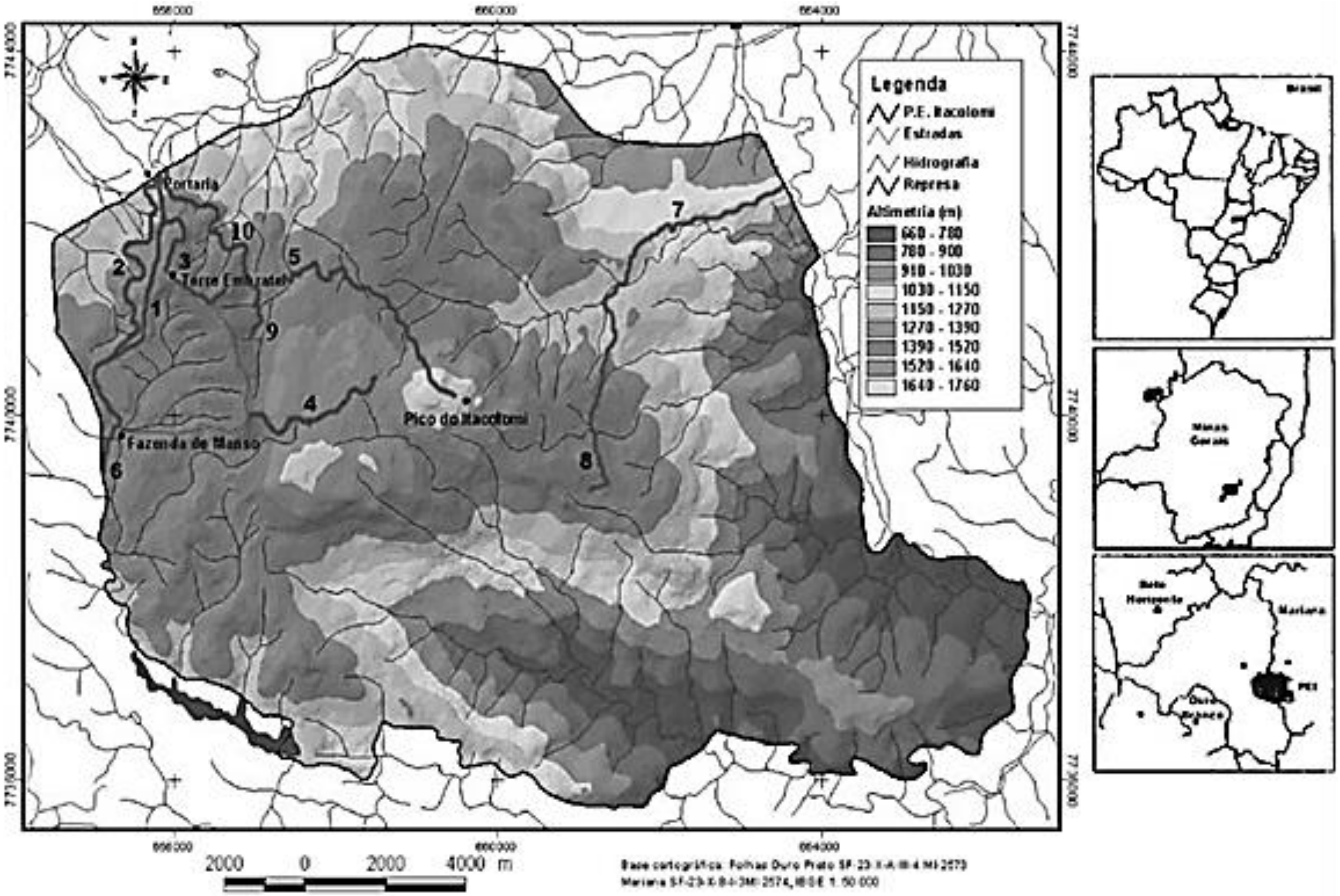
Figura 2. Panorâmica da Floresta Estacional Semidecidual (Inferior) e dos Campos Rupestres (Superior). Imagem capturada na região da Fazenda do Manso, interior do Parque Estadual do Itacolomi, entre Ouro Preto e Mariana (MG, Brasil). (Foto: Fagundes R, 2008). Barra de escala: aproximadamente 1m (objeto mais próximo).

Figura 3. Panorama dos diferentes tipos de Campo Rupestres contemplados no presente estudo. Imagens capturadas no interior do Parque Estadual do Itacolomi, Ouro Preto (MG, Brasil). (a) Floresta Estacional nos vales e Campo Rupestre nos topos de morro. (b) Região de transição entre o Campo Graminoso (Superior) e o Escrube sobre filito (Inferior). (c) Detalhe da Campo Rupestre do Tipo Escrube. (Fotos: Fagundes R, 2012). Barra de escala: aproximadamente 1m (objeto mais próximo).

Figura 4. Espécies de plantas portadoras de nectários extraflorais estudadas entre 2011 e 2014 em Campo Rupestre, Ouro Preto (MG/Brasil). Detalhe do nectário extrafloral no canto superior esquerdo. Barra de escala: 1cm. Siglas: (BAC) Banisteriopsis campestris, (BAM) *B. malifolia*; (CHD) *Chamaecrista desvauxii*; (CHM) *C. mucronata*; (CRM) *Crotalaria micans*; (INV) *Inga vulpina*; (HEU) *Heteropterys umbellata*; (PET) *Peixotoa tomentosa*; (SEP) *Senna pendula*; (SER) *S. reniformis*; (STG) *Stachytarpheta glabra*; (INS) *I. sessilis* (Foto: Fagundes R, 2014).

Figuras

Figura 1



Base cartográfica: Folhas Ouro Preto SF-23-X-A-III-4 MI-2573
Mariana SF-23-X-B-I-3MI-2574, IBGE 1: 50 000

Figura 2

Figure 3

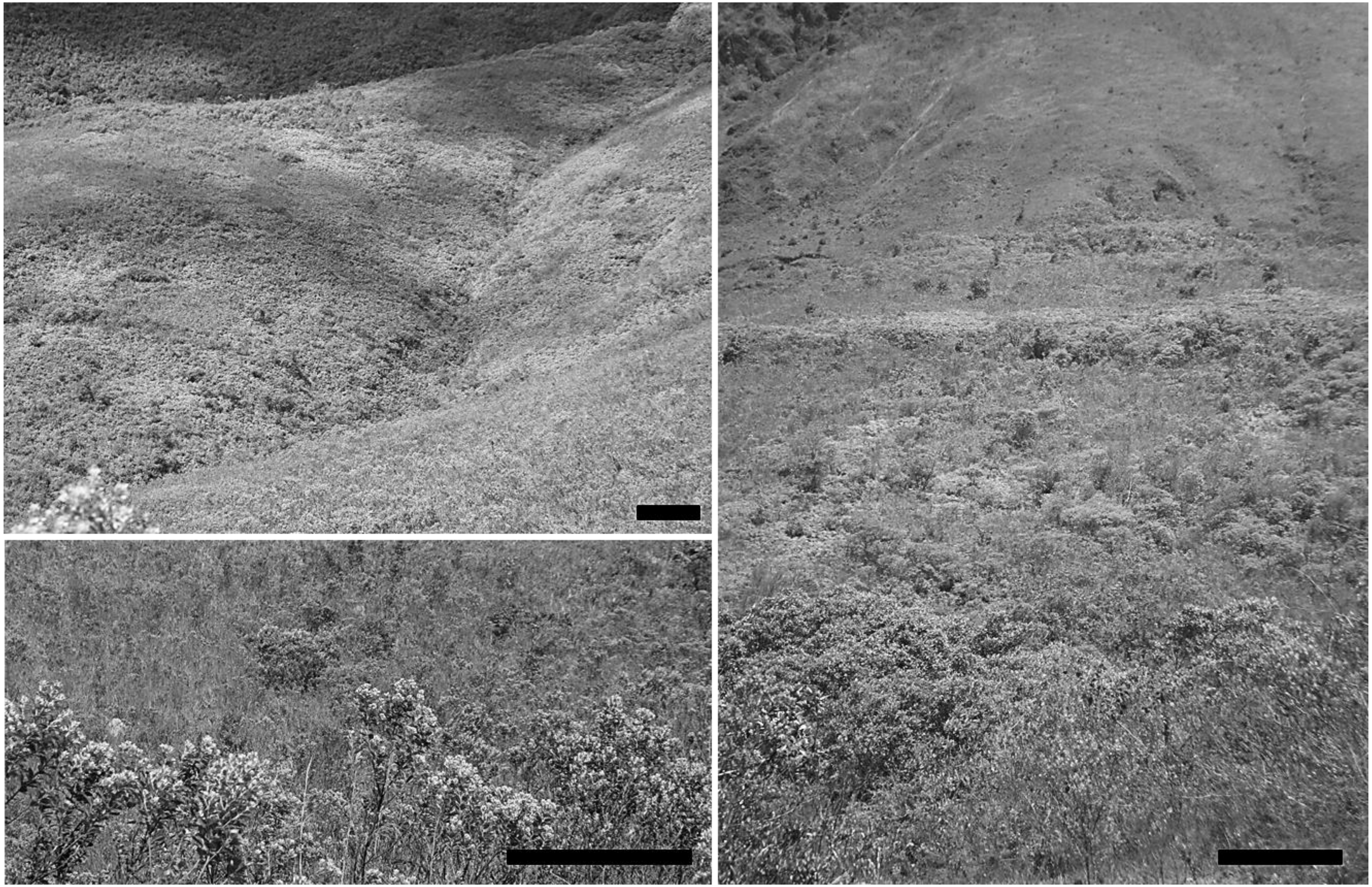
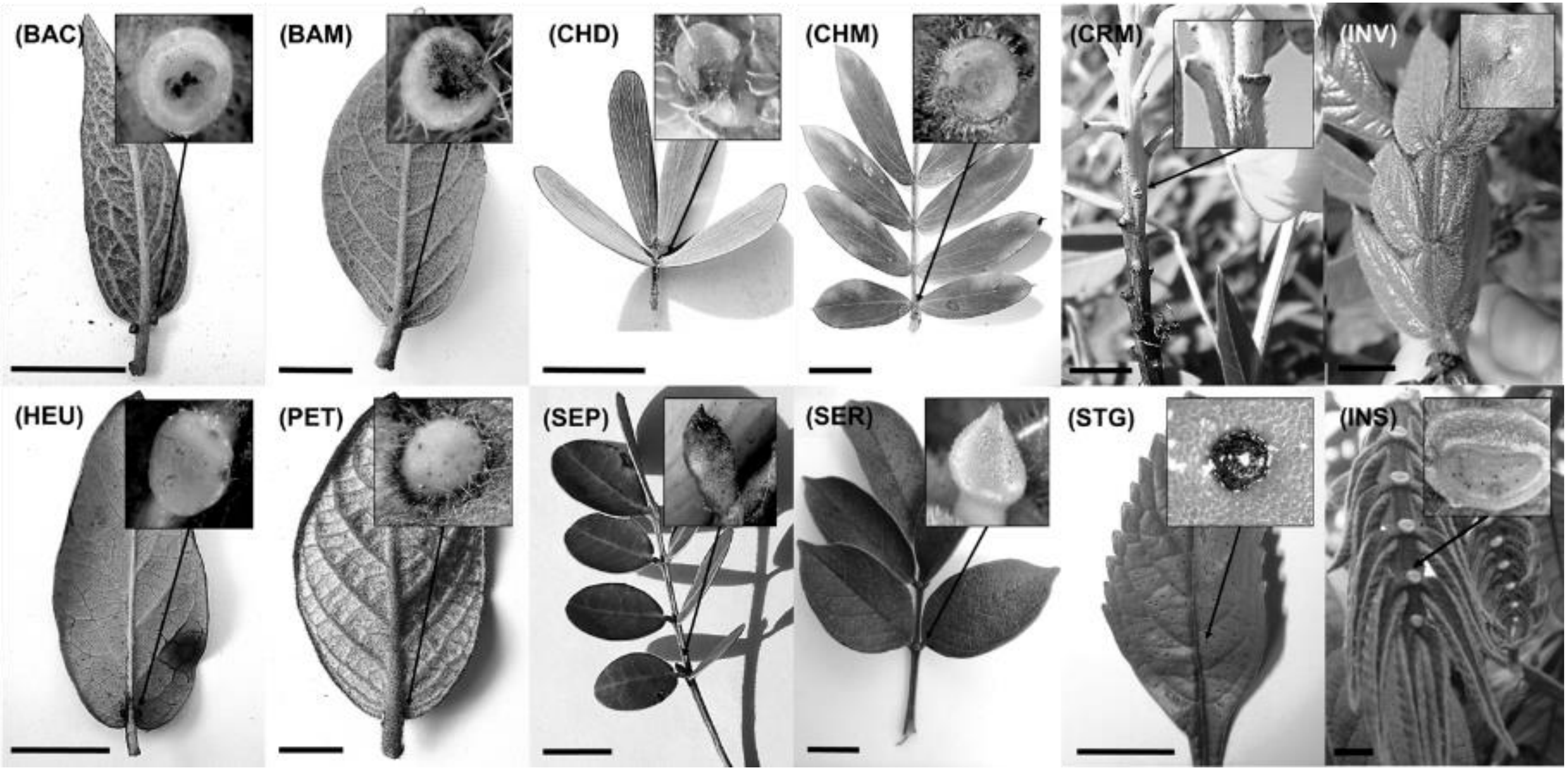


Figure 4



Capítulo 1

Disponibilidade de recursos e dominância interespecífica como mecanismos de estruturação de redes multitróficas formiga-planta-hemíptero

Resource availability and interspecific dominance as structural mechanisms of ant-plant-hemipteran multitrophic networks

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Resumo

Neste estudo, avaliou-se a disponibilidade e a dominância de recursos (nesse caso néctar e *honeydew*) como fatores reguladores das interações formiga-planta-hemíptero. Foram estudadas 12 espécies de plantas (240 arbustos) e 12 espécies hemíptero (240 agregações) em interação com formigas em uma área de Campo Rupestre, Brasil. Observou-se que espécies de formigas que coletam *honeydew* (7 espécies) são um subconjunto das espécies de formigas que se alimentam de néctar (25). Especificamente, a sub-rede de interações entre formigas e plantas exibiu um padrão aninhado de interações e baixo grau de especialização, como esperado para mutualismos. No entanto, a rede de interações entre formigas e hemípteros apresentou padrão aninhado mas revelou-se altamente especializada. Observamos que o núcleo central de espécies altamente interativas foi o mesmo para ambas as sub-redes, apesar das sub-redes apresentarem diferentes estruturas. Por conseguinte, estas duas interações baseadas em recursos devem ser consideradas como uma única rede multitróficas. Além disso, verificou-se uma forte relação entre a disponibilidade de NEFs e a quantidade de interações com formigas. No entanto, hemípteros abundantes não interagiram com mais espécies de formigas, provavelmente devido dominância numérica da formiga protetora. Tomados em conjunto, nossos resultados mostram a importância da disponibilidade de recursos para a estruturação de redes multitróficas de interações formiga-planta-hemíptero.

Palavras-chave: comportamento de dominância, aninhamento, partilha de recursos, especialização, trofobioses, competição interespecífica.

Abstract

In this study, we assessed the availability and dominance of sources of sugar-based resources, nectar of extrafloral nectaries and honeydew of sap-sucking hemipterans, as structural factors of ant-plant-hemipteran multitrophic networks. We studied 12 plant species (240 shrubs, 20 per species) and 12 hemipteran species (240 aggregations, 20 per species) interacted with 26 ant species in an area of Rupestrian Fields (Rocky Montane Savannah), Brazil. We observed that ant species that collected honeydew were a subset of the species of ants that fed on nectar. Specifically, the ant-plant subnet exhibited a nested pattern of interaction with low degree of specialization. However, the ant-hemipteran subnet presented lower nestedness but higher specialization than ant-plant subnet. Moreover, we observe that, in spite of the ant-plant and ant-hemipteran subnets being structurally different, the central core of highly interacted species was the same for both subnets. Therefore, these two resource-based interactions should be considered as a single multitrophic network. In addition, we found a strong relationship between the offer of EFNs and the amount of interactions with ants. However, hemipteran species that were most abundant did not interacted with more species of ants, probably due to the numerical dominance of the tending species. Moreover, 12 ant species accessed host plants but did not interacted with hemipterans due to inference by the dominant tending specie. Indeed, segregation between ant species interacting with plants and hemipterans was higher than expected by chance. Taken together, our results show the importance of the resource offer structuring networks of ant-plant-hemipteran interactions since availability of food sources increased frequency of interactions, although it also increased species dominance and reducing interaction sharing.

Key words: dominance behavior, nestedness, resource partitioning, specialization, trophobiosies, interspecific competition.

Introduction

The activity of ants on plants can be higher in the presence of predictable and highly nutritious food resources, such as nectar secreted by plants bearing extrafloral nectaries (Blüthgen et al. 2000; Davidson et al. 2003) and honeydew excreted by some sap-sucking Hemiptera (Blüthgen et al. 2000; Del-Claro 2004). Extrafloral nectar, and possibly honeydew, enhances the survivorship and growth of ant colonies (Byk and Del-Claro 2011). Thus, an advantageous resource for ants. In return, ants can capture natural herbivores of the plants, as well as predators of hemipterans, therefore increasing the reproductive success of plant and hemipteran partners (Moreira and Del-Claro 2005; Nascimento and Del-Claro 2010; Fagundes et al. 2013). At the community level, ants interact with EFN-bearing plants generating complex networks of species interactions (Bascompte and Jordano 2007). In these networks, species are represented as nodes and the interactions among them are represented as links. Usually, networks of interactions between EFN-bearing plants and ants are described by a low degree of network specialization and a highly nested pattern of interactions (i.e., species with fewer interactions are connected to species with a larger number of interactions in cohesive subgroups) (Bascompte and Jordano 2007; Guimarães-Jr et al. 2007; Chamberlain et al. 2010; Dáttilo et al. 2013). However, the structure of interactions between ants and honeydew–hemipterans are poorly known, although it may follow the nested pattern expected for mutualistic interactions (Staab et al. 2014).

Plants with EFNs and honeydew–producer hemipterans are ecological equivalents in creating mutualisms with ants (Delabie 2001; Del-Claro 2004; Rico-Gray and Oliveira 2007), although honeydew and nectar are available in very distinct forms for ants. EFNs are small and scattered sources of food that secrete nectar occasionally over the whole plant crown, which becomes a large but unpredictable patch of food sources (Heil et al. 2000; Rico-Gray and Oliveira 2007). In this case, ant species able to quickly find active nectaries and remove the nectar, discovery competitors, are more successful in competing for the resource and stablish stable interactions (Davidson et al. 2003). In contrast, honeydew–hemipterans live in aggregations of adults and broods limited to meristems and plant reproductive structures (Del-Claro 2004; Blüthgen et al. 2006), and represent patches of a fixed food source that produce honeydew continuously and regulated by ant tending behavior (Delabie 2001; Del-Claro 2004). In this case, territorial species that are more efficient in defending constant sources of renewable resources, interference competitors, would be better successful in forming stable interactions with hemipterans. Therefore, the compatibility of resource type offered and the foraging behavior of Ant species would regulate the composition of ants interactive. While the competition for the resource would set the variance between ants in the frequency of interactions. Thus, plants and Hemiptera should interact with a particular composition of Ant species.

Moreover, the composition of the resource may also affects the interactions. Extrafloral nectar is a solution that mostly contains sugars (especially fructose) mixed with small portions of amino acids, lipids, phenols, alkaloids and volatile compounds (Blüthgen et al. 2004). Honeydew, on the other hand, can be a more nutritious food, composed of undigested sap (mainly glucose, sucrose and fructose), the hemipteran-specific sugar melazitose, and a mixture of amino acids, amides, fatty acids, alcohols and minerals (Blüthgen et al. 2004; Blüthgen and Fiedler 2004). The nutrient composition of the reward is an important filter for the formation of interactions because some ant species have preferences and specializations to consume certain compounds (Blüthgen and Fiedler 2004). Few studies compared ant-plant with ant-hemipteran interaction within the same area as components of multitrophic networks (Becerra and Venable 1989; Blüthgen et al. 2000; Campos and Camacho 2014). This approach is important to understand ant mutualistic interactions, since ant species could explore both resources but competition for valuable and limited resources regulated by bottom-up effects of resource availability and quality may regulate ant interactions (Blüthgen et al. 2000; Davidson et al. 2003; Blüthgen and Fiedler 2004).

Ant–plant and ant–hemipteran interactions are resource-based, and it is our understanding that resource availability might have stronger effects structuring mutualism outcomes involving ants (Lange et al. 2013, Lange & Del-Claro, 2014). Differences in quality, quantity and availability of food resources might reflect in the amount and diversity of interacting ant species (Blüthgen et al. 2000; Del-Claro 2004), driven the network structure and regulating the mutualism outcomes (Chamberlain et al. 2010; Lange et al. 2013; Lange and Del-Claro 2014). Competitively superior ants could monopolize the most limited, nutritious and energetic food resources and allow the coexistence of only a few other submissive ant species (Blüthgen and Fiedler 2004; Fagundes et al. 2012). Different interacting species presents different protection capabilities that may change the outcomes of the mutualism (Heill & McKey 2003; Lange and Del-Claro 2014), reducing or increasing the benefits, and consequentially regulating co-evolutionary processes (Lange and Del-Claro 2014; Diaz-Castelazo et al. 2004). Therefore, it is necessary to determine the mechanisms that structure mutualistic interactions in order to understand the regulatory processes of mutualism efficiency and its evolutionary potential.

In this study, we analyzed the interactions among ants, EFN–plants and hemipterans. Specifically, we evaluated how the availability of food resources affects the structure of ant-plant-hemipteran network. We hypothesized that EFN-bearing plants and honeydew-producer hemipterans do not equally provide food sources for ants. Therefore, the network of ant-plant and ant-hemipteran interactions should not have the same structure with differences in the composition of the interacting species and its interaction frequency. We hypothesized that ant species will dispute the least available resource, i.e. more limited, leading to low sharing of interactions between ant species. This mechanism would lead to a lower number of interactions and a network structure less nested but more modular and specialized regarding the most limited resource, nectar or honeydew. Thus, we seek to demonstrate how variation between mutualists in investment and specific characteristic of ant rewards may affect the structure of multitrophic ant-plant-hemipteran networks.

Methods

Study area

We recorded ant-plant and ant-hemipteran interactions at the Parque Estadual do Itacolomi (PEIT), Ouro Preto (MG, Brazil). The study site occupies an area of approximately 7,000 ha of Atlantic Forest and Savanna, at an elevation of 700 to 1,770m a.s.l. (Giuletti *et al.*, 1997). The climate in the region is subtropical humid (Cwb, based on Köppen classification), with a mean annual temperature of 21°C and mean annual precipitation of 2,018 mm (Giuletti *et al.*, 1997). We conducted the survey in Rupestrian Fields (rocky montane savanna); an ecosystem with rock outcrops surrounded by continuous grassland with small, tortuous bushes and woody vegetation both in rock crevices or nested within large vegetation patches (Giuletti et al. 1997; Alves et al. 2014).

Ant-plant-hemipteran interactions

We searched for interactions between ants and EFN–plants or honeydew–hemipterans between August and September of 2011. We sampled an area of approximately 6ha (20°24'S and 43°30'W, elevation 1, 150m a.s.l). We walked the area for eight consecutive hours to identify the hemipteran and plant species interacting with ants, which resulted in 12 species of plants and 12 species of hemipterans (Table 1 and 2, Appendices 1 and 2). After that, we marked

20 individual shrubs bearing EFNs and 20 shrubs hosting hemipteran aggregations (a group of adults and immatures) for each species found to equalize sampling effort (total of 240 plants and 240 hemipterans host-plants). Shrubs were at least 10 m apart. We observed each plant once in three periods at day: 8:00–11:00 h, 12:00–15:00 h and 16:00–19:00 h. For each observation, we followed the foraging ant until it collected nectar or honeydew (interaction), or left the plant (no interaction). We repeated these observations four times, each seven days. In every shrub, we identified the ant species (number of interactions) and quantified the number of workers per species (recruitment).

Resource-consumer dependency

We measured the availability of resources by the number of plants with EFNs or hemipterans in the area (large scale) and by the mean number of EFNs per plant and hemipterans per aggregation (small scale). First, we counted the number of plants and hemipteran aggregations in the area inside three parallel transects 300 × 1 m, spaced for 500 m within the area used to sample the interactions. Second, we measured the number of EFNs on each plant and hemipterans on each aggregation using the same 20 plants and 20 aggregations sampled for interactions. For hemipterans, we count all hemipteran individuals on per aggregation of each hemipteran species. For plants, we estimated the number of EFNs per plant based on the method proposed by Blüthgen et al. (2000). We counted the number of leaves of three random branches to extract a mean value and then multiplied it by the number of total branches on the plant. We multiplied the estimated number of leaves by the number of EFNs per leaf to estimate the number of EFNs per plant. We also measured the height of the shrub, because plant size might affect EFN number. We divided the estimated number of EFNs by the plant height to access an EFN–density measure. Note that density of EFNs and hemipterans might be a good approximation of resource offer, but it does not estimate nectar or honeydew production directly (Chamberlain et al. 2010).

We identified ants and hemipterans by comparison with specimens from the entomological collection of the Department of Biodiversity, Evolution and Environment of Federal University of Ouro Preto (UFOP, Brazil). We identified the plants at the herbarium José Badini (OUPR) of the same university (UFOP).

Data analysis

For network analyses, we defined each type of interaction (ant–plant or ant–hemipteran) as an adjacency matrix ***"A"***, where "A_{ij}" is the number of interactions between a plant or a hemipteran species “i*”* with an ant species “*j”*, and zero is the absence of interactions. For each sub-network, we calculated the following network descriptors: the number of interactions per species (average degree), network specialization (H_2’ index, Blüthgen *et al.*, 2006), nestedness, and modularity. To evaluate nestedness we used the NODF-metric in the ANINHADO software (Guimarães-Jr and Guimarães 2006). We tested modularity using index (M), based on Simulated Annealing (SA) using the software MODULAR (Marquitti *et al.,* 2014). We estimated the significance of nestedness and modularity in the ant–plant and ant–hemipteran networks using the Null Model II (n= 1,000 randomizations for each network). We characterized the generalist core (i.e., those with the most interactions) and peripheral components (i.e., those with few interactions) according to Dáttilo et al. (2013).

We tested the difference in interactions frequency between ant-plant and ant-hemipteran interactions using Pearson’s correlation. If plant and hemipterans interacts with ants differently, we expected that ant species with more interactions with plants had less interaction with hemipterans, and vice-versa. Otherwise, the same species could interacted with both plants and hemipterans; therefore, we expected that ants with more interaction with plants would have more interactions with hemipterans. Additionally, we evaluate if the interactions of ants with individual plants or hemipteran aggregations would be overlapped, indicating interaction sharing, or segregated, indicating competition for interactions. For this, we use the analysis of co-occurrence based on C-score and tested by iteration of Monte Carlo (Gotteli 2000). In this analysis, we grouped species that shared interactions with each plant individuals or hemipteran aggregations and then calculated the C-score index of co-occurrence as an index of co-interaction. Then, 1,000 randomizations of the co-occurrence matrices generated a random C-score. If the observed C-score is statistically higher than the random C-score, one can consider that the community organization is probably not random but deterministic and species segregation is higher, such as in communities structured by competition, as we hypothesized. If the observed C-score is lower than expected, ant species share high number of interactions and co-exist on the same plant or tending a hemipteran aggregation.

We evaluated the limitedness of nectar and honeydew by comparing the number of food sources offered by plants and hemipteran species with t-test. For this, we used the average number of EFNs per plant and the average number of hemipterans per aggregation. We compared ant recruitment and interactions between plants and hemipteran species using t-test (n = 12 for EFN–plants and n = 12 for hemipterans). For this, we used the total number of ant workers (recruitment) and species (interactions) observed in each EFN–plant or hemipteran species as independent cases (n = 12 for EFN–plants and n = 12 for hemipterans). We evaluated the effect of resource availability on the number of interactions with ants and ant recruitment (ants per plant or ants per aggregation) using General Linear Model (GLM). We used the local abundance of EFN–plants and the average number of EFNs per plant as predictors for the number of interactions with ants (dependent variable). We used the same analysis for the number of hemipterans aggregations and the average number hemipterans per aggregation as predictors of ant interactions. Factors were not self-correlated (Pearson: $p > 0.05$).

We transformed variables using log transformation (base 10) to meet the assumptions of the statistical analyses. All statistical analyses were performed in R (R development Core Team 2009).

Results

Ant-plant-hemipteran interactions

We observed 11,268 ants distributed among 38 species (Table 3), in which 2,521 ants (22% of total abundance, 2 species) collected nectar from 240 shrubs of 12 plant species (Table 1; Figure 1), and 7,938ants (70% of the total, 7 species) were observed harvesting honeydew from 12 hemipterans species (Table 2, Figure 1). The remaining 809 ants (8%, 12 species) climbed plants but did not accessed EFNs or hemipterans (Table 3). Six ant species interacts with both plants and hemipterans, 19 were exclusively interacted with plants and only one species exclusively interacted with hemipteran. *Camponotus crassus, Camponotus novogranadensis* and *Brachymyrmex heeri* were the most interacted species (*i.e.*, components of the generalist core) in the ant–plant subnetwork, whereas only *C. crassus* comprised the generalist core of the ant–hemipteran subnetwork (Figure 1). The number of interactions per species (average degree = 2.48) were higher in ant-plant compared to ant-hemipteran subnetwork (1.21). The ant–plant subnetwork was more generalized ($H_2' = 0.31$) compared to the ant–hemipteran subnetwork ($H_2' = 0.54$). We observed that the ant–plant subnetwork exhibited higher nestedness ($NODF = 44.81$, $p = 0.03$) than ant–hemipteran sub-network ($NODF = 37.25$, $p = 0.21$), but lower modularity ($M = 0.314$, $p = 0.38$) than ant-hemipteran subnetwork ($M = 0.412$, $p = 0.58$). Although, only ant-plant network presented a nested pattern of interactions higher than expected by null models.

We observed that ant–plant and ant–hemipteran subnetworks were structurally different but linked by the same network core of ant species (Figure 1). We found that ant species that interacted with more plant species also interacted with more hemipteran species (Pearson r =

0.74, *p* = 0.001, n=26) (Figure 1). However, we observed that different ant species partitioned the interactions with EFN-plant more often than expected by chance (C-score: $C_{observed} = 3.87$, $C_{random} = 3.53$, $p < 0.001$). C-score were also higher than expected by chance for ant-hemipterans interactions ($C_{observed} = 0.7$, $C_{random} = 0.66$, $p < 0.001$), indicating that ant species partitioned many hemipterans aggregations. Therefore, both interactions may be structured by deterministic factors regulating ant interactions leading to low interaction sharing between ant species. This segregation could be weaker for ant-plant interactions, because we found several ant species sharing individual plants at the same time, especially for EFN-rich species (Table 3). On the other hand, we observed only one or two ant species tending hemipteran aggregations and the species did not change over time. *Camponotus crassus* monopolized 128 aggregations (53.3% of 240), while the other six species monopolized 61 aggregations (25.4%) or coexisted submissively with *C. crassus* (51 aggregations, 21.3%; Table 3).

Resource availability regulating interactions

Plants with EFNs represented 41% of the shrub abundance in the area, and aggregations of honeydew–hemipterans occurred on 24% of the shrubs (204 samples; Tables 2 and 3). We did not found EFN-bearing plants hosting hemipterans. In general, plant species provided more food sources (Mean ± SE: 691.3 ± 397.9 EFNs per plant, n = 12 plant species) than hemipteran species (58.3 ± 24.5 hemipterans per aggregation, n = 12; t-test: t = 3.37, d.f. = 22, p = 0.003 (Figure 2). Moreover, the mean number of ant species that interacted with plants (8 ± 0.9, n = 12) was higher compared to that for hemipterans (1.9 ± 0.2, n = 12; t-test: t = 7.8, d.f. = 22, $p < 0.001$) (Figure 3). However, the mean number of ant workers recruited for plant species (17.6 ± 3.7 ants per plant species, n = 12) was not different compared to that for hemipteran species (55.1 ± 17.2, n= 12; t = 0.1, d.f. = 22, $p < 0.9$) (Table 1 and 2).

We observed that plant species most abundant in the area (GLM: $R^2 = 0.61$, $\beta = 0.01$; $F_{1,9} = 5.9$, p = 0.03) and with largest number of EFNs per plant ($\beta = 0.17$; $F_{1,9} = 8.3$, p = 0.01) interacted with most ant species (Figure 2). Accordingly, ant recruitment was higher in most abundant plants (GLM: $R^2 = 0.76$, $\beta = 5.31$, $F_{1,9} = 14.9$, p = 0.003) with greater number of EFNS ($\beta = 0.08$; $F_{1,9} = 13.9$, p = 0.004). Nevertheless, hemipteran species with the largest abundance in the area and with higher number of individuals per aggregation did not have a greater recruitment (GLM: R2 = 0.16, $F_{1,9} = 0.8$, p = 0.4; $F_{1,9} = 0.9$, p = 0.4) and number of interactions with ant species (GLM: $R^2 = 0.06$, $F_{1,9} = 0.04$, p = 0.8; $F_{1,9} = 0.5$, p = 0.5) (Figure 2).

Discussion

In this study, we show that hemipterans are less common than EFN bearing plants and the availability of honeydew and nectar sources varied among species affecting the whole structure of ant-plant-hemipteran network. Ant species that fed on honeydew were part of the ant species assemblage that also fed on nectar, and these shared species were the most connected ants. This result confirmed our hypothesis that different characteristics of nectar and honeydew would lead to a different composition of ant species interacting with plants or hemipterans. Plants presented more interactions with ants than hemipterans, because all ant species that climbed EFN-plants collected nectar at least once, while ant recruitment was higher for hemipterans, because few interacted species monopolized aggregations by numerical dominance. In fact, ant species commonly observed collecting nectar had visited the host plant but were not able to access honeydew due to interference of tending-ants. Consequentially, as we predicted, variation in frequencies of interactions with ant species leads to structural differences between ant–plant and ant-hemipteran subnetworks. Ant-plant subnet was more nested than modular, highly generalized presenting few interactions with dominant ants and several interactions with low frequent species. On the other hand, ant-hemipteran subnet was more modular than nested, highly specialized, with few interactions per species and only one core species, *C. crassus*, that monopolizes more than half aggregations. Therefore, EFN availability increased the number of interactions between ants and plants while territoriality around hemipterans prevented interaction sharing between ant species. Ant dominancy is common for tree-dwelling ants collecting honeydew, but rare for ant-plant interactions (Majer et al. 1994; Blüthgen et al. 2000; Chamberlain et al. 2010; Campos and Camacho 2014).

In ant-plant network, we observed that plant species with more EFNs attracted more ant workers and interacted with more ant species. Three complementary hypothesis explained this scenario. Firstly, the whole plant can be considered as a large-fixed patch of food resources for ants, and the greater the amount of food, the greater the number of ants needed to collect nectar (Parr and Gibb 2010). Secondly, advantageous resources (energetic and nutritionally), but shortly limited, allow the coexistence of many species due to low competition (Davidson et al. 2003; Blüthgen et al 2004; Blüthgen and Fiedler 2004). The high availability of nectar source may attract to plants several ant species avoiding aggressive encounter with hemipteran-tending species since ants may change between these two resources according with this limitedness (Moya-Raygoza & Larsen 2001; Katayama and Suzuky 2003). Third, temporal unpredictability in the production of nectar (Lange et al. 2013; Falcão et al. 2014), does not induces forage fidelity and territoriality observed for stable fixed resources (Fagundes et al. 2013; Del-Claro 2004), which reduces even further the effects of interference competition and allows species to share interactions (Davidson et al. 2003; Blüthgen et al. 2006). However, discovery competition for nectar may be more important than interference in regulating ant-plant interactions (Davidson et al. 2003). Ant species that quickly found nectar droplets might had competitive advantage, reducing co-occurrence of species in plants with low EFN offer (Davidson et al. 2003).

In ant-hemipteran interactions, on the other hand, interference competition might be more important than discovery competition (Cushman and Addicott 1989, Fisher et al. 2001) because it is more energetically economical to maintain dominance of a fixed source that produces food continuously than search for new sources (Blüthgen et al. 2000; Parr and Gibb 2010). Hemipteran tending-ants presents forage fidelity and territoriality (Cushman and Addicott 1989; Fischer et al 2001; Del-Claro 2004), reducing the number of species sharing hemipterans (Davidson et al. 2003; Fagundes et al. 2012), even in species with large aggregations. In fact, we observed that few numerical dominant species monopolized the hemipteran aggregations. For example, *C. crassus* monopolized more than half of hemipteran aggregations and interacting with almost all species. Few other ant species co-occur with *C. crassus* in the same hemipteran aggregation acting as opportunists, what explain hemipteran species interacting with more than one ant specie. This segregation has been observed for ant-hemipteran interactions (Blüthgen et al. 2000; Fagundes et al. 2012; Campos and Camacho 2014), and may be an evidence of intense interference between ants to collect honeydew compare to nectar (Davidson et al. 2003; Blüthgen et al. 2007). Competitive mechanisms, based on resource dominance hierarchies (Dáttilo et al 2014a), might had created the simpler structure observed for ant-hemipteran networks and not coevolutionary processes of specialization because tending ants were highly generalists.

Several factors, including temperature, precipitation (Rico-Gray et al. 2012), phenology (Lange et al. 2013), abundance and body size (Chamberlain et al. 2010) can explain patterns in ant-plant interactions (Vazquez et al. 2009; Dáttilo et al. 2013; Dáttilo et al. 2014a). In this study, we show that resource availability is an important additional mechanism determining the number of interactions between plants and ants, but not between hemipterans and ants. Plants bearing EFNs were more abundant than hemipteran and plants presented ten times more food sources (i.e. EFNs per plant) than hemipterans (i.e. individuals per aggregation). This higher limitation of honeydew sources compare to nectar sources may had increased competition for hemipterans, and thus reduced hemipteran interactions to few ant species. On the other hand, the high availability of nectar sources attracted to plants several ant species avoiding aggressive encounter with hemipteran-tending species since ants may change between these two resources according with this limitedness (Moya-Raygoza & Larsen 2001; Katayama and Suzuky 2003). Moreover, partitioning of interactions between ant species was stronger for ant-hemipteran than ant-plant interactions although it was higher than expected by chance for both interactions. Ant species may compete for plants and hemipterans interactions because both are high valuable and limited resources, but competition should be stronger for most limited resources (Blüthgen et al. 2000; Delabie 2001; Del-Claro 2004), therefore context-dependent (Chamberlain et al. 2010).

In conclusion, this study showed that ant–plant and ant–hemipterans networks are structurally different, although both are essentially resource-based mutualistic interactions (Rico-Gray and Oliveira 2007). We suggest (1) that ant–plant networks are structured by resource availability, leading to highly connected and generalized networks, and resource competition (mainly discovery competition), since plants with fewer EFNs interacts basically with highly interacting and abundant species (resource dominants). In contrast, (2) ant–hemipteran networks are a result of resource dominance and territoriality, with few ants monopolizing almost all hemipterans aggregations, leading to a very specialized network with few interactions. More interesting, (3) we observed that both network are related since the ant species most connected with hemipterans was also highly connected with plants and those species are the most abundant in the area as they access more resources. This results support the hypothesis that the ant numerical dominance in the foliage is based on mutualistic resource use (extrafloral nectar and honeydew, Blüthgen et al 2000, 2006, Blüthgen and Fiedler 2004a, b). Therefore, our study provides basic information in how ant-plant-hemipteran networks are structured and suggest that both sub-networks should be considered as a single multitrophic network to better understanding of ant mutualistic interactions.

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Tables

Table 1. Characteristics of the EFN–plant species found associated with ants. The measures of resource availability and the properties presented in the interaction network are shown.

Plant species	Code	Local abundance	EFN per plant (mean ± S.D.)	Interactions with ants	Ants per plant (mean ± S.D.)
Banisteriopsis campestris	BAC	8 (10%)	301.4 ± 201.2	12	4.3 ± 1.7
Banisteriopsis malifolia	BAM	2 (2%)	42.1 ± 21.9	5	4 ± 1.9
Chamaecrista desvauxii	CHD	6 (7%)	177.9 ± 100.4	6	3.4 ± 1.4
Chamaecrista mucronata	CHM	14 (17%)	517.3 ± 521.1	13	2.8 ± 2.4
Crotalaria micans	CRM	13 (16%)	417.1 ± 309.6	6	6.9 ± 5.6
Heteropterys umbellata	HEU	8 (10%)	683.2 ± 608.8	9	4.9 ± 2.1
Inga sessilis	INS	2 (2%)	847.9 ± 592.9	9	4.6 ± 1.7
Inga vulpina	INV	6 (7%)	33.3 ± 13.9	8	5.6 ± 1
Peixotoa tomentosa	PET	5 (6%)	22.1 ± 12.5	5	3.7 ± 1.5
Senna pendula	SEP	4 (4%)	15 ± 6.4	3	3.8 ± 1.8
Senna renniformis	SER	1 (1%)	259.5 ± 125.8	7	6.8 ± 2.7
Stachytarpheta glabra	STG	15 (18%)	4,890.9 ± 4,374.8	13	12 ± 5

[1] The number of EFNs is an estimate based on the mean number of EFNs per branch multiplied by the number of branches (mean ± standard deviation, n = 20 plants).

Table 2. Characteristics of the twelve honeydew-producing Hemiptera tended by ant species. The measures of resource availability and the properties presented in the interaction network are shown.

Hemiptera species	**Code**	**Local abundance**	**Hemipterans per aggregation (mean ± S.D.; n = 20)**	**Interactions**	**Ants per aggregation (mean ± S.D.; n = 20)**
Achonophora sp. 1	AC1	4 (8%)	111.5 ± 70.5	2	5.1 ± 2.2
Aphis sp. 1	AP1	2 (4%)	305.9 ± 190.9	1	6.9 ± 1.9
‘’Bolbonata sp. 1	BO1	2 (4%)	7.8 ± 6.2	3	1.2 ± 2
Calloconophora sp. 1	CA1	12 (25%)	20.6 ± 20.3	2	2.6 ± 4.8
Ceroplastes sp. 1	CE1	1 (2%)	1.00	1	10.5 ± 1.9
Ceroplastes sp. 2	CE2	13 (27%)	16.1 ± 2.8	1	30.1 ± 23.9
Ceroplastes sp. 3	CE3	3 (6%)	2.4 ± 0.7	2	20.3 ± 3.9
Ceroplastes iheringi	CEI	2 (4%)	76.3 ± 28.1	2	3.1 ± 2.5
Ceroplastes lucidus	CEL	4 (8%)	15.5 ± 2.8	1	2.0 ± 0.9
Coccus viridis	COV	3 (6%)	18.2 ± 4.7	3	1.4 ± 0.9
Enchenopa sp. 1	EN1	1 (2%)	39.7 ± 42.6	3	3.2 ± 2.2
Pseudococcus sp. 1	PS1	2 (4%)	87.9 ± 24.5	2	12.7 ± 1.2

[1] Mean number of individuals of twelve colonies from each species (mean ± standard deviation).

Table 3. Ant species observed interacting with EFN–plants (EFN) and honeydew-producing hemipterans (HDW) and their properties in ant–plant and ant–hemipteran sub-networks. Ant species with no interactions represent species observed climbing shrubs (EFN-plants or hemipteran-host plants) but not collecting nectar or honeydew.

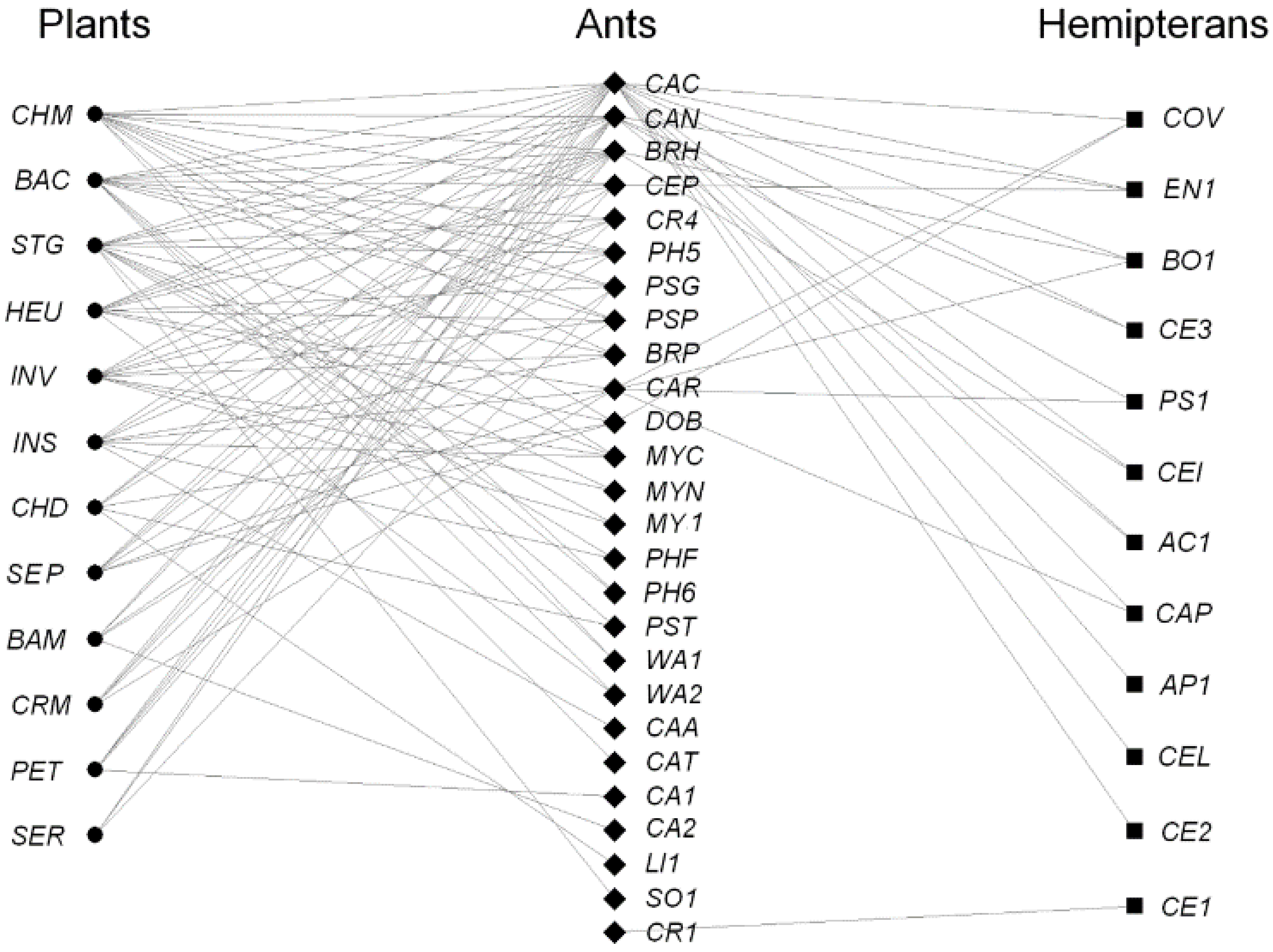
		Recruitment		**Interactions**	
Ant species	**Code**	EFN	HDW	EFN	HDW
Brachymyrmex heeri	BRH	98	29	9	2
Brachymyrmex pictus	BRP	41	0	4	-
Camponotus ager	CAA	28	0	1	-
Camponotus atriceps	CAT	8	0	1	-
Camponotus crassus	CAC	1159	4724	12	10
Camponotus novogranadensis	CAN	313	1761	10	4
Camponotus rufipes	CAR	96	1029	4	4
Camponotus sp. 1	CA1	44	15	1	-
Camponotus sp. 2	CA2	26	3	1	-
Camponotus sp. 3	-	0	1	-	-
Cephalotes pusillus	CEP	151	82	5	1
Crematogaster sp. 1	CR1	0	850	-	1
Crematogaster sp. 2	-	0	73	-	-
Crematogaster sp. 3	-	0	1	-	-
Crematogaster sp. 4	CR4	86	0	5	-
Dorymyrmex brunneus	DOB	31	12	4	1
Gnamptogenys sulcata	-	2	0	1	-
Linepithema sp. 1	LI1	5	0	1	-
Myrmelachista catharinae	MYC	121	0	4	-
Myrmelachista nodigera	MYN	40	0	2	-
Myrmelachista sp. 1	MY1	8	0	2	-
Pachycondyla sp1	-	0	3	-	-
Pheidole fallax	PHF	28	0	2	-
Pheidole sp. 1	-	0	10	-	-
Pheidole sp. 2	-	0	11	-	-
Pheidole sp. 3	-	0	3	-	-
Pheidole sp. 4	-	0	1	-	-
Pheidole sp. 5	PH5	36	0	4	-
Pheidole sp. 6	PH6	5	0	2	-
Pseudomyrmex gracilis	PSG	114	65	5	-
Pseudomyrmex pallidus	PSP	51	2	5	-
Pseudomyrmex termitarius	PST	9	0	2	-
Pseudomyrmex sp. 1	-	0	54	-	-
Pseudomyrmex sp. 2	-	0	1	-	-
Pseudomyrmex sp. 3	-	0	1	-	-
Solenopsis sp. 1	SO1	0	16	-	-
Wasmannia sp. 1	WA1	0	19	-	-
Wasmannia sp. 2	WA2	2	0	2	-

List of Figures

Figure 1. The tritrophic ant–plant–hemipteran network in the Rocky Montane Savanna (Brazil). Nodes depict plants (circles), ants (diamonds) and hemipterans (squares) and lines depict interactions based on ants collecting nectar from EFN–plants or honeydew from hemipterans. Ant species CAC, CAN and BRH composed the generalist core of ant–plant network, but only CAC composed the generalist core of ant–hemipteran network. Species codes are presented in Tables 1, 2 and 3.

Figure 2. Linear relationship between the number of interacting ant species and the number of sources of food (mean ± standard error): EFNs per plant (circle) or hemipterans per aggregation (square) ($R2 = 0.61$; $F_{1, 9} = 8.3$, $p = 0.01$) (a). Difference in the mean number of ant species interacting with EFN–plants and hemipterans ($t = 7.8$, $p < 0.001$) (b). Difference in the mean number of resources offered by plants and hemipterans (t-test: $t = 3.37$, d.f. = 22, $p = 0.003$) (c). Species codes are presented in Tables 1, 2 and 3. Note the log scale used.

Figure 1


Plants
Ants
Hemipterans
CHM
BAC
STG
HEU
INV
INS
CHD
SEP
BAM
CRM
PET
SER
CAC
CAN
BRH
CEP
CR4
PH5
PSG
PSP
BRP
CAR
DOB
MYC
MYN
MY1
PHF
PH6
PST
WA1
WA2
CAA
CAT
CA1
CA2
LI1
SO1
CR1
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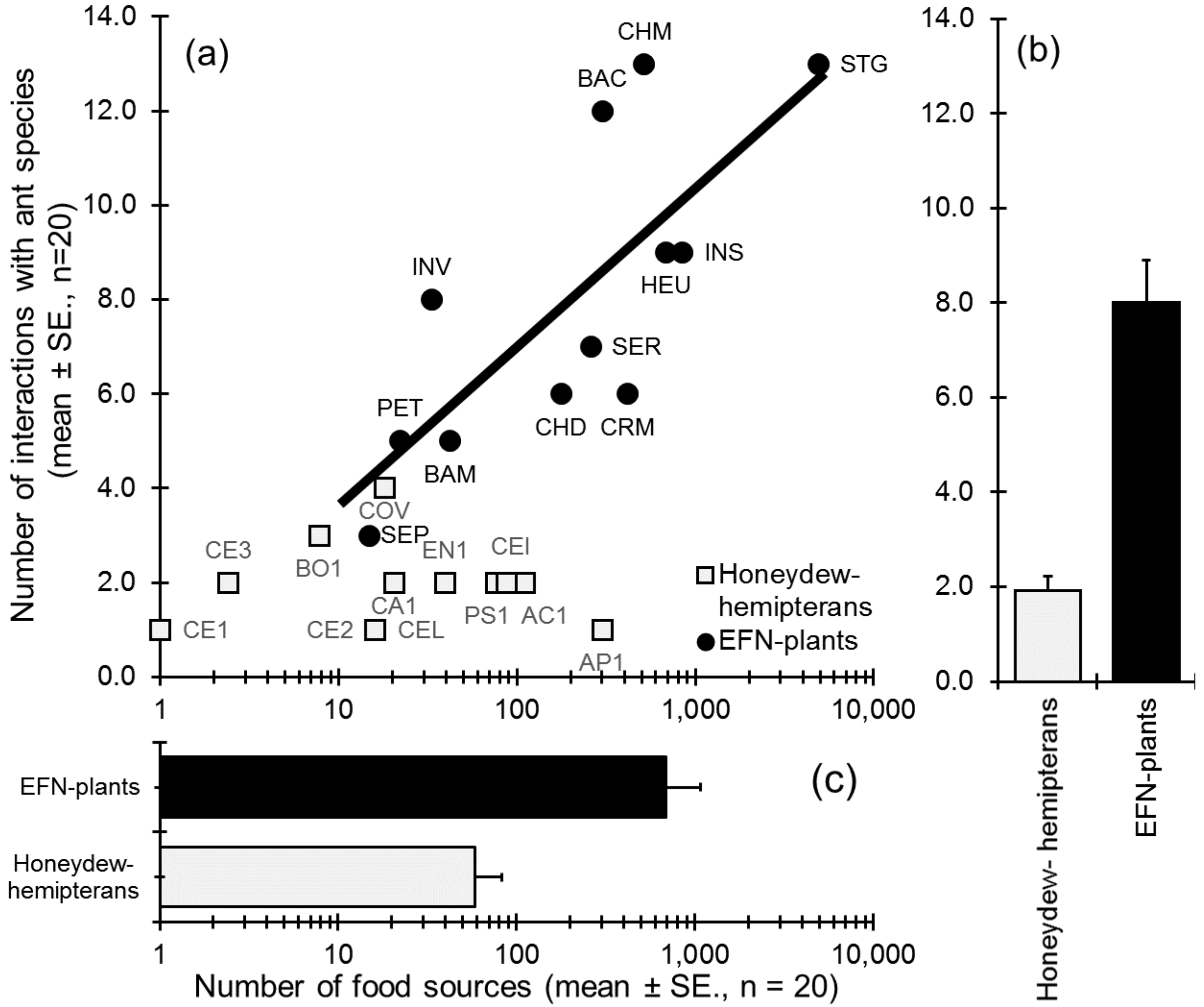
BO1
CE3
PS1
CEI
AC1
CAP
AP1
CEL
CE2
CE1

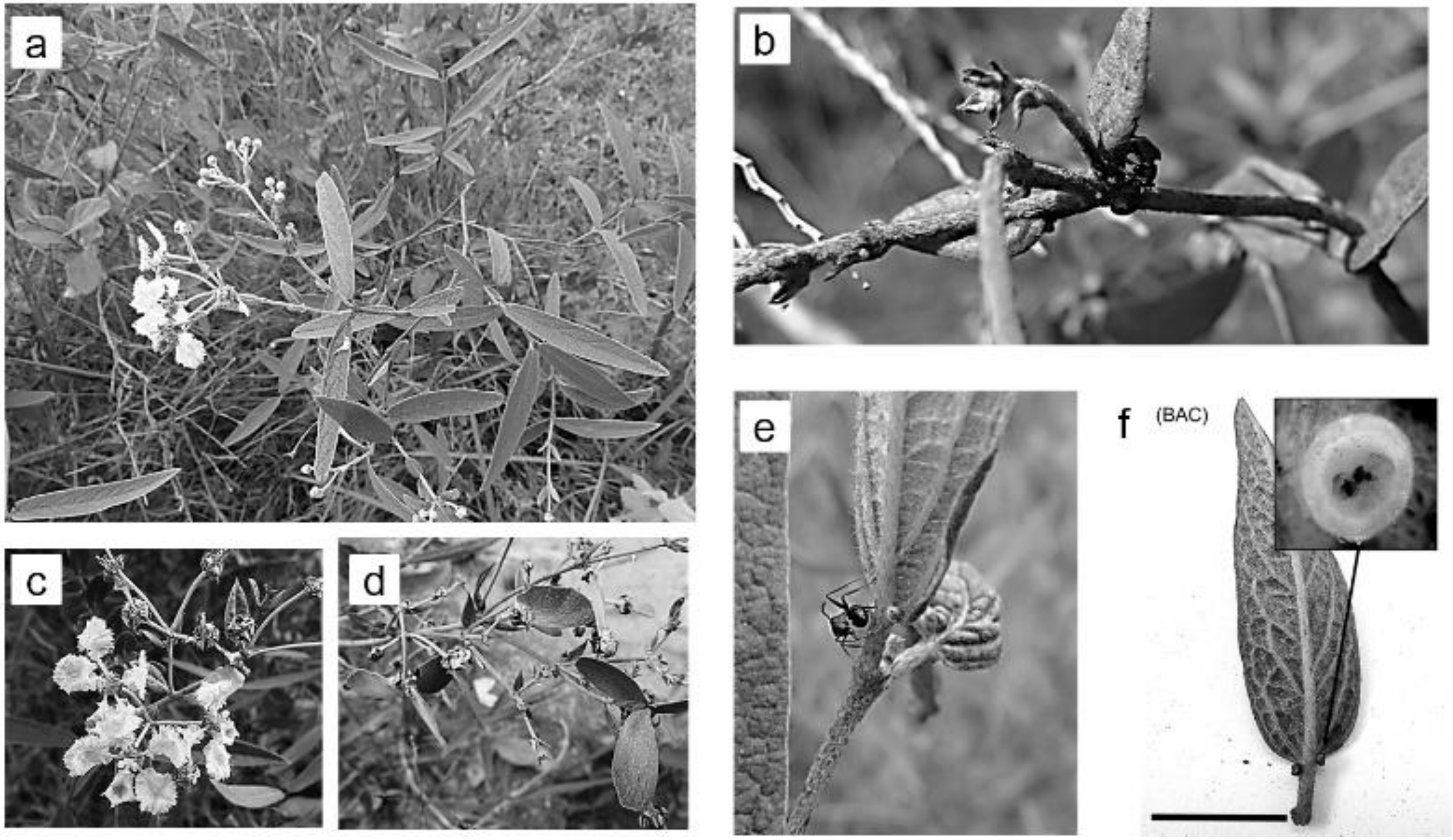
Figure 2


(a)
(b)
(c)
Number of interactions with ant species (mean ± SE., n=20)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
CHM
STG
BAC
INS
HEU
INV
SER
CHD
CRM
PET
BAM
COV
SEP
CE3
BO1
EN1
CEI
CE1
CE2
CA1
CEL
PS1
AC1
AP1
Honeydew-hemipterans
EFN-plants
1
10
100
1,000
10,000
Number of food sources (mean ± SE., n = 20)
Honeydew- hemipterans
EFN-plants

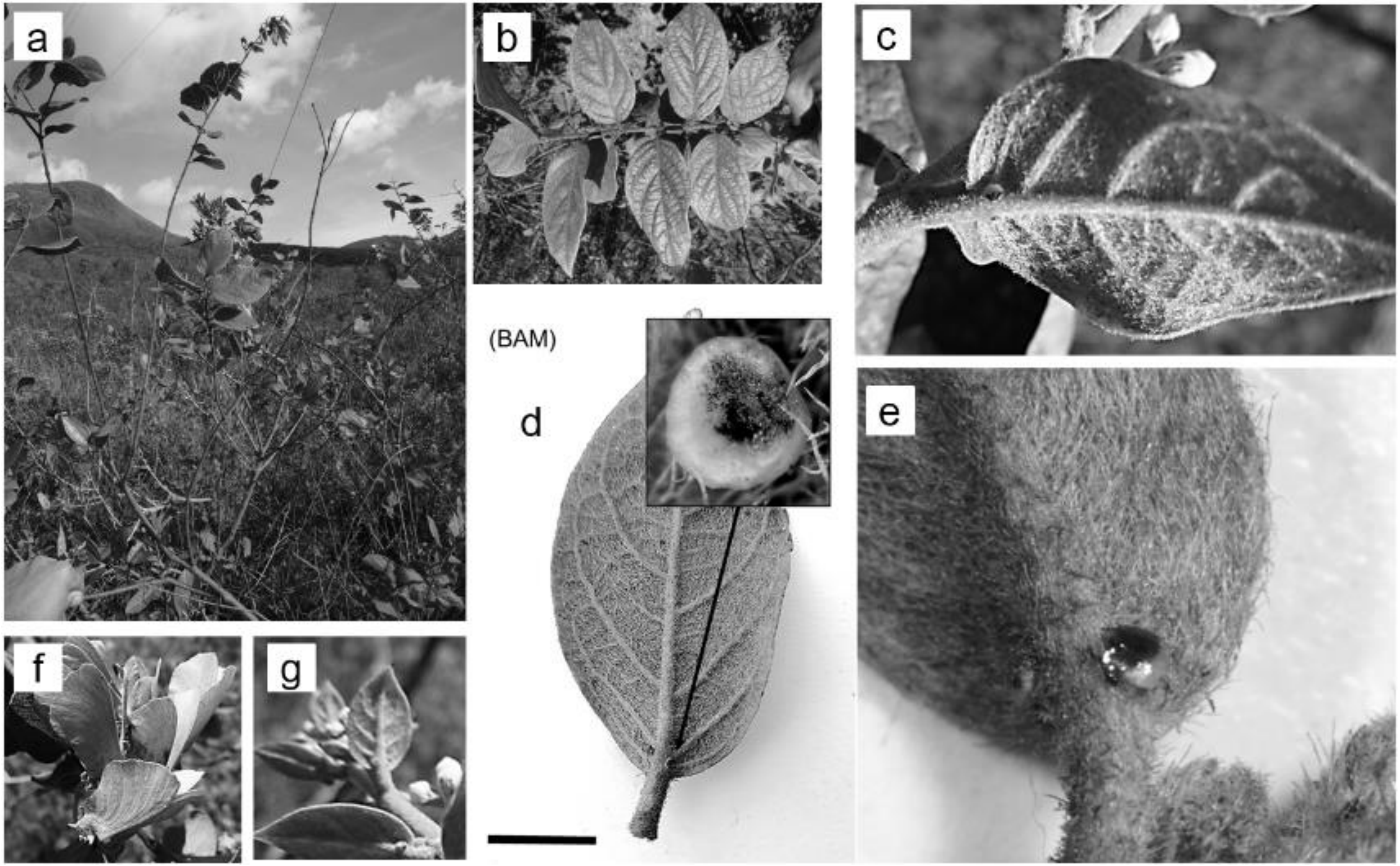
Appendices

Appendix 1. Plant species with extrafloral nectary observed in Rupestrian Fields, at Ouro Preto, Brasil. (Fotos: Fagundes R).

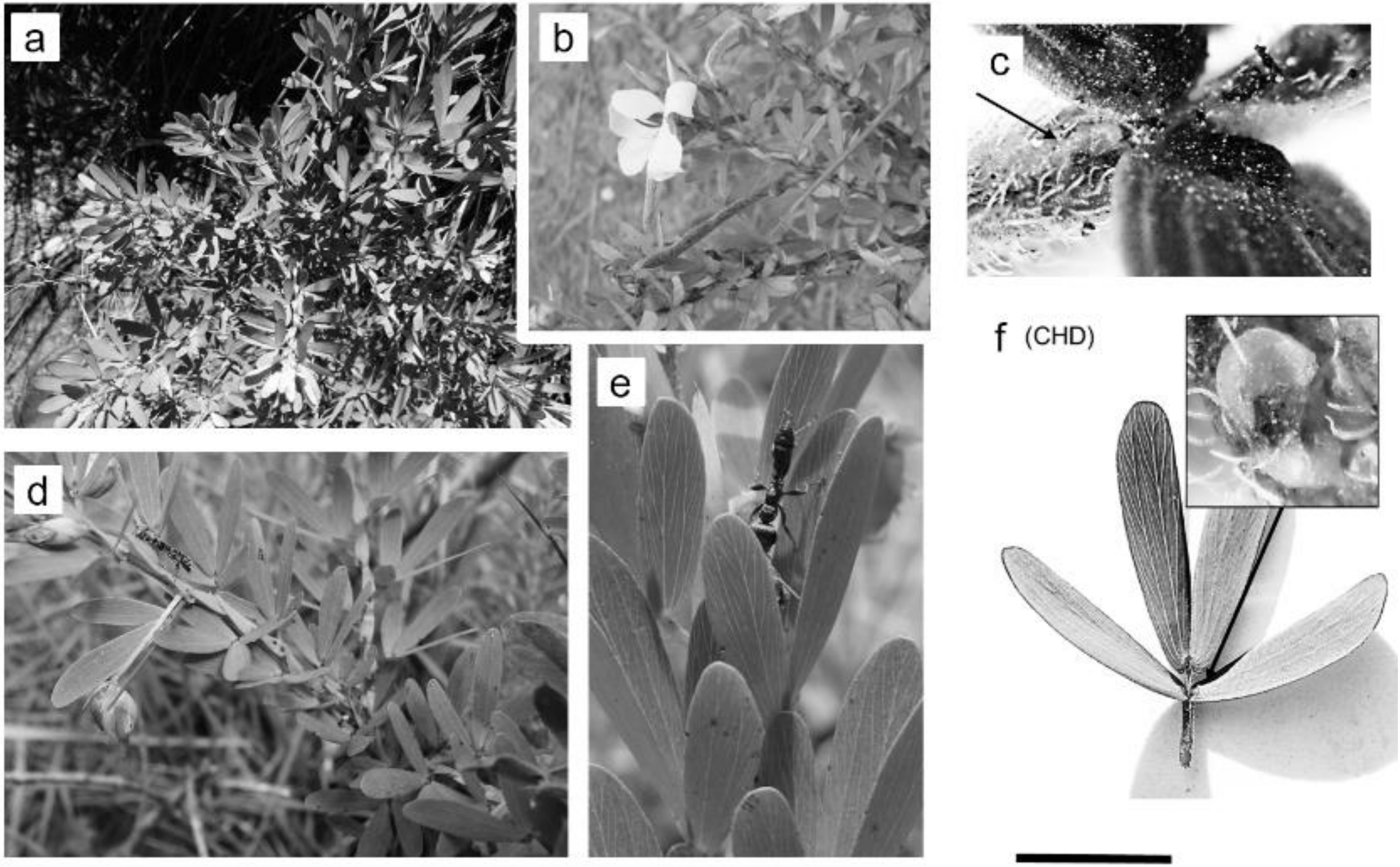
Banisteriopsis campestris (a. Juss.) Little (Malpighiaceae) (code: BAM). (a) Individual shrub with flowers. (b) *Cephalotes pusillus* collecting nectar from an extrafloral nectary. (c) Flowers. (d) Fruits. (e) *Camponotus crassus* defending the plant. (f) Leaf with focus on the nectary.



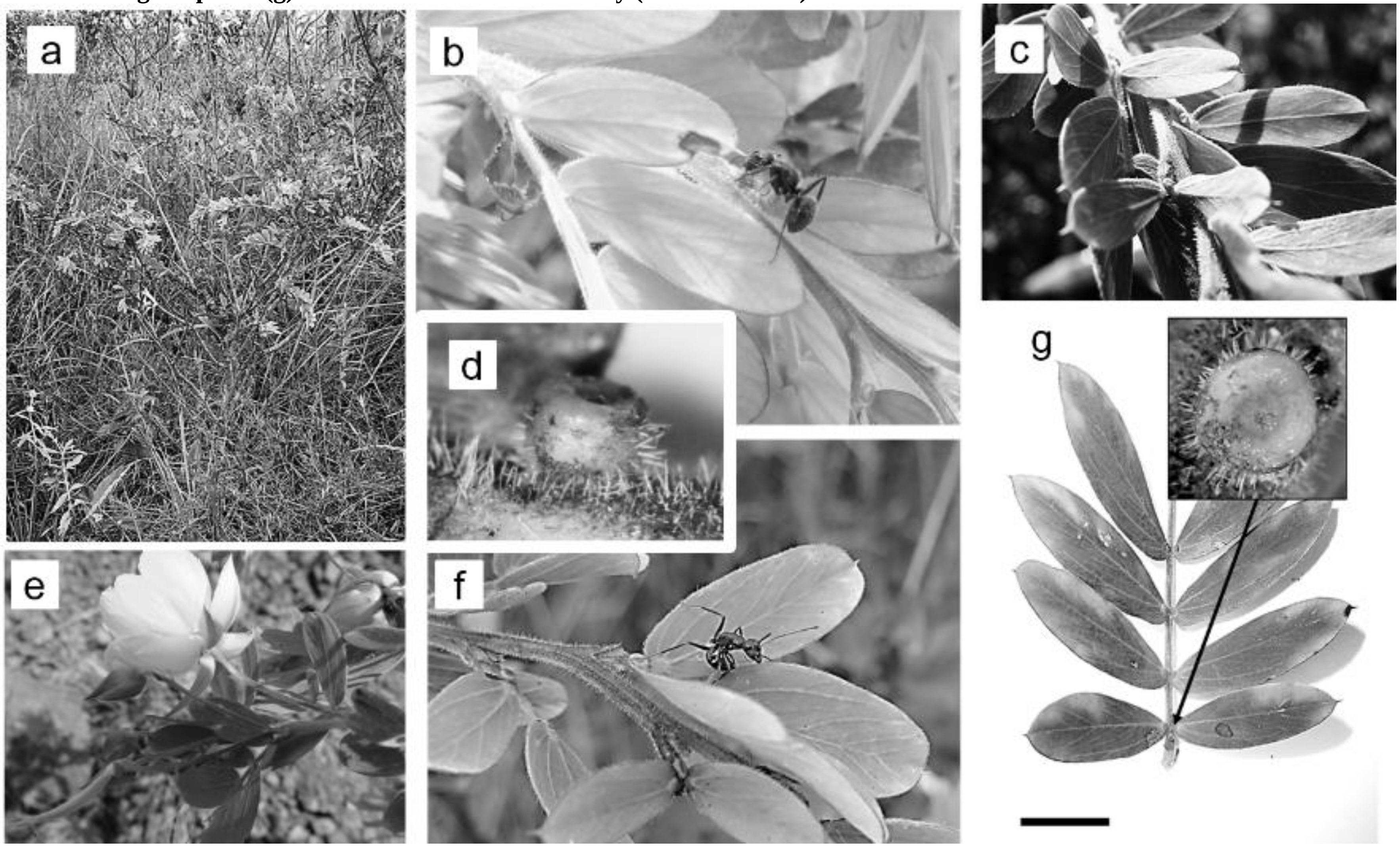
Banisteriopsis malifolia (Nees & Mart.) B. Gates (Malpighiaceae) (BAM). (a) Individual shrub. (b) Branch. (c) Leaf. (d) Leaf with focus on nectary (Scale bar: 1cm). (e) Nectary with cumulated nectar. (f) Fruits. (e) Meristem with flower buds.



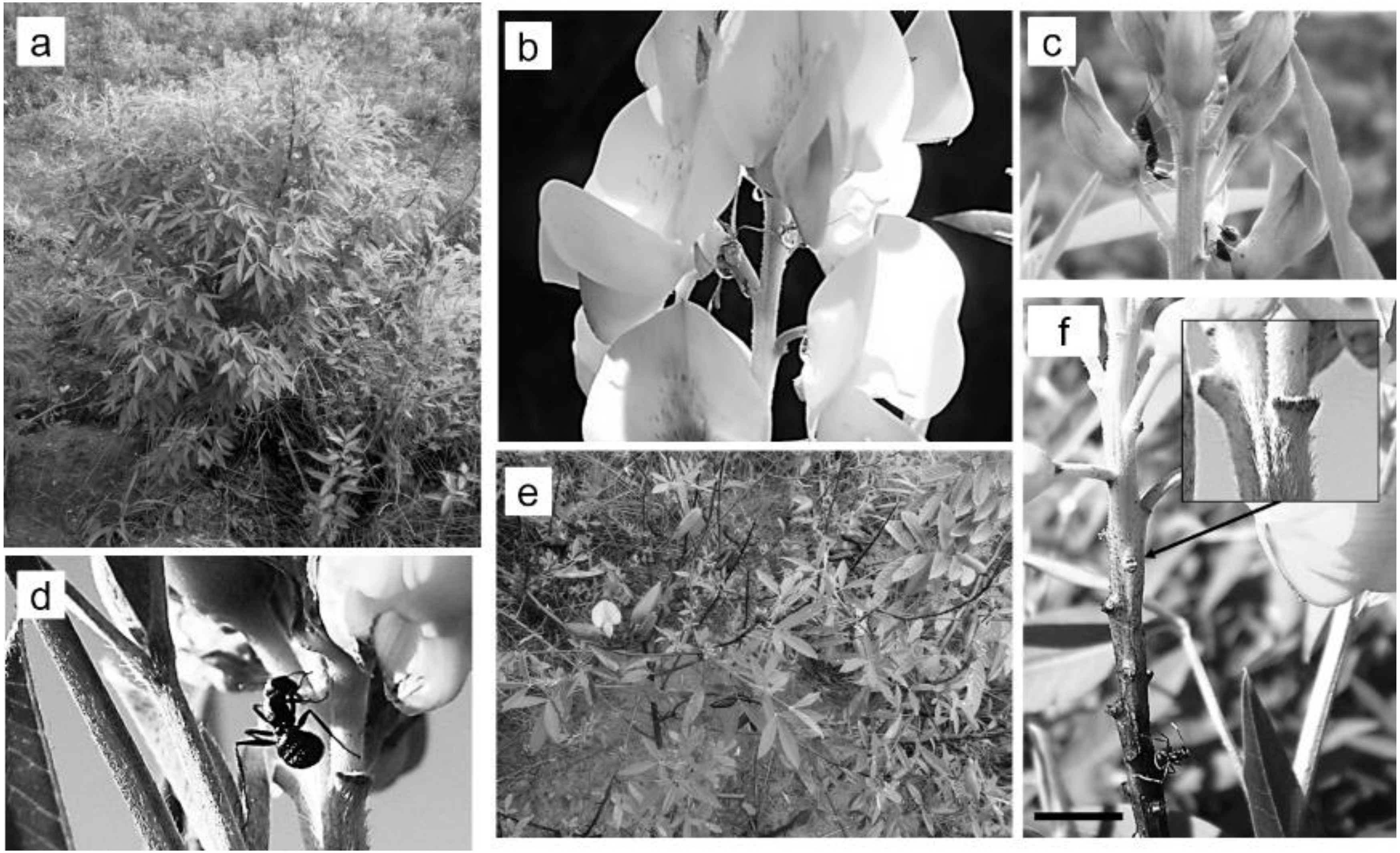
Chamaecrista desvauxii **(Collad.) Killip. (Caesalpinioideae) (Code: CHD). (a) Individual shrub. (b) Flower. (c) Detail of EFN inside leaf stipule (arrow). (d)** ***Pseudomyrmex gracillis*** **foraging for nectar. (e)** ***P. gracillis*** **capturying a prey. (f) leaf with focus on EFN (scale bar: 1cm).**



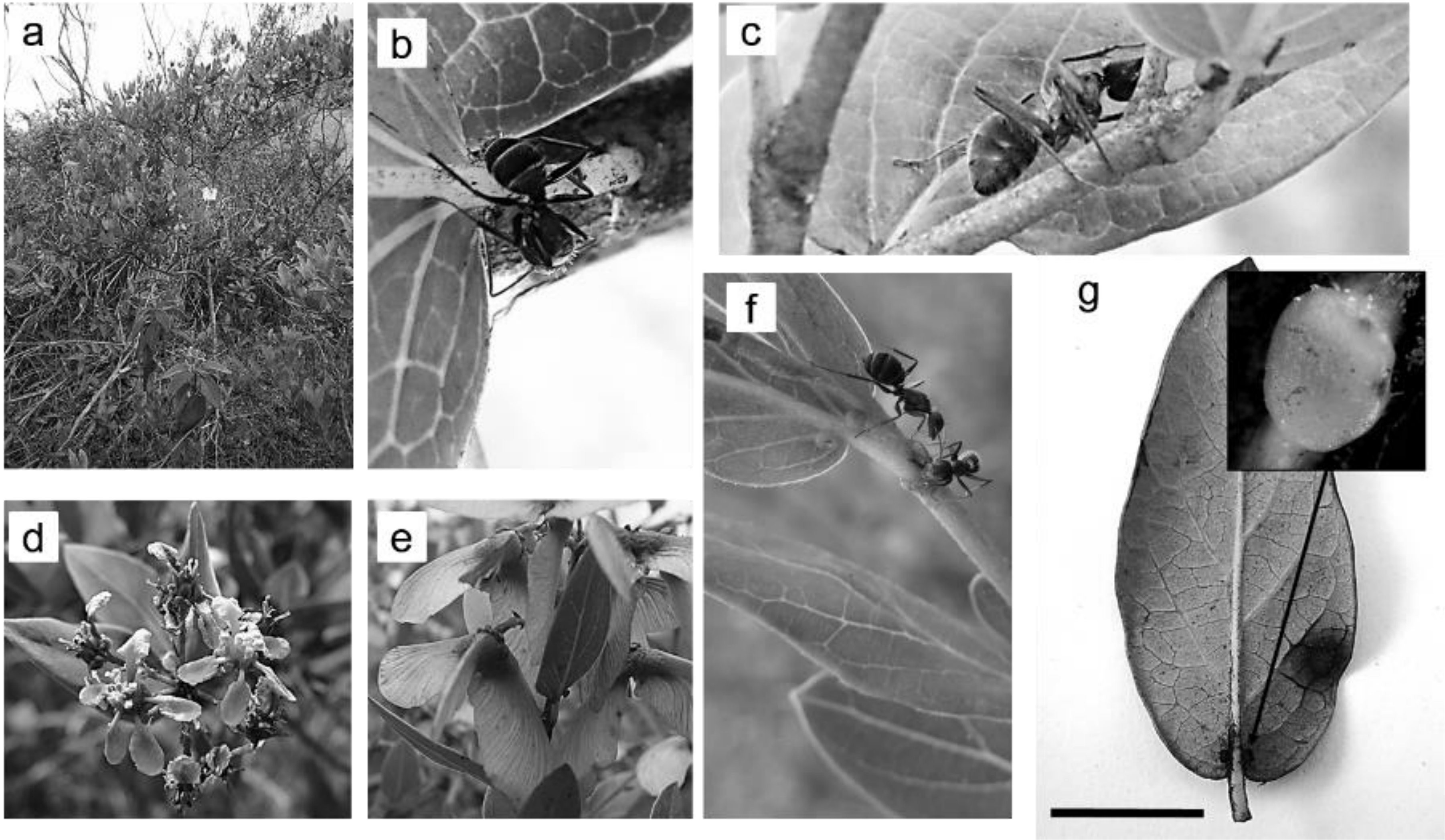
Chamaecrista mucronata **(Spreng.) H.s. Irwin & Barneby (Caesalpinoideae) (Code: CHM). (a) Plant individual. (b) *Camponotus crassus* eating nectar from nectary. (c) Leves with cumulated nectar in the nectaries. (d) Detail of extrafloral nectary. (e) Flower and fruit. (f) C. crassus defending the plant. (g) Leaf with focus on the nectary (scale bar: 1cm).**



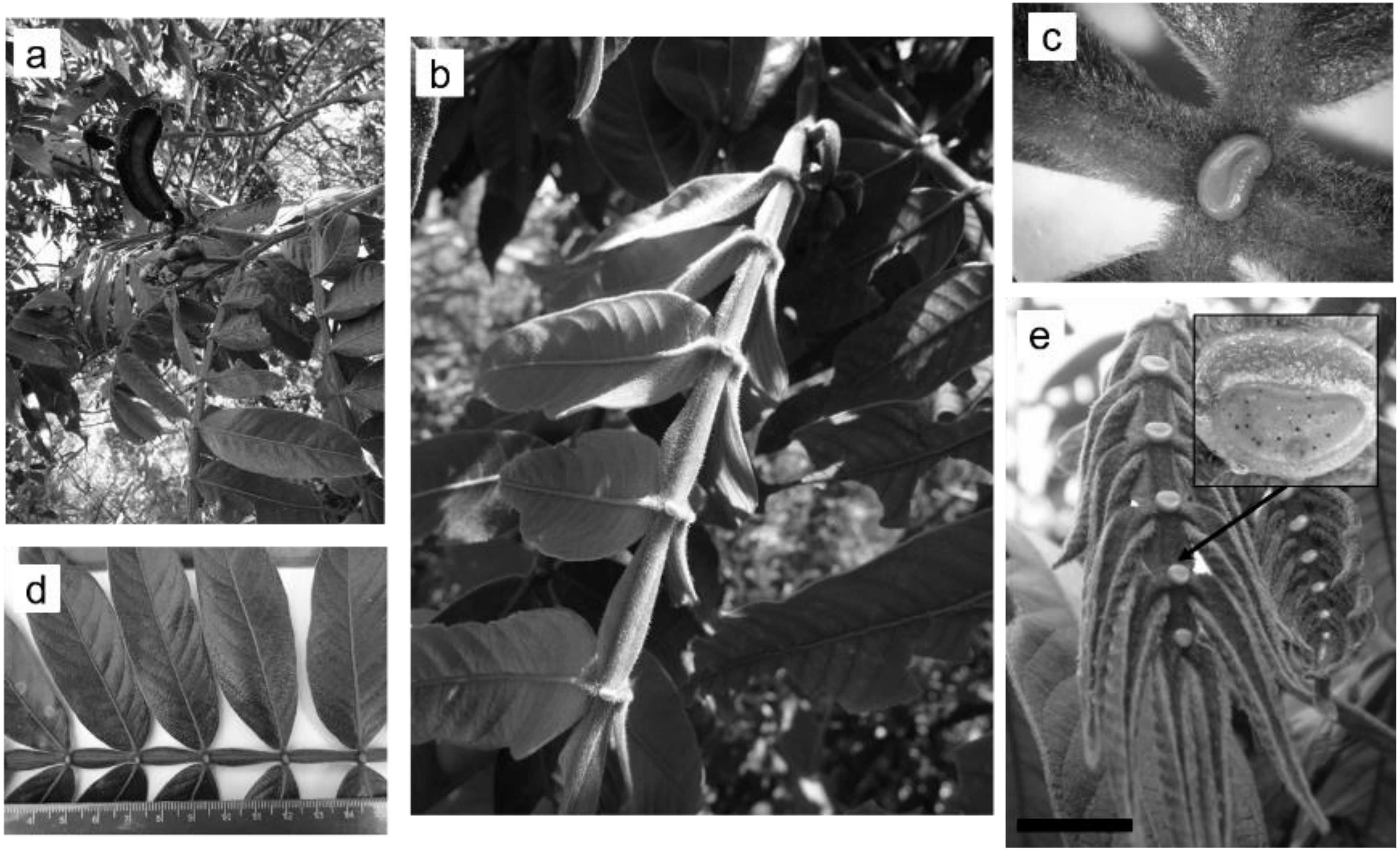
Crotalaria micans Link (Fabaceae) (code: CRM). (a) Plant individual. (b) Inflorescence with accumulated nectar in the nectaries. (c) *Camponotus rufipes* foraging for nectar. (d) *Camponotus crassus* collecting nectar from nectary. (e) Plant with flowers and Fruits. (f) Inflorescence with focus on the EFN. Note C. *crassus* defending the plant. (scale bar: 1cm).



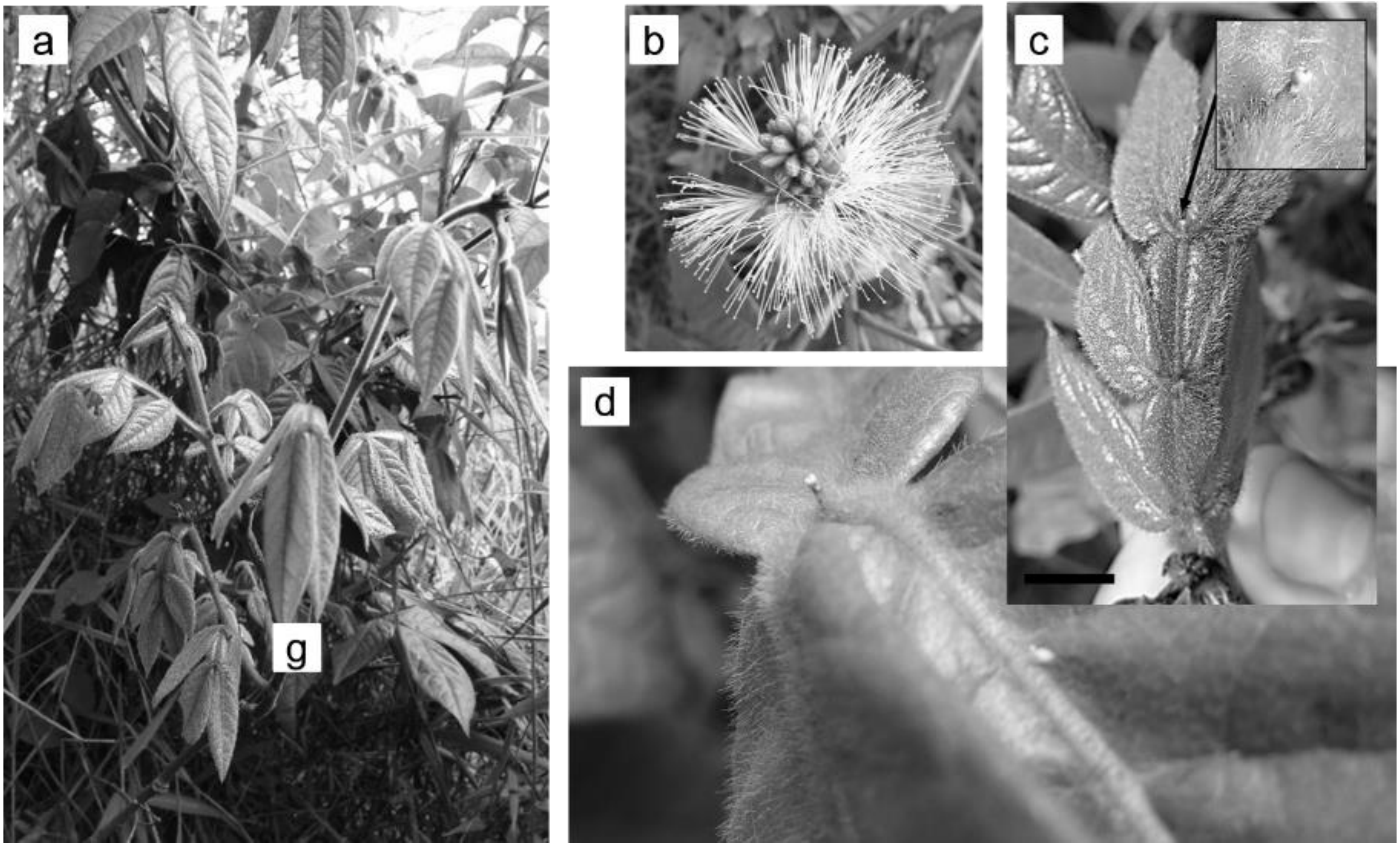
Heteropterys umbellata **A. Juss. (Malpighiaceae) (code: HEU). (a) Plant individual. (b)** ***Camponotus novogranadensis*** **collecting nectar. (c)** ***Camponotus rufipes*** **collecting nectar. (d) Flowers. (e) Fruits. (f)** ***Camponotus novogranadensis*** **foraging for nectar. (g) Leaf with focus on the nectary. (scale bar: 1cm).**



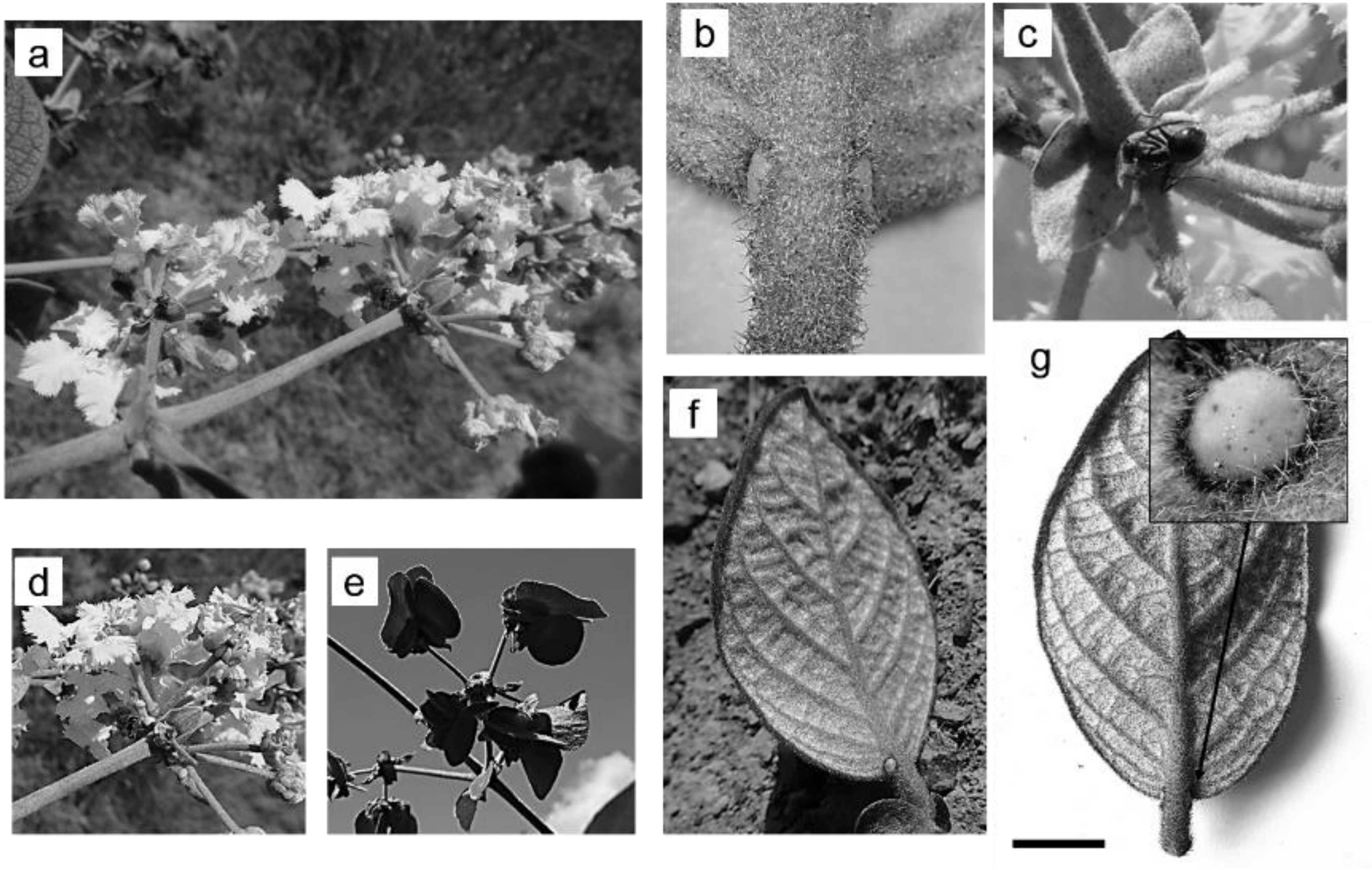
Inga sessilis **(Vell.) Mart. (code: INS). (a) Branch with fruits. (b) Leaf with nectaries between leaflets. (c) Extrafloral nectary. (d) Leaf. (e) Leaf with focus on the nectary (scale bar: 1cm).**



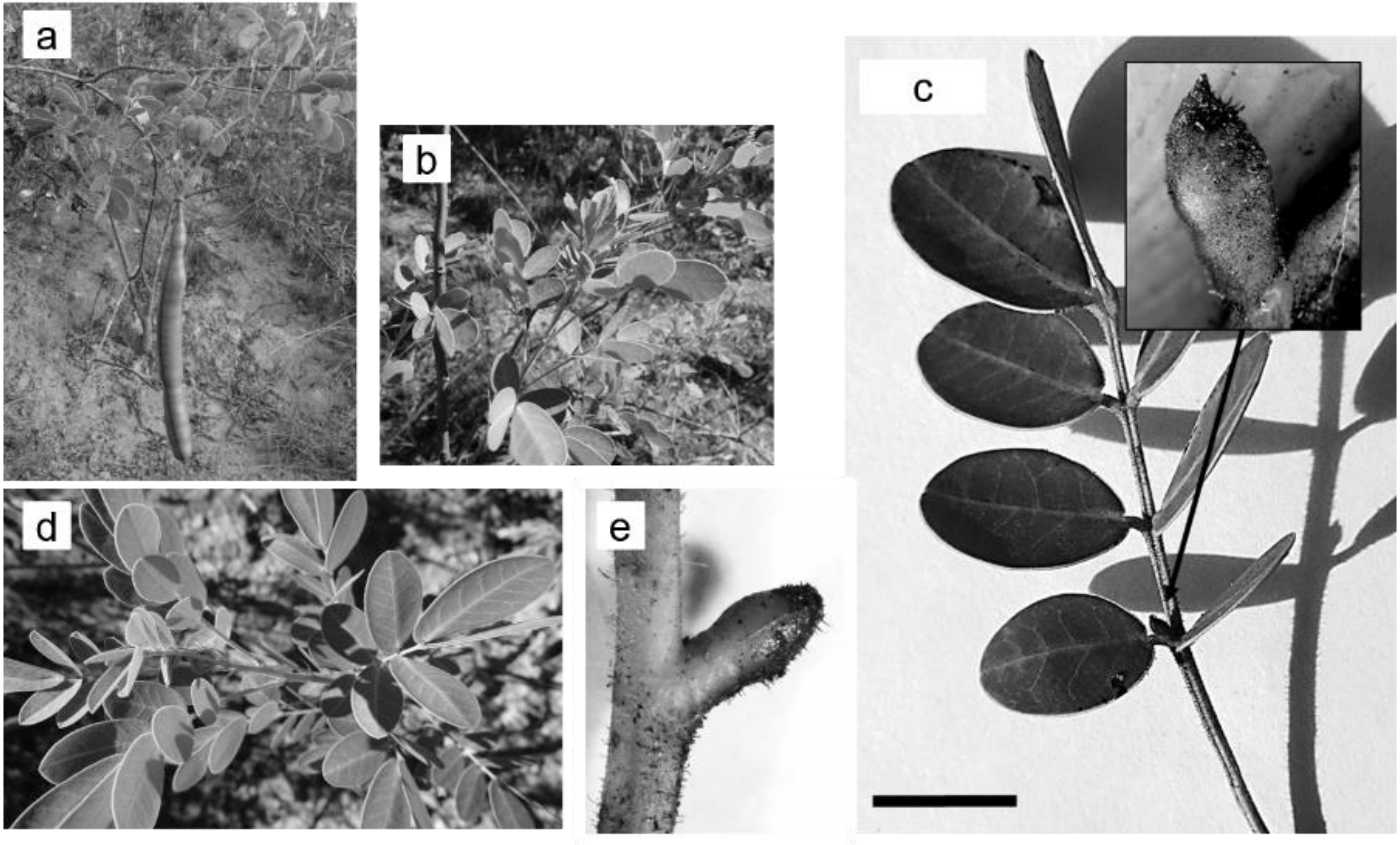
Inga vulpina Mart. ex Benth (code: INV). (a) Individual shrub. (b) Flower. (c) Leaf with focus on the nectary (scale bar: 1cm). (d) Detail of the nectary.



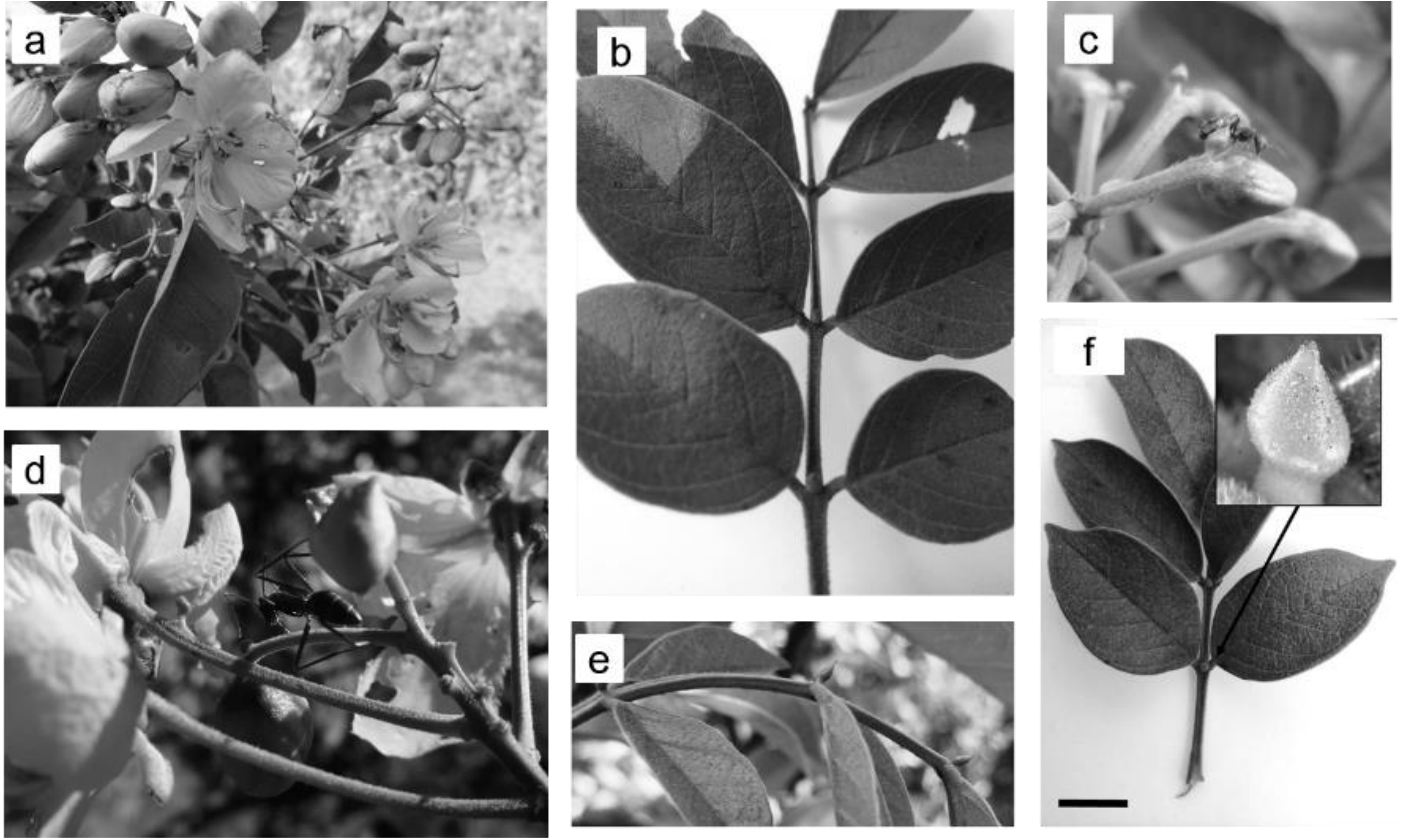
Peixotoa tomentosa a. Juss. (Mapighiaceae) (PET). (s) Inflorescence. (b) Detail of nectaries in both sides of leaf stem. (c) *Camponotus* crassus feeding on the nectarie. (d) Flowers. (e) Fruits. (f) Leaf. (g) Leaf with focus in the EFN (scale bar: 1cm).

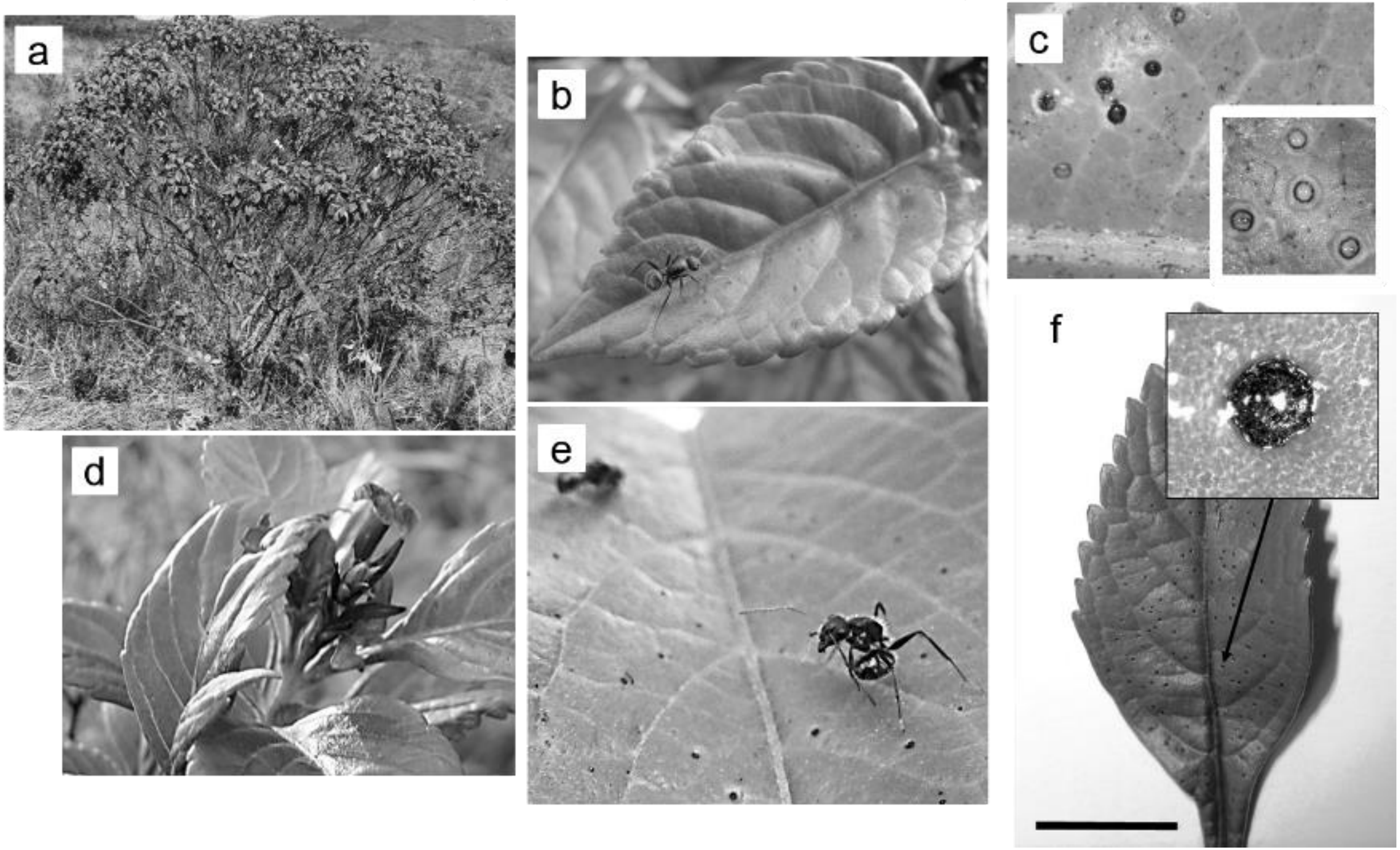


Senna pendula (Willd.) Irwin & Barneby (Fabaceae) (code: SEP). (a) Brach with fruit. (b) Branch resprouting. (c) Leaf with focus in the EFN (scale bar: 1cm). (d) Individual plant. (e) Detail of the nectary.



Senna reniformis (g. Don) H.S.Irwin & Barneby (Fabaceae) (SER). (a) Brach flowering. (b) Brach with nectaries between leaflets. (c) *Camponotus novogranadensis* feeding nectar. (d) *Camponotus ager* foraging. (e) Young leaf with nectaries. (f) Leaf with focus on the nectary (scale bar: 1cm).

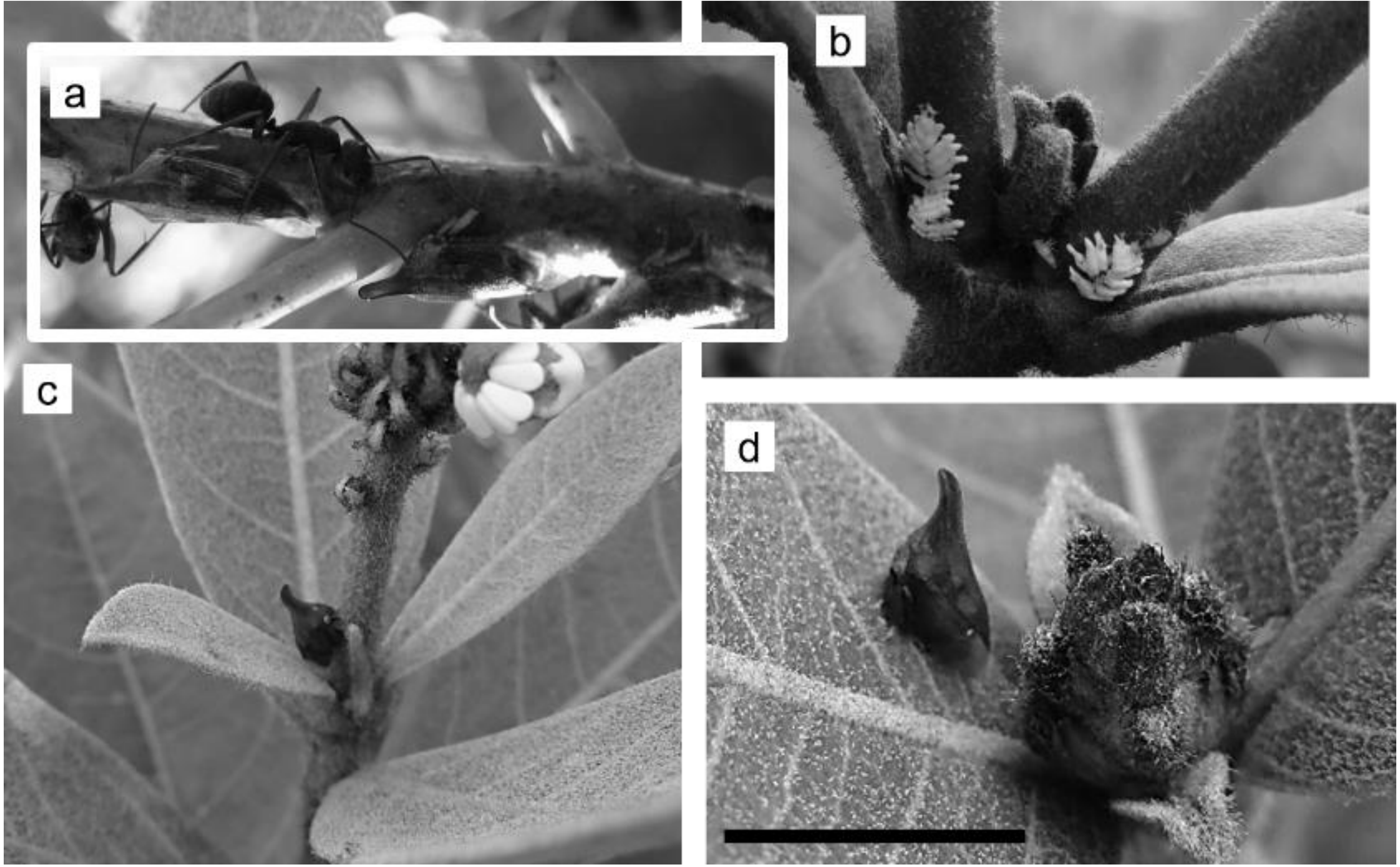




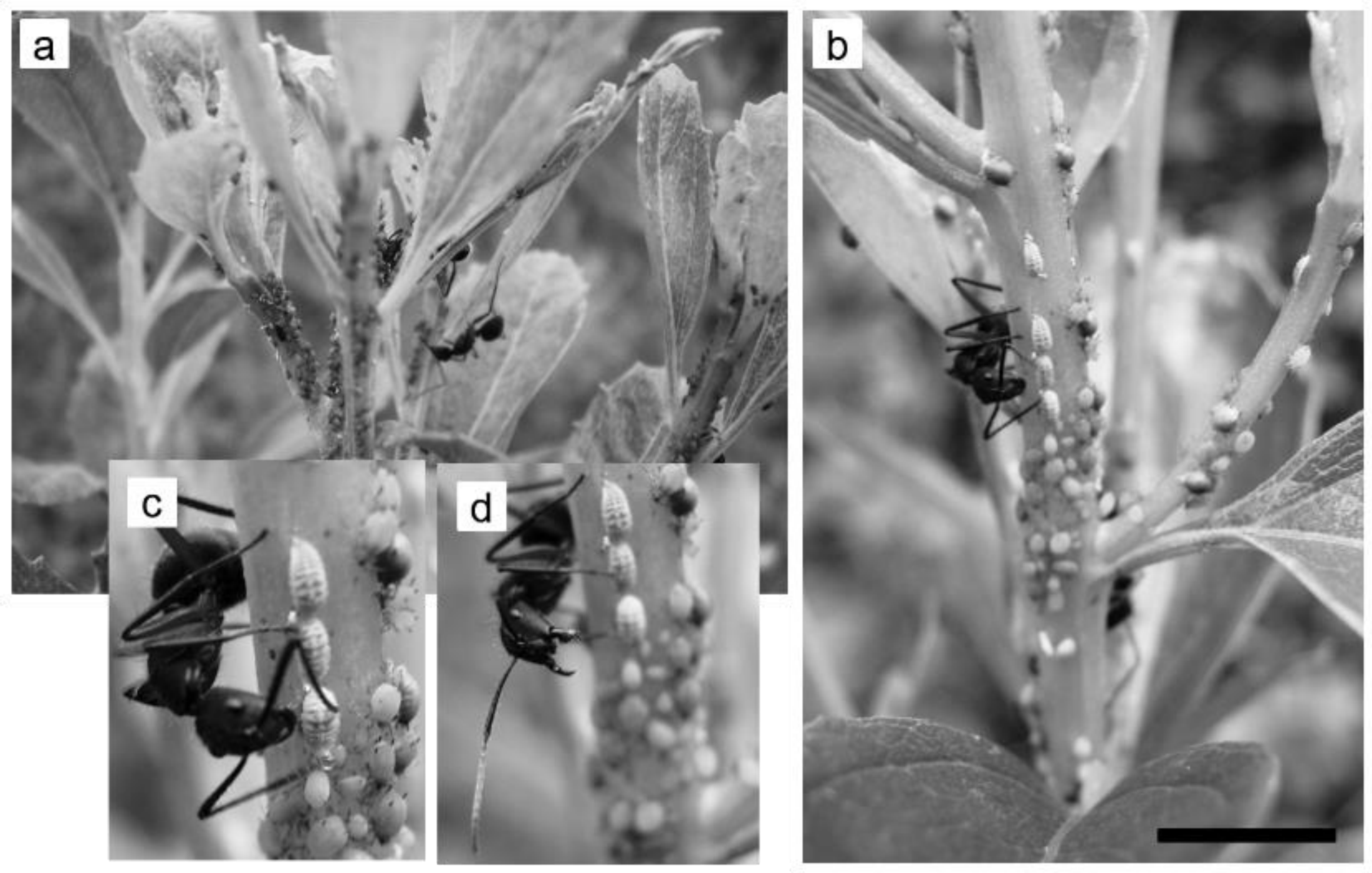
Stachytarpheta glabra Cham. (Verbenaceae) (code: STG). (a) Indivudual plant. (b) *Camponotus crassus* foraging for nectar. (c) Detail of nectaries (black circles) on the leaf blade with focus in tree nectaries. (d) Inflorescence. (e) *Camponotus crassus* and *Camponotus novogranadensi*s foraging for nectar. (f) Leaf with focus on the nectary (scale bar: 1cm).

Appendix 2. Species of honeydew-producer hemipterans collected by ants in Rupestrian Fields, Ouro Preto, Brazil. (Foto: Fagundes R.)

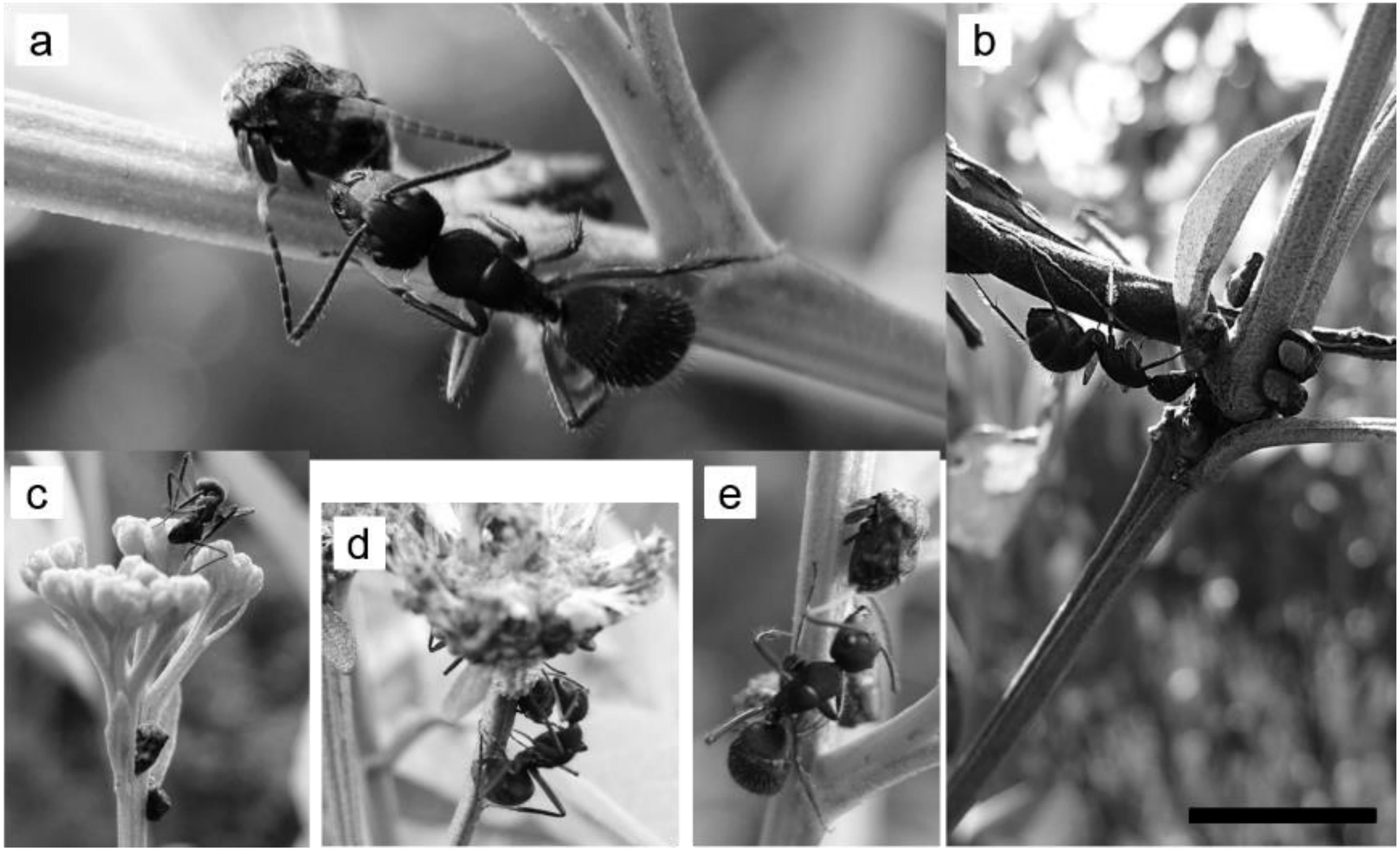
Achonophora sp. 1 (Membracidae) (code: AC1). (a) *Camponotus rufipes* interacting with an aggregation in *Acacia sp.* (b) Egg mass in *Byrsonima variabillis*. (c) Adult starting an aggregation in *B. variabillis.* (d) Adult infesting a meristem of *B. variabilis.*



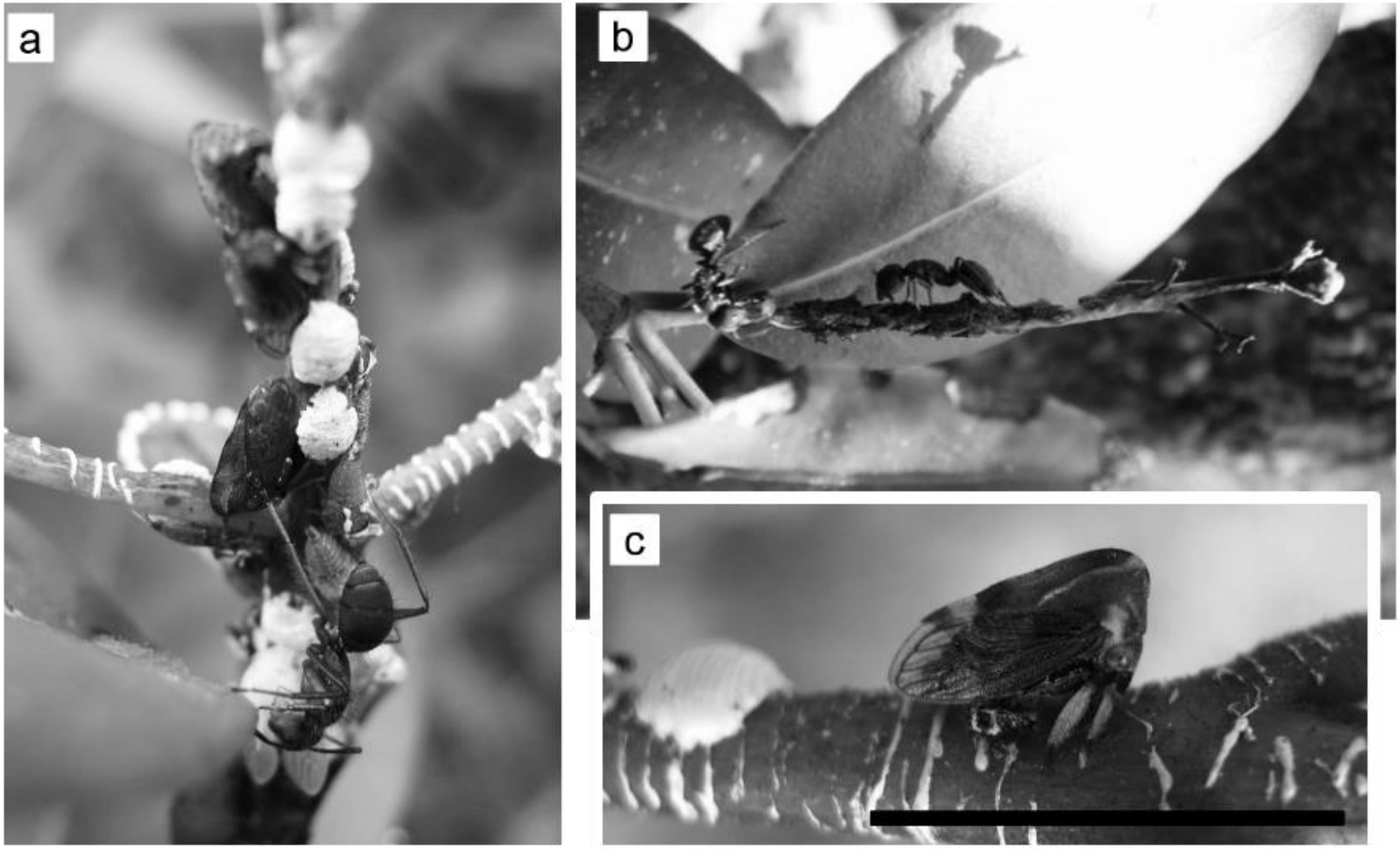
Aphis sp.1 (Code: AP1) (Aphididae). (a) Aggregation in *Baccharis reticularia* interacting with *Camponotus rufipes* (b) *C.rufipes* tending and aggregation of *Aphis* associated with a species of Coccidae in *Baccharis reticularia* (scale bar: 1cm). (c) C. rufipes collecting a drop of honeydew. (d) *C. rufipes* displaying aggressive behaviour: opening mandibles.



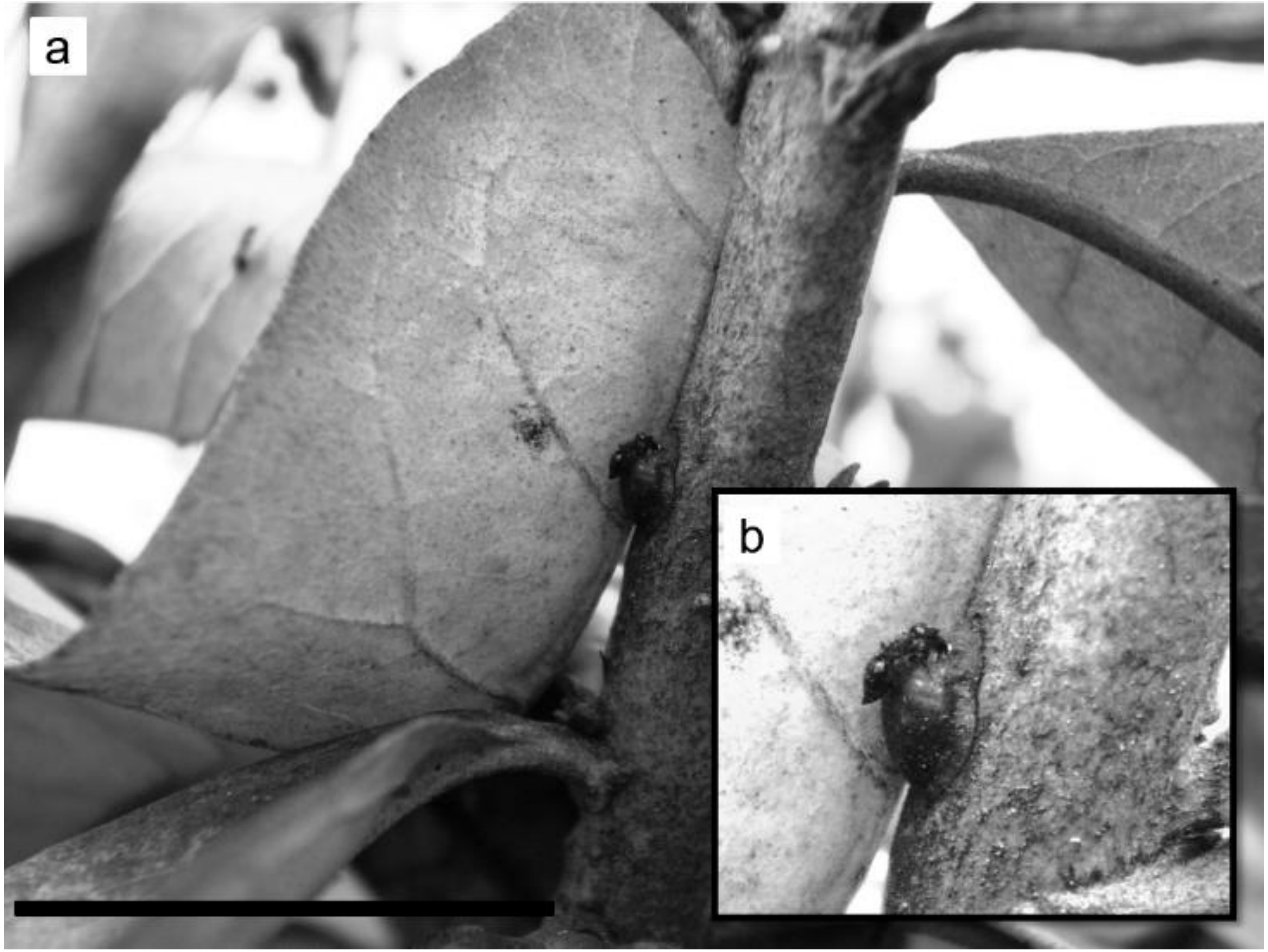
Bolbonota sp.1 (BO1) (Membracidae). (a) *Camponotus rufipes* interacting with an adult in *Baccharis* sp. (b) *C. rufipes* patrolling na aggregation in *Eremanthus erithropappus* (scale bar: 1cm).(c) *C. rufipes* patrolling an aggregation in inflorescences of *Baccharis reticularia*. (d) *C. rufipes* tending an adult in fruits of *Baccharis reticularia.* (e) *C. rufipes* collecting honeydew released by an adult.



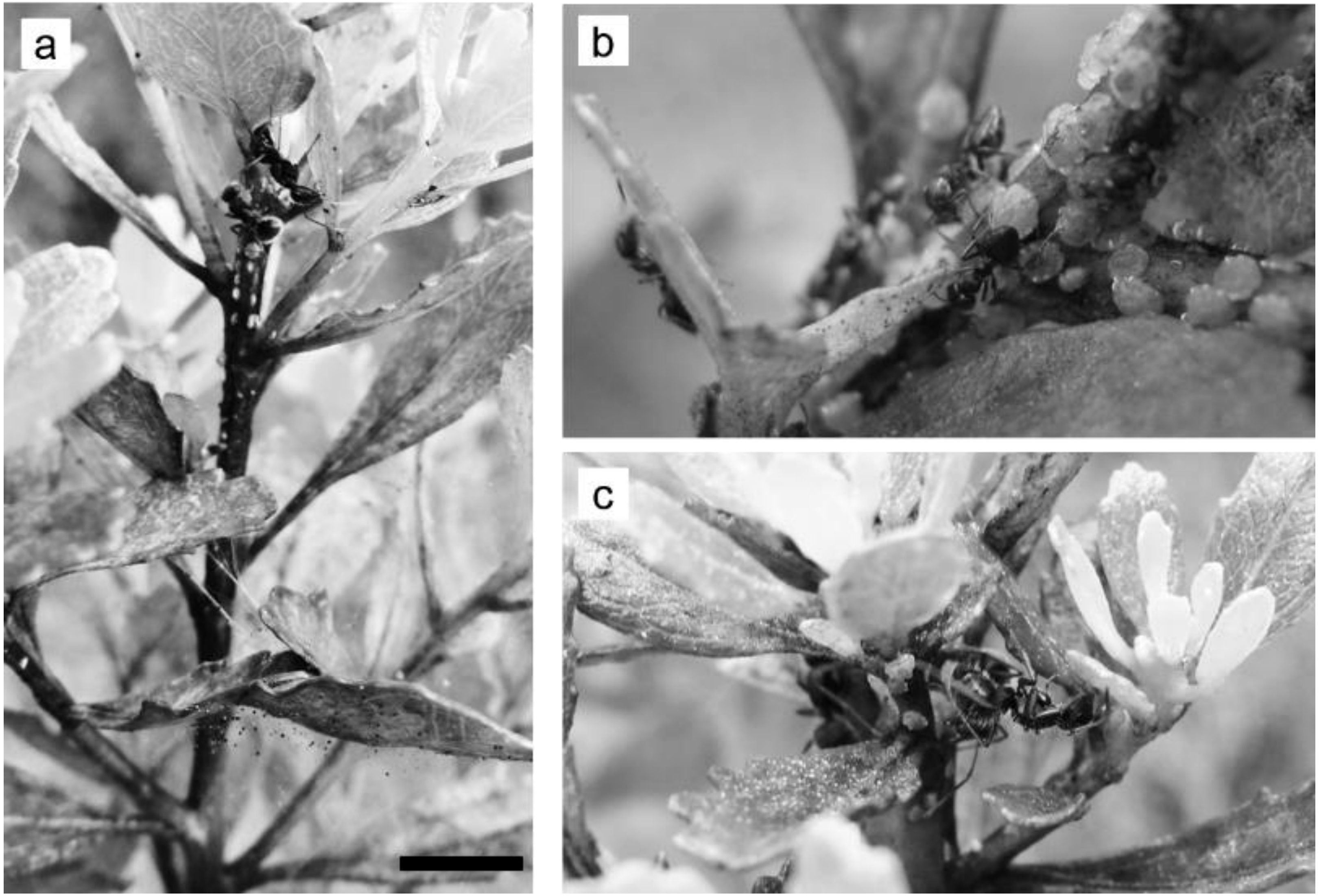
Calloconophora sp. 1. (Membracidae) (code: CA1). (a) *Camponotus rufipes* patrolling an infestation in *Myrcia* sp. (b) *Camponotus senex* patrolling an infestation in *Myrcia* sp. (c) an adult laying eggs in *Myrcia* sp. (scale bar: 1cm).



Ceroplastes sp. 1. (Coccidae) (code: CE1). (a) *Crematogaster* sp.1 interacting with an adult of *Ceroplastes* sp.1 in *Eremanthus incanus* (scale bar: 1cm). (b) *Crematogaster* sp.1 collecting honeydew secreted by an adult.

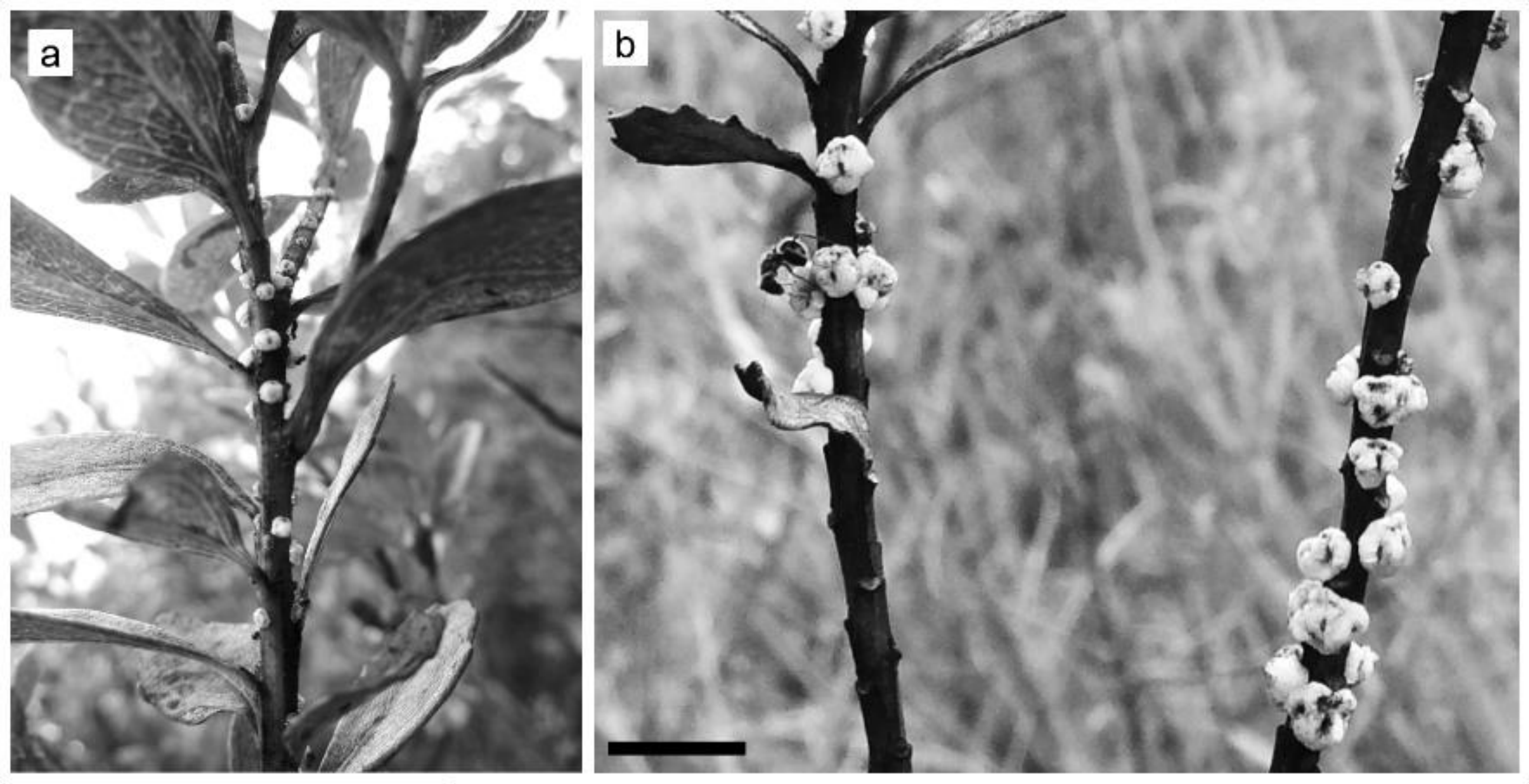


Ceroplastes sp2. (Coccidae) (code: CE2). (a) Aggregation in *Baccharis reticularia* interacted with *Camponotus crassus* (scale bar: 1cm). (b) Infestation in *B. reticularia* interacted with *Cephalotes* sp. (c).

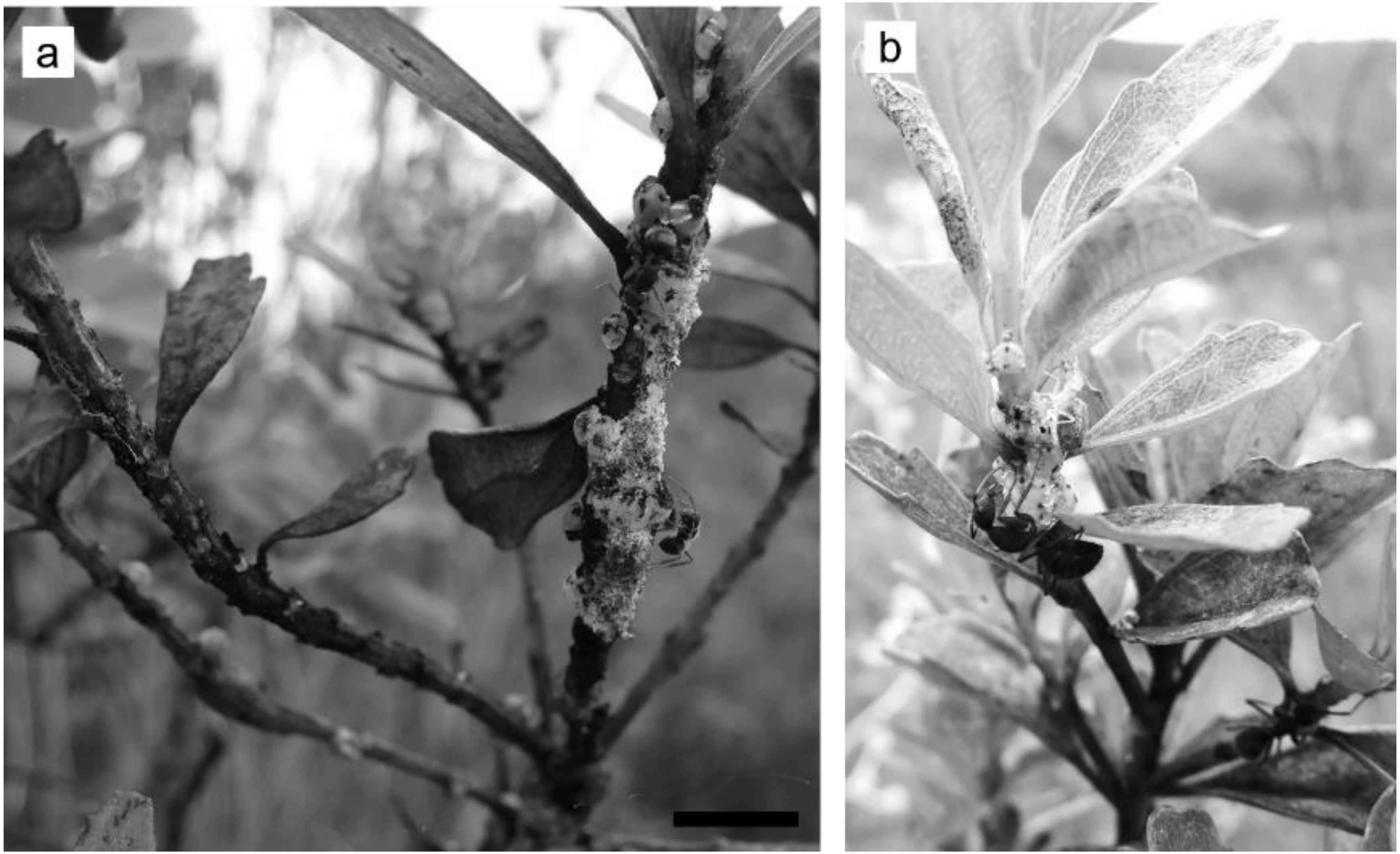


Ceroplastes sp. 3 (Coccidae) (code: CE3). (a) Agreggarion of *Ceroplastes* sp. 3 interacted with *Crematogaster* sp. in *Baccharis reticularia* (scale bar: 1cm). (b) *Crematogaster* protecting *Ceroplastes* sp. 3.

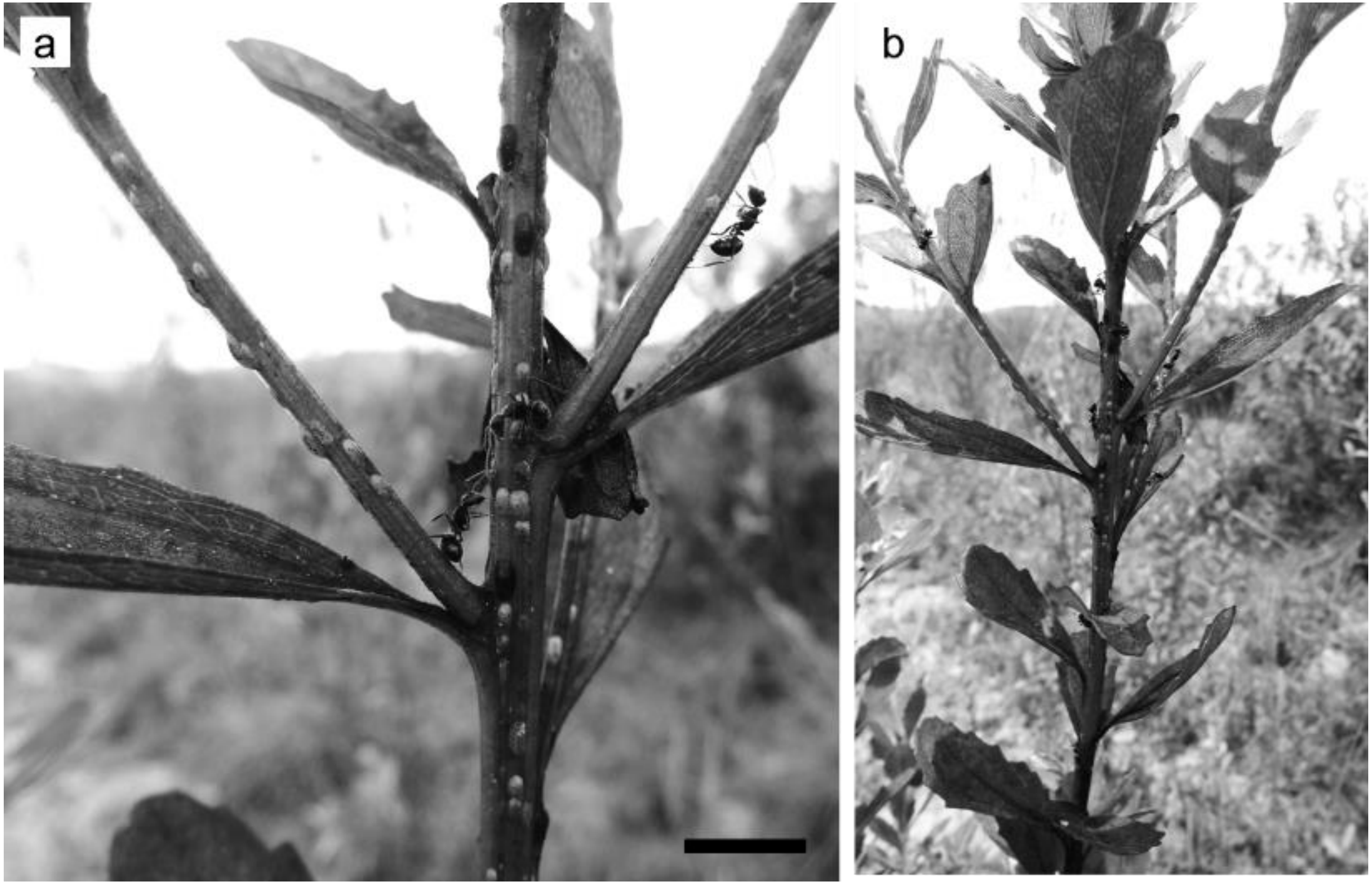
Ceroplastes iheringi Cockerell 1985 (Coccidae) (code: CEI). (a) Aggregation of *C. iheringi* interacted with *Brachymyrmex heeri* in *Baccharis reticularia*. (b) Aggregation of *C. iheringi* interacted with *Camponotus crassus* in *Baccharis reticularia* (scale bar: 1cm).



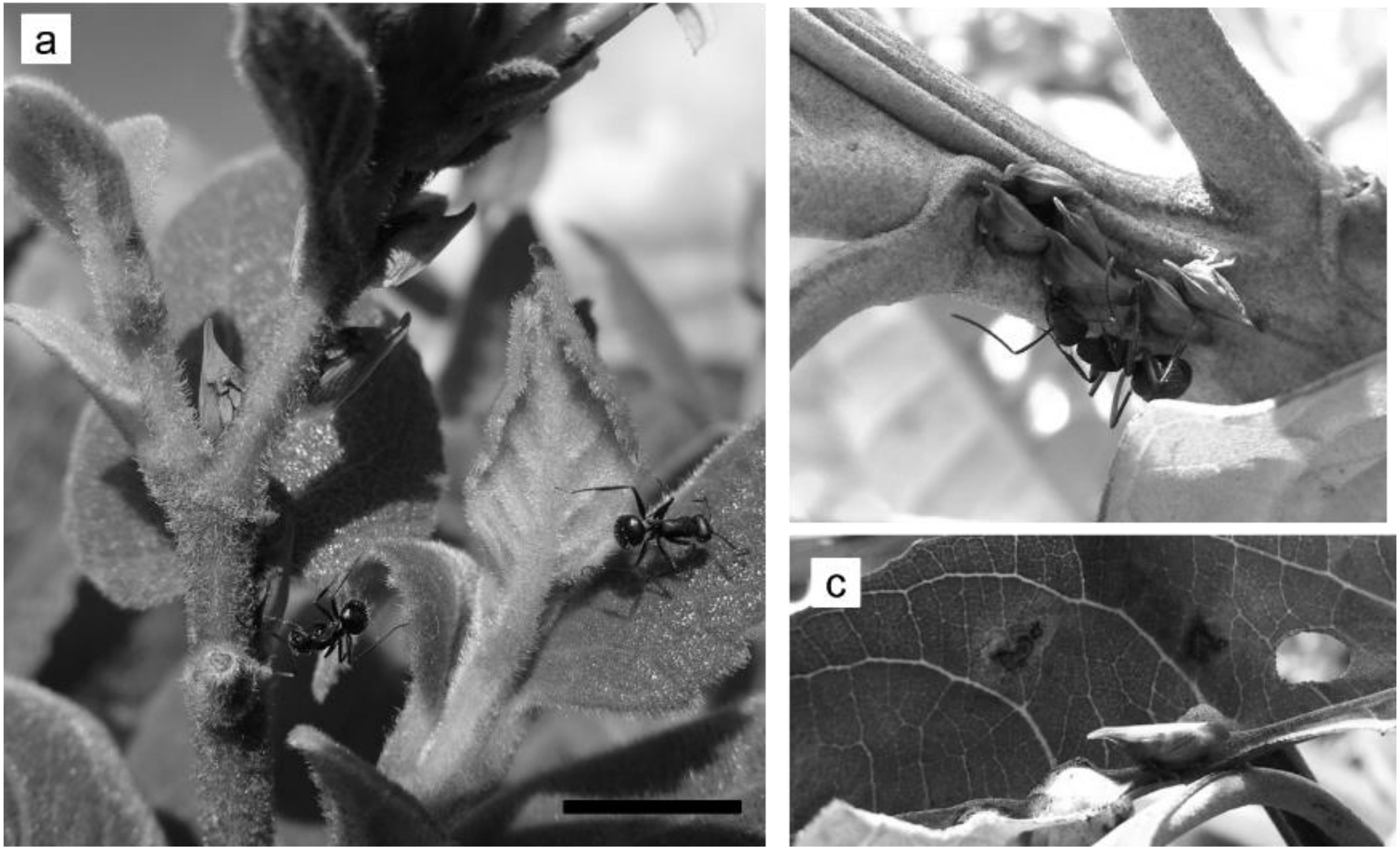
Ceroplastes lucidus Hempel 1900 (Coccidae) (code: CEL). (a) Aggregation of *C. lucidus* interacted with *Camponotus crassus* in *Baccaris reticularia* (scale bar; 1cm). (b) *Camponotus rufipes* tending an aggregation of *C. lucidus* in *B. reticularia*.

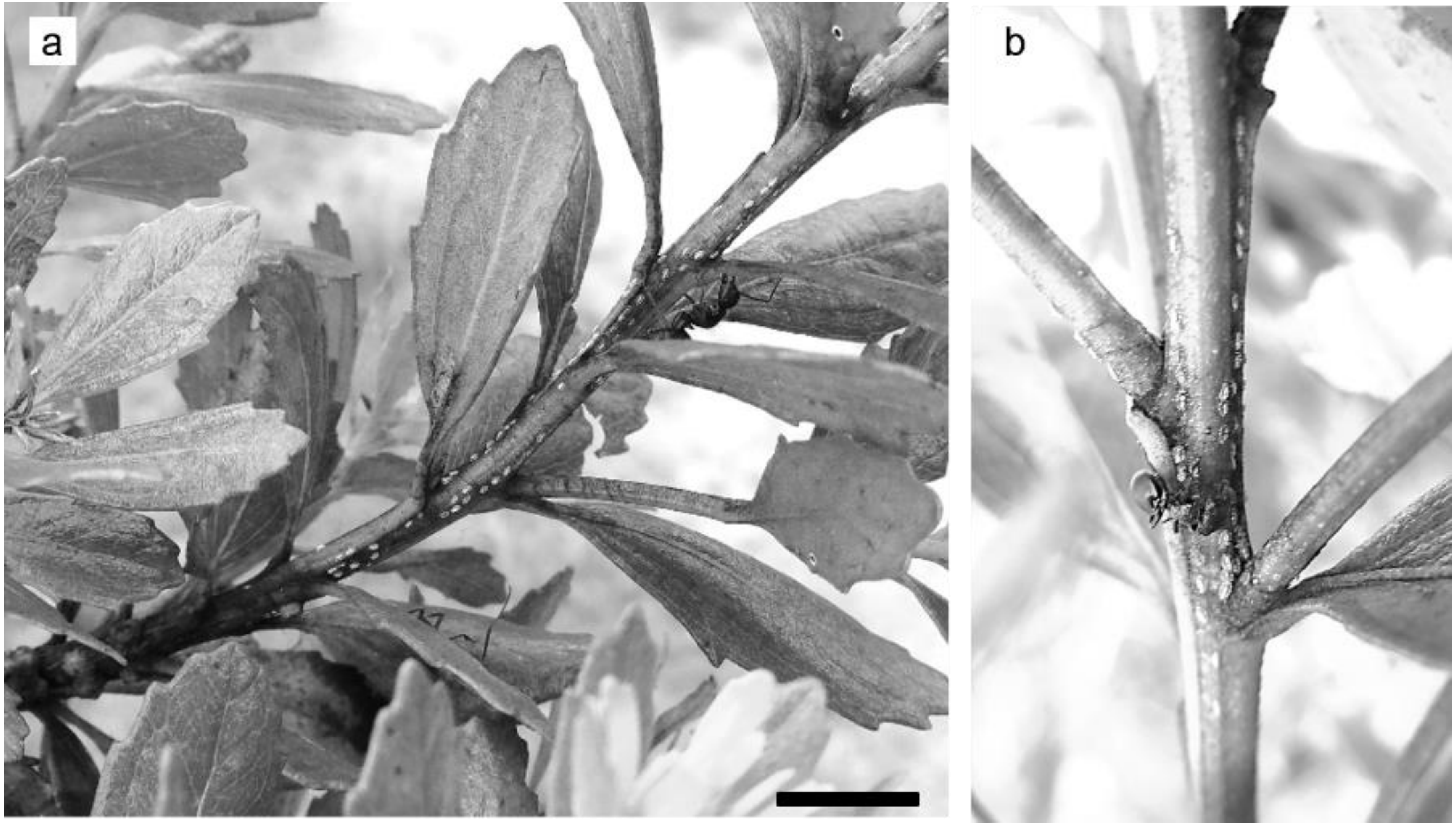


Coccus viridis Green 1889 (Coccidae) (code: COV). (a) Aggregation interacted with *Camponotus crassus* in *Baccharis reticularia* (scale bar: 1cm) (b) *C. crassus* patrolling an aggregation of *C. viridis* in *B. reticularia*.



Enchenopa sp. 1 (Membracidae) (code: EU1). (a) Aggregation of *Enchenopa* sp.1 interacted with *Camponotus crassus* (scale bar: 1cm). (b) *C. crassus* tending an adult of *Enchenopa* sp. 1





Pseudococcus sp. 1 (Pseudococcidae) (code: PS1). (a) Aggregation of *Pseudococcus* sp. 1 interacted with *Camponotus rufipes* in *Baccharis reticularia* (scale bar: 1cm). (b) *Cephalotes pusillus* collecting honeydew excreted by a nymph.

Capítulo 2

Contexto-dependência em redes mutualistas formiga-planta: resultados de interação condicionados a qualidade do néctar e agressividade da formiga

Context dependency in ant-plant mutualistic networks: interaction outcomes conditioned to nectar quality and ant aggressiveness

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Resumo

Interações entre formigas e plantas são geralmente facultativas, com interações pouco especializadas e alto compartilhamento de plantas (sobreposição de nicho). Inúmeros casos dessas interações já foram descritos e foi observada uma grande variação na frequência de interação entre as espécies de plantas. Porém, permanecem incompreendidas as causas dessas variações e suas consequências para os benefícios do mutualismo (resultados condicionais). Dentro de dois anos de observações em Campos Rupestres (Brasil; 2011 - 2013) e dois experimentos manipulativos nós abordamos a cadeia mutualista completa; desde a recompensa (néctar) fornecida pela planta para a formiga interagente até o benefício recebido pelas plantas (defesa contra herbívoros). Todas as 10 espécies de plantas com nectários extraflorais atraíram formigas e interagiram de forma generalista, com alta sobreposição mas diferente frequência de interação. A remoção da formiga mostrou que plantas associadas às formigas apresentam menor perda de área foliar por herbivoria do que plantas sem formigas. Além disso, mostramos que os benefícios líquidos das interações dependem da qualidade do néctar, que atrai formigas maior número de formigas e espécies mais agressivas. Assim, os benefícios do mutualismo estão condicionados mais à presença de formigas agressivas do que a grande quantidade de formigas interagentes, que por sua vez depende de um néctar vantajoso (mutualismo condicional). Em suma, nós fornecemos evidências da importância da qualidade do recurso-recompensa e a agressividade da formiga interagida como fatores-chave para a efetividade do mutualismos e estrutura das interações mutualísticas formiga-planta. Mais importante, mostramos que o caráter facultativo dessas interações não representa baixo benefícios do mutualismo desde que hajam formigas agressivas envolvidas. Essas formigas agressivas interagem com todas as plantas, em diferentes frequências resultantes da atratividade do néctar, e garantem a estabilidade da rede de interação ao protegerem todas as espécies de plantas.

Palavras-chave: condicionalidade, néctar extrafloral, herbivoria, eficácia da interação, proteção vegetal.

Abstract

Interactions between ants and plants are usually facultative, with low specialized interaction and high sharing of plant interactions (niche overlap). Several interactions has been described with great variation in the frequency of interaction between ants and plants. However, the causes of these variations e its consequences for the benefits of mutualisms (conditional outcomes) remain unclear. Within two years of field observation (2011 – 2013) and two manipulative experiments with ant-plant interaction at Rupestrian Fields ecosystem (Brazil), we address the complete mutualistic chain; from the reward (nectar) offered by the plant to the ants, to the benefits received by the plants (herbivory defense). The ten species of EFN-bearing plants interacted with 32 ant species in generalized networks of highly overlapped interactions, but at different interaction frequencies. The removal of the ants showed that plants received less herbivory when interacted with ants. However, the net benefits of the mutualism depended on the quality of the nectar provided by the plant (context-dependency), which attracts aggressive species (resource-consumer relationship). Therefore, interactions with aggressive species, more than the total number of interactions, regulates the mutualism benefits, which in turn depended on better quality nectar (context dependency). In conclusion, we provided empirical evidences for the hypothesis of resource-reward quality and ant species aggressiveness are key-factors to the mutualism effectiveness and the structure of ant-plant interactions. More importantly, we show that the facultative character of ant-plant mutualism did not represent low benefits if aggressive ant species are involved. These aggressive species interact with all plants and guarantees the stability of the interaction by effectively protecting the plants accordingly with the value of rewards.

Keywords: conditionality, extrafloral nectar, herbivory, interaction effectiveness, plant protection.

Introduction

Interactions between ants and plants through extrafloral nectaries (EFNs) are mutualistic relationships based on resources offered by plants (i.e. nectar) in exchange for protection provided by ants (i.e. defense against herbivores) (Bronstein 1998; Heill & McKey 2003; Nascimento & Del-Claro 2010). The ecological relationship between plants bearing EFNs and ants is a key model in current studies of mutualistic interactions (Bronstein 1998; Heill & McKey 2003; Vilela *et al.* 2014). Recently, network theory allowed studying interactions at community scale, instead of paired relationships, and many studies have been published describing the nested and highly generalized interaction pattern of networks between ants and EFN-bearing plants (Bascompte *et al.* 2006; Lange *et al.* 2013; Dáttilo *et al.* 2013a, c; 2014a, b). However, the current main challenges of Interaction Ecology is to understand the nature of the interactions within the network (mutualism, commensalism or exploitation) and the variation on the mechanisms structuring network under different contexts (Bronstein 1998; 2001; Chamberlain & Holland 2008; Meyer *et al.* 2014).

The production of EFNs is a defense strategy present in 25% of Angiosperm species, with ant-plant interactions varying from facultative to obligatory mutualism (Koptur 1992). Protection against herbivory is a key benefit of the ant-plant mutualisms (Heill & McKey 2003), but several studies suggest that this benefit is highly variable between plant species (Bronstein 1998; Rosumek *et al.* 2009; Chamberlain & Holland 2008), and may not even occur for some paired interactions (Lange & Del-Claro 2014). This conditionality has been systematically documented in several ecosystems (Thompson 1988, Cushman & Addicott 1991; Bronstein 1998), and may result from the dependency between resources (plants) and consumers (ants) (Chamberlain & Holland 2008). In this context, the outcomes of the ant-plant interactions may be conditioned to the interactive effect of nectar quality (reward for ants) and the protective effectiveness of the ants (benefits for plants).

As a resource consumer relationship, the stability and efficiency of the interaction depends on the availability and attractiveness of the resource offered (Bronstein 1998; Chamberlain & Holland 2008). Therefore, the density of the consumer will be higher when there is greater availability of resources. Indeed, ants recruit more workers for plants with numerous EFNs (Chamberlain & Holland 2008), greater volume of nectar and higher concentration of sugar (O’Dowd 1979). This mechanism must regulate the outcomes of mutualisms (bottom-up effect; Blüthgen *et al.* 2004); since plants with the highest number of protective ants would suffer less herbivory damage (Rosumek *et al.* 2009; Nascimento & Del-Claro 2010; Vilela *et al.* 2014). In fact, some arboreal ant species are territorial, especially towards highly advantageous resources, and the aggressiveness might be related with resource quality and limitedness (Blüthgen & Fiedler 2004). Nectar attractiveness to ants is important for plant protection (Rosumek *et al.* 2009), and the mutualism outcome may not occur when plants produce low-quality rewards (Bentley 1977; O'Dowd 1979, Alves-Silva & Del-Claro 2013, Del-Claro *et al.* 2014).

The mechanism of better resource attracting consumers that are more protective may drive co-evolutionary processes in ant-plant mutualisms (Bronstein 1998). Plants with high-quality rewards had high reproductive success (Nascimento & Del-Claro 2010) and ants that collect more nectar had higher colony growth (Byk and Del-Claro 2011). Moreover, competition between ants for sugar rich nectar, as well, plants competing for highly aggressive ants may be critical since high-quality nectar and effective ants are limited resources (e.g. ant-hemipteran interaction, Cushman & Addicott 1989; Del-Claro & Oliveira 2000; Fagundes *et al.* 2012), which affects the distribution of interactions between plants (Dáttilo et al. 2014a). Therefore, understanding the structure, maintenance and evolution of interactions between ants and plants depends on the comprehension of the relationship between the nectar quality (cost to plant) and the ant defensibility (benefit). Though quite logical, this mechanism has been empirically explored by very few studies on the scale of community (Heill & McKey 2003; Heill & McKey 2003; Lange & Del-Claro 2014).

In this study, for the first time, we evaluated the structure of ant-plant interactions in Montane Rocky Savanna predicting an interaction pattern typical of mutualistic networks: asymmetric, nested and generalized. Moreover, we evaluated patterns of interactions between ants and plants, and its mutualistic outcomes, in light of resource-consumer dependency hypothesis (Chamberlain & Holland 2008). We specifically evaluated the effect of resource quality (EFN density, volume and sugar concentration of nectar) in the number of interactions with ants. We hypothesized that plants producing high-quality nectar present higher interaction frequency and, more importantly, interactions with ants that are more aggressive. Based on this, we hypothesized that nectar-rich plants will present less herbivory. This study provides empirical evidences suggesting that the outcomes of ants-plants interactions depend on the quality of the resource offered by the plant and consequential protection by highly aggressive ants, confirming the hypothesis of resource-consumer dependency on ant-pant interactions.

Methods

Study area

We performed the study at Itacolomi State Park (PEIT / IEF), located between the cities of Ouro Preto and Mariana (MG / Brazil, 20°22'30”S 43°32'30"W). PEIT is a conservation unity covering an area of proximately 7,500ha of Atlantic Rainforest and Rupestrian Fields. We conducted the observations and experiments between July 2011 and July 2013 in an area of Rupestrian Fields (rocky montane savanna). Rupestrian Field is an ecosystem belonging to the Cerrado (Brazilian savanna) composed by shrubs and small trees scattered in grass fields or aggregated into large patches of vegetation interspersed with rocky outcrops (Alves *et al.* 2014). The study area is located between 1,100 and 1,300m altitude and the climate is temperate humid (Cwb, Koeppen). The average annual temperature is 21° C and the average rainfall is 2,018mm.

Ant-plant interactions

We sampled the interactions in an area of approximately 4ha. Firstly, we walked the area for eight consecutive hours to identify the EFN-bearing plant interacting with ants, which resulted in 10 plant species (Table 1). Then, we tagged 20 shrubs of each species. We kept a minimum distance of 10m between the shrubs to avoid overlapping of ant nests. We observed each plant in three periods: 8:00-11:00 h, 12:00-15:00 h and 16:00-19:00 h. In each observation, we identify the ant species collecting nectar (interactions) and count the number of workers foraging in each shrub (recruitment). We repeat these observations in four days separated by one week (sample). We repeated the samples every 3 months, starting in July 2011 and ending in July 2013. For each plant species, the variable denoting ‘ant interactions’ was the average number per month of ant species interacted with each plant species, considering the two years of study (20 shrubs per plant species, 200 total). We did the same for the variable treated as ant recruitment using the number of ant workers per shrub of each plant species.

Resource attractiveness

We also evaluated the plant investment in attracting ants by the number of EFNs per plant and quality of nectar (volume and sugar concentration). For each shrub, we covered an apical branch of the plant (near the meristem) with a voile bag for 24 hours. This allowed the accumulation of enough nectar to measure the concentration of sugar and reduced the effect of day-night variations in the production of nectar. At the end, we collect nectar accumulated in 10 EFNs of each shrub. We repeated this procedure in all eight samplings. We also quantify the number of EFNs present in each shrub according to Dáttilo *et al.* (2014b); we multiplied the average number of EFN/branch (N = 3) by the number of branches of the shrub. We also measured the plant height to use as covariate for nectar quality and ant density on each shrub.

Consumer protective effectiveness

We measured the aggressiveness of the ants with the classical method proposed by Oliveira *et al.* (1987). We selected 10 shrubs of each plant species. Then, we stick 20 termites in every bush with non-toxic glue. We stick termites as far away from each other as possible to make it difficult to ants found two termites at once. The termites were glued with legs upwards, making it difficult to self-detach and fall. After this, we quantified the number of termites removed over three hours. We apply this procedure on 10 shrubs per day (one of each plant species) due to time constraints. The whole experiment last 10 days within a period of one month. The interval between the days ranged 1-5 days. We calculated the percentage of termites removed per shrub for each plant species and used this value as indicative of ant aggressiveness.

Conditional outcomes of mutualism

Finally, we conducted a classic experiment of ant removal to assess the ant protection and the variation in protective efficiency between plant species with different nectar quality (context dependency). In late November 2012, we selected 20 shrubs of each plant species. Plants possessed similar height and were at reproduction period. Out of these 20 shrubs, we randomly selected 10 shrubs to be treated with ant removal. We blocked the access of ants to the crown. We wind a plastic tape (10cm wide) at the base of the trunk and apply a layer (5cm wide) of non-toxic resin Tanglefoot® over the tape. The resin prevents the passage of ants into the shrub crown. We pruned the branches of neighboring plants and leaves of grass that could serve as a bridge for the ants. Then, we randomly selected three branches of each shrub (farthest possible) and six apical leaves on each branch (18 leaves per shrub). We maintained the blockade for three months. After that, we used a millimeter plastic grid disposed over each of the marked leaf to measure leaf loss. Then, we quantified the number of squares corresponding to the intact leaf area and leaf area lost. The values treated as indicative of herbivory correspond to the average (per shrub) of the percentage of leaf area lost.

Data analysis

Ant-plant interactions

We started the analysis evaluating the structure the interaction network. We built one matrix “A_{ij}”, where “A” is the interaction frequency of an ant species “i” in harvest nectar from a plant species “j”. Interaction frequency corresponds to the sum of ant workers (recruitment) observed on the 20 shrubs from each plant species during all eight quarterly samplings (2011-2013). We used the Bipartite package (Dormann et al. 2008) in R software (R Development Core) to build the network graph and calculate the follow metrics that describes interaction sharing: connectance (percentage of possible interactions actually performed), the average number of shared partners (for both ant species and plant species) and niche overlap (also for ants and plants). The pattern of interaction was assessed using the ANINHADO software (Guimarães & Guimarães 2006), in which we calculate the metric NODF of nestedness and its significance based on comparison with random NODF generated by Null Model Type I. In addition to NODF, we calculate the H2’ level of network specialization and its significance compared to H2’ randomly generated by Monte-Carlo’ iterations. All descriptor metrics were useful to interpreting the structure of interactions between ants and plants from the perspective of resource-consumer relation since they are all based on overlap degree of resources usage.

We used ANOVA to test the difference between plant species (fixed factor) in the average number of foraging ants observed per shrub (i.e. recruitment) and the average number of ant species collecting nectar on each shrub (i.e. number of interactions). Since ant occurrence in the shrubs can be related to the size of the plant, we used plant height as a covariate. We tested paired differences with LSD-tests.

Resource attractiveness

We compare the plant attractiveness through the follow dependent variables calculated for each shrub: average number of nectaries per shrub; nectar volume per EFN; and the average concentration of sugar. We tested the difference between plant species (fixed factor) for the three parameters using a multivariate analysis of variance (MANOVA), since they were self-correlated. The 20 shrubs of each plant species were used as independent cases (N = 200). Since plant size may affect nectar production, we use the height of the shrubs as a covariate. To test the attractiveness of resource quality we summarize the three measured parameters using Principal Component Analysis (PCA), since they were correlated. The first axis resulting from the analysis was used as a ‘resource quality index” against the number of interactions with ants by two simple linear regression analyzes (SLR); one for the dependent variable 'ant recruitment' and one for the dependent variable 'number of interactions'.

Consumer protective effectiveness

We use the percentage of captured termites (aggressiveness index) as a measure of ant aggressiveness against potential herbivores (protective effectiveness) on each plant species. We compared the ant aggressiveness between the ten plant species (fixed factor) using ANOVA (N = 20 shrubs per plant species). To explain the variation in ant aggressiveness between plant species we use the resource quality index (quantity and quality of nectar). We also test the effect of interaction frequency with an index for 'ant interactions'. Resource quality and ant interactions indices were used in a simple linear regression model (SLR) as explanatory factor for the variation in the ant aggressiveness between plant species.

Conditional outcomes of mutualism

We evaluated the mutualistic nature of the ant-plants relationship by comparing the percentage of leaf area loss (dependent variable) between plants with and without ant interactions (fixed factor) for all ten plant species studied (fixed factor), using analysis of variance (ANOVA). For each plant species, we calculated the difference in average herbivory between plants with and without ants as a 'protective outcome index'. We divided the average herbivory of shrubs without ants by the average herbivory of shrubs protected by ants. The resulting value is a 'protective outcome index', i.e. how much more herbivory plants not interacted with ants suffer compared to protected conspecifics. This index is dimensionless, which enables comparisons between different species regarding its biological specifics in leaf area. Additionally, we performed a PCA to summarize the amount of recruited ants and the number of ant species interacted into a single 'ant interaction index'. Then, using multiple linear regression (MLR), we evaluated the ant interaction index and the ant aggressiveness index as explanatory factors for differences in the protective outcomes between plant species.

All dependent variables were linearized by logarithmic function to attend assumptions of linear models and ANOVA. Linear models and analyses of variance were performed in Action (Equipe Estatcamp, 2014), a free statistical software based on R platform (RCore Team, 2014). PCA analysis were performed on PAST free statistical software. All descriptive values in the text and figures depict the mean value as central measure and standard deviation as dispersion measure.

Results

Ant-plant network

The ant-plant network presented a nested pattern of interaction (NODF: 57.8, $p < 0.001$) and low degree of specialization (H2' = 0.17, $p < 0.001$; Figure 1). The network possessed 147 interactions (45.9% of possible interactions). Ant species interacted with 4.6 ± 3.7 plant species (mean ± standard deviation, n = 10), sharing on average 2.1 plant species (niche overlap: 21%). *Campontous crassus*, *C. novogranadensis*, *C. senex*, *Cephalotes pusillus*, *Pseudomyrmex gracillis*, *P. pallidus*, *Brachymyrmex heeri* and *Pheidole* sp.1 (25% of ant species) were the highest interacted species (10 interactions) (Figure 1, Table 1). On the other side, plant species interacted with 14.7 ± 1.5 ant species and they shared an average of 10.6 ant specie partners (niche overlap: 76.3%). The total number of interactions for plant species varied between 12-16 ant species (Figure 1, Table 2).

We observe 13, 888 ants from 32 ant species foraging on 10 EFN-bearing plant species (Table 1 and 2; Figure 1). We observed an average recruitment of 8.7 ± 9.2 ant workers and 5.3 ± 1.9 ant specie interactions per shrub (mean ± standard deviation; N = 200). The average recruitment differ between plant species (ANOVA: $F_{9,\ 189} = 48.7$, $p < 0.001$; Table 1). Plant species had also differed in the average number of interactions per shrub ($F_{9,\ 189} = 9.1$, $p = 0.0001$, Table 2). The PCA' first axis, representing the 'number of ant interactions' with plants explained 80.4% (PCA: Eigenvalue = 1.6) of the variance observed for recruited workers (correlative coefficient: 0.56) and for interactions with ant species (0.55; Table 2).

Resource attractiveness

All plant species showed different number of EFNs, nectar volume and sugar concentration (MANOVA: $F_{27,\ 547} = 122.9$, $p < 0.001$; Table 3). The PCA' first axis, representing the 'resource quality', explained 65.7% (Eigenvalue = 1.97) of the variance observed in the quantity of EFNs (correlative coefficient: 0.35), volume of nectar (0.39) and sugar concentration (0.48) between plant species (Table 3). We observed that plants offering nectar with higher quality, attracted more ant workers to its crown (SLR: $R = 0.70$, $\beta = 0.84$, $F_{1,\ 8} = 19.2$, $p = 0.002$) and interacted with more ant species (SLR: $R = 0.64$, $\beta = 0.8$, $F_{1,\ 8} = 14.2$, $p = 0.005$; Figure 2). *Stachytarpheta glabra*, *Chamaecrista mucronata* e *Banisteriopsis campestris* were the most nectar-rich plants and the most interacted plant species, while *Chamaecrista desvauxii* and two species of *Senna* produced a poor nectar and were the least interacted plants

(Table 2 and 3; Figure 2). The average recruitment ($F_{1, 189}$ = 1.52, p = 0.2) and the average number of interactions with ant species ($F_{1, 189}$ = 0.3, p = 0.6) did not respond to plant size.

Consumer protective effectiveness

Ant species captured on average 46.4 + 38.3% (N = 4000 termites). The ant aggressiveness (percentage of termites captured) was different between plant species ($F_{9, 190}$ = 7.19, p < 0.001). S. glabra was the most protected plant (95 ± 22% of termites captured), followed by *C. mucronata* (53 ± 26%), while *Banisteriopsis malifolia* was the least protected (20 ± 28%). This difference in aggressiveness was explained by the resource quality (SLR: β = 0.16, t = 3.43; Figure 3). Thus, ants were highly aggressive on plants providing nectar with better quality, such as *S. glabra* and *C. mucronata* (MLR: R^2 = 0.59, $F_{1, 8}$ = 11.8, p = 0.01; Figure 3).

Conditional outcomes

The results of our experiment show that plants without ants had greater loss of leaf area (23 ± 15%) compare to plants protected by ants (13 ± 9%; $ANOVA_{factor}$: $F_{1, 180}$ = 45.6 , p < 0.001). This difference in the rate of herbivory was consistent for all plant species ($ANOVA_{interaction}$: $F_{9, 180}$ = 1.8, P = 0.07; Figure 4). The results of our experiment show that plants without ants had greater loss of leaf area (23 ± 15%) compare to plants protected by ants (13 ± 9%; $ANOVA_{factor}$: $F_{1, 180}$ = 45.6 , p < 0.001). This difference in the rate of herbivory was consistent for all plant species ($ANOVA_{interaction}$: $F_{9, 180}$ = 1.8, P = 0.07; Figure 4). On average, plants interacted with ants had 1.9 ± 0.9 times less herbivory than plants unprotected by ants. This difference between the plants in the quantity of leaf area lost (protective outcome index) was explained by the cumulative effect of the number of interactions with ants (interaction index; MLR: β = 0.32; t = 1.05) and the aggressiveness of ant partners (aggressiveness index; MLR: β = 2.52; t = 1.77; Figure 5). Thus, plants interacted with a great number of ants, and more importantly, defended by highly aggressive protectors, presents great beneficial outcome from the mutualism (MLR: R^2 = 0.58, $F_{2, 7 =}$ 4.9, p = 0.04; Figure 5).

Discussion

Corroborating our first hypothesis, the network of interactions between ants and plants with EFNs in Rupestrian fields, for the first time studied, did not differ from the nested and low specialized pattern of interaction observed in mutualistic networks studied in Brazilian Cerrado (Lange *et al.* 2013; Lange & Del-Claro 2014) and Amazon Forest (Dáttilo *et al.* 2013a, c) or other tropical ecosystems (Diaz-Castelazo *et al.* 2004; Guimarães *et al.* 2007; Bascompte *et al.* 2006; Vazquez *et al.* 2009). Our results shown that plants interactions were highly generalized (mean of 14.7 ant species interacted per plant species) and with high overlap of interaction (mean of 10 ant species shared per plant species). Conversely, ant species were more specialized (mean of five plant species interacted per ant species) but shared only 2.1 species. This generalist networks are very cohesive which increases resistance against disturbances (Dáttilo 2012; Passmore 2012) but suggest high competitive pressure caused by large niche overlap between species involved (Dáttilo *et al.* 2014a). These results is the first evidence supporting our hypothesis that ant-plant interaction may be based in consumer response to resource attractiveness, leading to high number of interactions for plants, but also consumer competition for nectar, leading to low number of interactions for ants and low overlap in interactions.

Our results shows that the variation in quantity and quality of the nectar offered by plants affected the number of interactions with ants. For example, *S. glabra* and *C. mucronata* had the highest mean number of interactions (average of 6.3) and ant recruitment (23 ants per shrub), and these species secreted voluminous nectar (1.7µL/EFN/24h) with high concentration of sugar (62% mg/mg). Thus, the number of ants foraging on plants in Rupestrian Fields is a result of food availability, as observed for Tropical Forests (Davidson *et al.* 2004; Blüthgen *et al.* 2004), Atlantic Forests (Campos *et al.* 2006) and Brazilian Savanah (Schoereder *et al.* 2010; Lange *et al.* 2013; Vilela *et al.* 2014) and Rupestrian Fields (Fagundes *et al.* 2012). However, at the crown level, a shrub with many scattered ENFs producing large amount of low-sugary nectar is able to accumulate many co-occurring species due to small-scale reduction of interspecific competition (Blüthgen *et al.* 2004), thereby allowing a greater number of interactions. For example, *B. campestris* and *H. umbellata* interacted with many species of ants (6.6) but with low recruitment (5.6 shrub ants), which may be due to the high number of EFNs (502.2 per shrub) producing voluminous nectar (1.4µL/EFN/24h) with relative low sugar content (39% mg/mg). Therefore, offers a large amount of nectar with better quality increases the number of interactions with ants because nectar is limited and high valuable at the same time attracting many ant species. However, when nectar is very sugary, it becomes more valuable and creates competitive pressures among ants (Davidson *et al.* 2003; Blüthgen & Fiedler 2004), leading to asymmetries in interaction frequency (Blüthgen & Fiedler 2004). As consequence of forage energetics, interspecific aggressiveness would be advantageous only towards highly energetic nectar (Koptur 2005).

High-sugary nectar is advantageous because it allows a low foraging cost for ants (Davidson *et al.* 2004). In this case, ants get much energy from small quantity of nectar by harvesting quickly and avoiding confrontations, reducing the effects of energy loss in finding and transporting nectar to the nest (Carroll & Janzen 1973; O'Dowd 1979). Moreover, ants that collect more nectar show higher colony growth (Byk & Del-Claro 2011), which may be enhanced by the consumption of sugar-rich nectar. Therefore, in plants with sugar-rich nectar, one can expected high numerical dominance. Note that the ant *C. crassus* interacted with more than half of the shrubs of the nectar-rich *S. glabra* and *C. mucronata*. *C. crassus* had also prevail in other four plant species, but did not in the four nectar-poor species. These dominant ants may interact with most of the plant species at the expense of lower competitive species (Blüthgen *et al.* 2004; Blüthgen & Fiedler 2004). In fact, the eight core ant species represented 91.6% of the 13,888 ants observed on plants, and *C. crassus* was the most common ant (56.6%). In this sense, we believe that the nested pattern of interactions within the ant trophic level is determined by resource dominance interactions (resource-consumer relationship), while plants achieve more interactions when offering best quality nectar. Taking together, our results indicates a balance between nectar quality and ant competition affecting the interaction patterns of ant-plant network. Thus, we confirmed our main hypothesis that a resource-consumer dependency mechanism structuring ant-plant interaction networks.

In general, plants bearing the ENFs in Rupestrian Fields were effectively defended against herbivores by ants, i.e. herbivory was higher in the absence of the protective ants. The interaction outcome depended on nectar quality, thus nectar-rich species as *S. glabra* and *C. mucronata* were better protected than nectar-poor species as *Senna reniformis* or *Crotalaria micans*. The difference in herbivory between plants was mainly determined by the aggressiveness of interacted ants, specially *C. crassus*, which in turn was enhanced by the quality of the offered nectar. This is even more interesting when combined with the result that all plants interacted with basically the same eight ant species (core network), which in turn were the most frequent and more interactive.. That is, aggressive behavior of ants are context-dependent and related to the quality of the offered nectar and not the identity of the ant specie, confirming our main hypothesis of context-dependency in ant-plant interactions.

The relationship between resource quality, aggressiveness and effectiveness of defense suggests an ecological mechanism of co-evolutionary processes between ants and plants. The emergence of a high quality nectar would be an important adaptation for plants in attracting aggressive partners, while a stable relation with territorial dominant ants would accelerate the evolution of specializations and reduction of exploitation by non-aggressive ants. For example, in the relationship between *Acacia* spp. and the dominant *Pseudomyrmex* spp., specialization occur by the presence of a sugar specifically digested by real mutualistic species, which considerably reduces the exploitation by bad-protective ants and increases net benefits (Heill *et al.* 2005; Kautz *et al.* 2009). Considering only mutualistic ant species, intraspecific competition may also play an important part, since ant colonies tend to exchange a less advantageous resource for a better one (Cushman & Addicott 1989; Blüthgen & Fiedler 2004). This could be another explanation to the context-dependency in aggressiveness, since high-aggressive colonies would dominate nectar-rich plants and provide better protection while nectar-poor plants may interact with low-aggressive colonies and receive less protection. This mechanism also explain why all plant species interacts with almost the same ant species but receive different protection. Thus, a competition among plants for the best protective ant (competition for mutualists) may be an important mechanism driven population dynamics of these plants, as observed for honeydew producers Hemiptera (Cushman 1981; Cushman & Addicott 1989). Studies at the population level consequences of ant-plant interactions are still rather scarce but suggest a positive effect of ants on the growth of plant populations (Bruna *et al.* 2014).

We observed an interesting pattern when we analized the differences in herbivory between plant species accodirng to nectar quality: (1) Plants that produces low quality nectar receives low herbivory damage and ants had low defensive effect. (2) Plants with high quality nectar also presents low levels of herbivory but ants had high defensive effect. (3) Plants with intermediate quality of nectar also suffer intermediate herbivory and ants had a medium impact in plant defense. Two mechanisms may be responsible for this pattern. First, as we show, ants may change its aggressive behavior depending on quality of nectar offered by the plant. Therefore, plants with low quality nectar would interact with poorly defensive ants. Secondly, plant species face different trade-off situations between fast growth investments versus defense investment that could be reflected in alternative defense strategies (Coley 1988; Herms & Mattson 1992). A week protection of a watering nectar production ought to produce some ant defense, enough to protect the plant against generalist herbivores, as expected to predominate on them (Coley 1988; Novotny et al. 2002; Ribeiro et al. 2005).

Evidences of existence and effects of species dominance hierarchies limiting the nectar consumption and filtering ant-plant interactions are growing steadily (Blüthgen & Fiedler 2004; LeBrun 2005; Diaz-Castelazo *et al.* 2010; Dáttilo *et al.* 2014a, b, c; Lange & Del-Claro 2014). The competitive mechanisms structuring ant assemblages are key factors to ecology of ants and plants (Gibb & Hochuli 2004; Parr 2008; Parr & Gibb 2010) and nectar play an important role (Davidson *et al.* 2003; Blüthgen *et al.* 2004; Blüthgen & Fiedler 2004). However the fully comprehension of competition effects on the ecology of interactions requires behavior observations on the field and experimental approach on the fitness of the interacted species. With these tools, we shown that resource-consumer dependency and competitive behaviors between ants may have great importance structuring relationships between ants and plants and regulating mutualistic outcomes. Therefore, this study contributed for the understanding of mechanisms structuring ant-plant mutualisms.

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Tables

Table 1. List of ant species observed interacting with plants in Rupestrian Fields (Ouro Preto, Brazil), between Jun/2011 and Jun/2013. 'Abundance' column presents the total number of ants observed foraging on plants, and 'Interaction' column presents the number of plant species interacted.

Ant Specie	Code	Abundance	Interactions
Brachymyrmex heeri	BRH	123	10
Brachymyrmex pictus	BRP	10	4
Camponotus alboanulatus	CAA	38	1
Camponotus crassus	CAC	7805	10
Camponotus melanoticus	CAM	171	4
Camponotus novogranadensis	CAN	2313	10
Camponotus rufipes	CAR	320	5
Camponotus senex	CAS	298	10
Cephalotes pusillus	CEP	1261	10
Crematogaster sp.1	CR1	103	8
Crematogaster sp.2	CR2	121	4
Crematogaster sp.3	CR3	2	1
Dorymyrmex brunneus	DOB	177	7
Gnamptogenys sulcata	GNS	9	3
Linepithema sp.1	LI1	3	1
Linepithema sp.2	LI2	2	1
Linepithema micans	LIM	10	2
Myrmelachysta catharinae	MYC	7	1
Pachycondyla sp.1	PA1	10	2
Pachycondyla sp.2	PA2	4	2
Pheidole sp.1	PH1	77	10
Pheidole sp.2	PH2	30	4
Pheidole sp.3	PH3	6	2
Pheidole sp.4	PH4	1	1
Pseudomyrmex gracillis	PSG	672	10
Pseudomyrmex pallidus	PSP	170	10
Pseudomyrmex termitarius	PST	100	8
Tapinoma sp.1	TA1	1	1
Wasmannia sp.1	WA1	32	2
Wasmannia sp.2	WA2	1	1
Wasmannia sp.3	WA3	9	1
Wasmannia sp.4	WA4	2	1

Table 2. List of EFN-bearing species observed in Rupestrian Fields (Ouro Preto, Brazil). The ‘ant recruitment’ column presents the total number of ants (also mean per shrub and standard deviation) observed foraging on 20 shrubs of each plant species quarterly observed from Jun/2011 to Jun/2013. The ‘ant interaction column’ presents the total number of ant species interacted (also mean and standard deviation). Plants differed in the number of ant interactions (ANOVA: $p < 0.0001$) and ant recruitment (ANOVA: $p < 0.001$). Different letters depict paired statistical difference (LSD test: $p < 0.05$). Species are classified according with interaction index (PCA axis combining the two interaction variables).

Plant species	**Code**	**Ant interactions**	**Ant recruitment**	**Interaction index (PCA Axis 1)**
Stachytarpheta glabra (Verbenaceae)	STG	15 (6.15 ± 1.8) A	4489 (28.1 ± 11.7) A	1.6
Chamaecrista mucronata (Leguminosae)	CHM	15 (6.4 ± 1.2) A	2877 (18 ± 7.4) B	1.3
Heteropteris umbellata (Malpighiaceae)	HEU	15 (6.3 ± 1.3) A	1065 (6.7 ± 2.3) C	0.5
Banisteriopsis campestris (Malpighiaceae)	BAC	15 (6.9 ± 1.6) A	721 (4.5 ± 1.7) D	0.5
Crotalaria micans (Fabaceae)	CRM	16 (4.7 ± 1.5) B	1383 (8.7 ± 4.8) C	-0.1
Banisteriopsis malifolia (Malpighiaceae)	BAM	16 (5.1 ± 1.8) B	822 (5.1 ± 3.2) D	-0.2
Senna pendula (Leguminosae)	SEP	12 (4.7 ± 1.9) B	1025 (6.4 ± 3.3) C	-0.3
Peixotoa tomentosa (Malpighiaceae)	PET	15 (4.4 ± 1.6) B	590 (3.7 ± 2.3) E	-0.9
Senna renniformis (Leguminosae)	SER	12 (4.2 ± 1.4) B	531 (3.3 ± 2.2) E	-1.0
Chamaecrista desvauxii (Leguminosae)	CHD	16 (3.8 ± 1.4) C	385 (2.4 ± 1.4) F	-1.5

Table 3. Differences in resource quality offered by the 10 plant species studied in Rupestrian Fields (Ouro Preto, Brazil). Values represent average values per shrub (N = 20 per plant species) measured in eight quarterly samplings between Jun/2011 and Jul/2013. Plant species shown different number of EFNs, nectar volume and sugar concentration (MANOVA: $p < 0.001$). Different letters depict paired statistical difference (LSD test: $p < 0.05$). Species are classified according with resource quality index (PCA axis combining the three resource quality variables).

Plant species	**Code**	**EFNs per shrub**	**Sugar concentration (mg/µL)**	**Nectar volume (µ/EFN/24h)**	**Resource quality index (PCA Axis 1)**
Stachytarpheta glabra (Verbenaceae)	STG	4,036 ± 4,414.3 A	1.04 ± 0.03 A	2.16 ± 0.83 A	1.8
Chamaecrista mucronata (Leguminosae)	CHM	581 ± 462.7 B	0.61 ± 0.06 B	1.15 ± 0.45 B	0.8
Banisteriopsis campestris (Malpighiaceae)	BAC	238.3 ± 166.8 C	0.52 ± 0.03 C	2.04 ± 0.64 A	0.8
Heteropteris umbellata (Malpighiaceae)	HEU	766 ± 557.8 B	0.47 ± 0.03 C	0.69 ± 0.42 C	0.5
Crotalaria micans (Fabaceae)	CRM	463.9 ± 428.7 B	0.22 ± 0.02 F	1.75 ± 1.9 B	0.2
Senna renniformis (Leguminosae)	SER	448.8 ± 331 B	0.25 ± 0.05 D	0.07 ± 0.02 D	-0.5
Banisteriopsis malifolia (Malpighiaceae)	BAM	64.6 ± 27 D	0.15 ± 0.03 D	1.33 ± 0.31 B	-0.5
Peixotoa tomentosa (Malpighiaceae)	PET	417.6 ± 413.4 B	0.18 ± 0.02 D	0.12 ± 0.02 D	-0.6
Senna pendula (Leguminosae)	SEP	464.6 ± 453.3 B	0.11 ± 0.03 D	0.09 ± 0.03 D	-0.9
Chamaecrista desvauxii (Leguminosae)	CHD	282.8 ± 165 C	0.05 ± 0.01 E	0.05 ± 0.02 D	-1.5

Figures

Figure 1. Network of interactions between ants and plants observed between Jul/2011 and Jul/2013 in Rupestrian Fields (Ouro Preto/Brazil). Right bars depict plant species, left bars depict ant species, and links depict paired interactions. Bar length, denote relative number of interaction performed by each species. Line width denote relative number of interaction between each pair of species. Species are sort top-bottom by decreasing number of interactions.

Figure 2. Linear relation between the resource quality index affecting ant recruitment and the ant interactions observed on plants. Plants providing higher volume of sugar-rich nectar recruited more ant workers (SLR: $p = 0.002$) and interacted with more ant species (SLR: $p = 0.005$).

Figure 3. Linear relation of resource quality (PCA Axis 1 combining 'number of EFNs', 'nectar volume' and 'sugar concentration') affecting ant aggressiveness (percentage of termites captured). Plants that offered nectar of better quality nectar interacted with ants that were more aggressive (MLR: $p = 0.01$).

Figure 4. Difference in the leaf area loss between shrubs with and without ants for 10 plant species bearing EFNs. Shrubs without ants lost more leaf area than interacted shrubs ($\text{ANOVA}_{\text{factor}}$: $p < 0.001$) regardless of plant species ($\text{ANOVA}_{\text{interaction}}$: $p = 0.07$). Labels presents the outcome protective index (interaction benefit), which indicates how much more herbivory a shrub without ants suffered in comparison to a protected shrub.

Figure 5. Number of interactions with ants and aggressiveness of ants as predictors for the mutualism outcomes in herbivory defence. Plants with more interactions with ants (MLR: $\beta = 0.32$), especially with highly aggressive species (MLR: $\beta = 2.52$), receive greater protection against herbivory (MLR: $R^2 = 0{:}58$, $p = 0{:}04$).

Figures

Figure 1

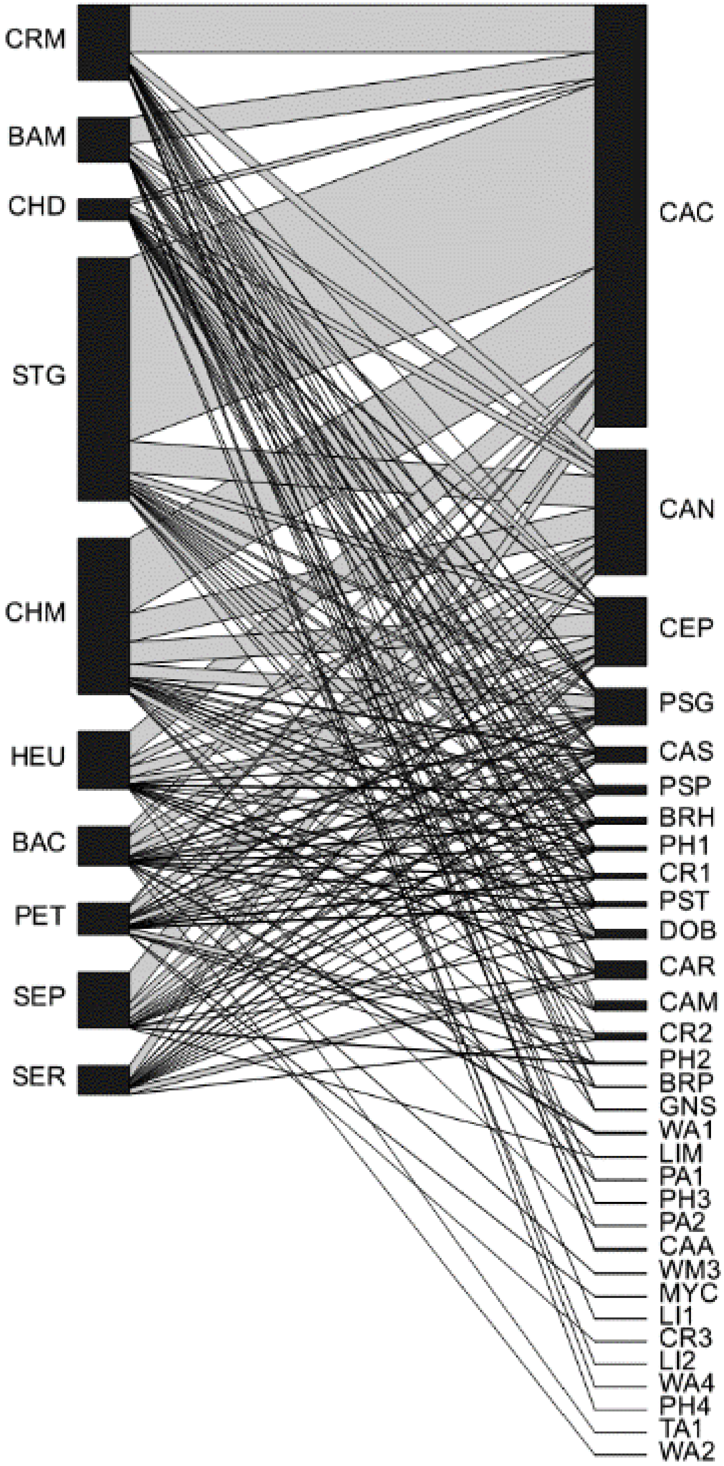


Figure 2

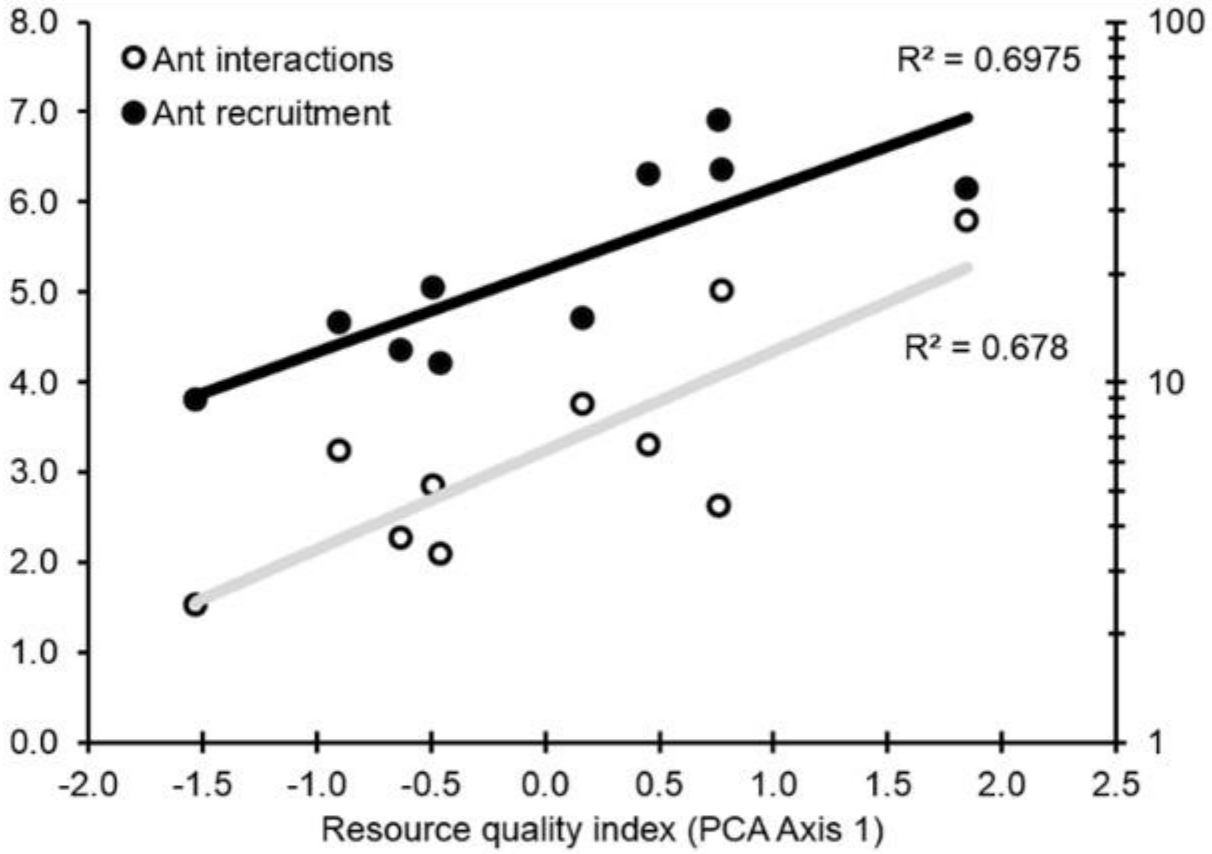

○ Ant interactions
● Ant recruitment
R² = 0.6975
R² = 0.678
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
100
10
1
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
Resource quality index (PCA Axis 1)

Figure 3

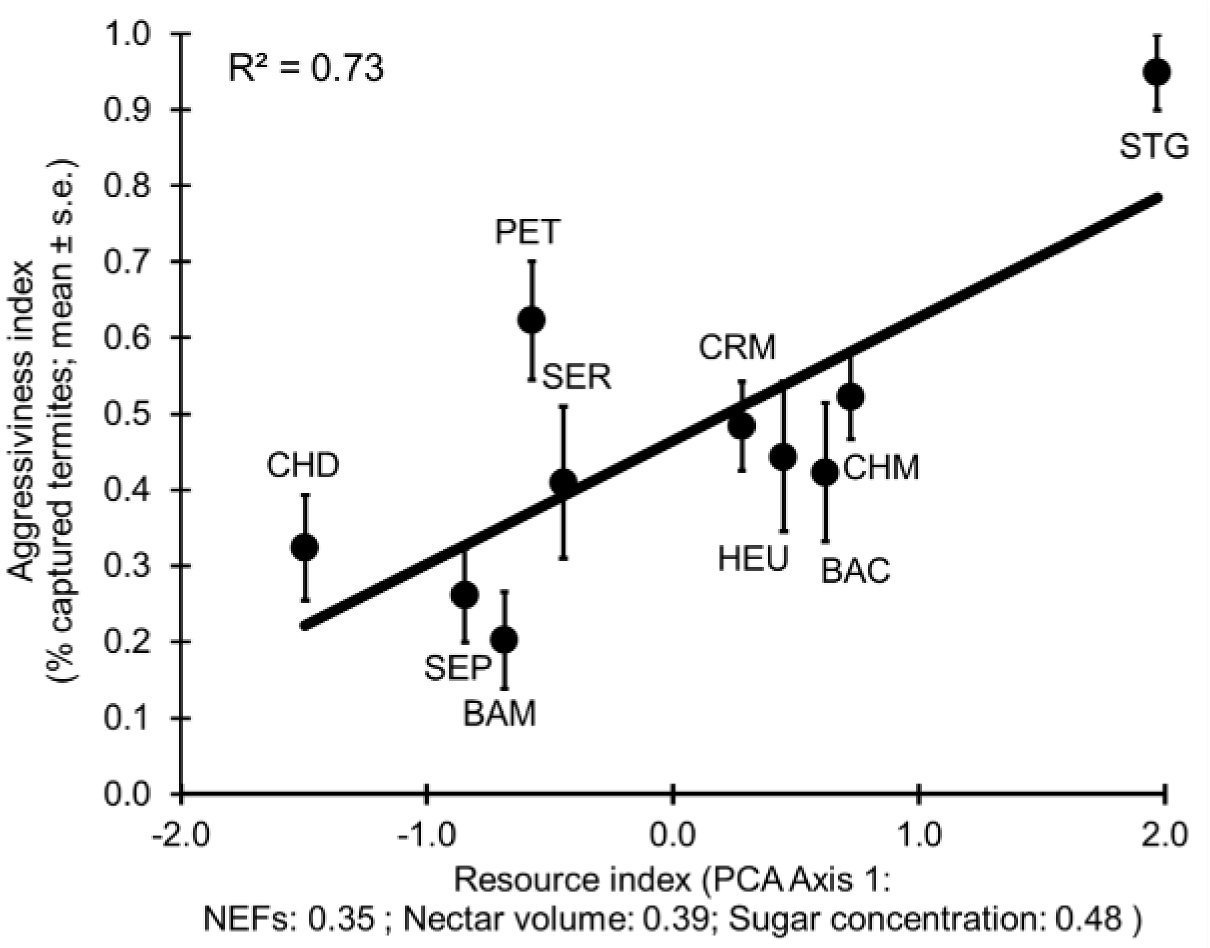
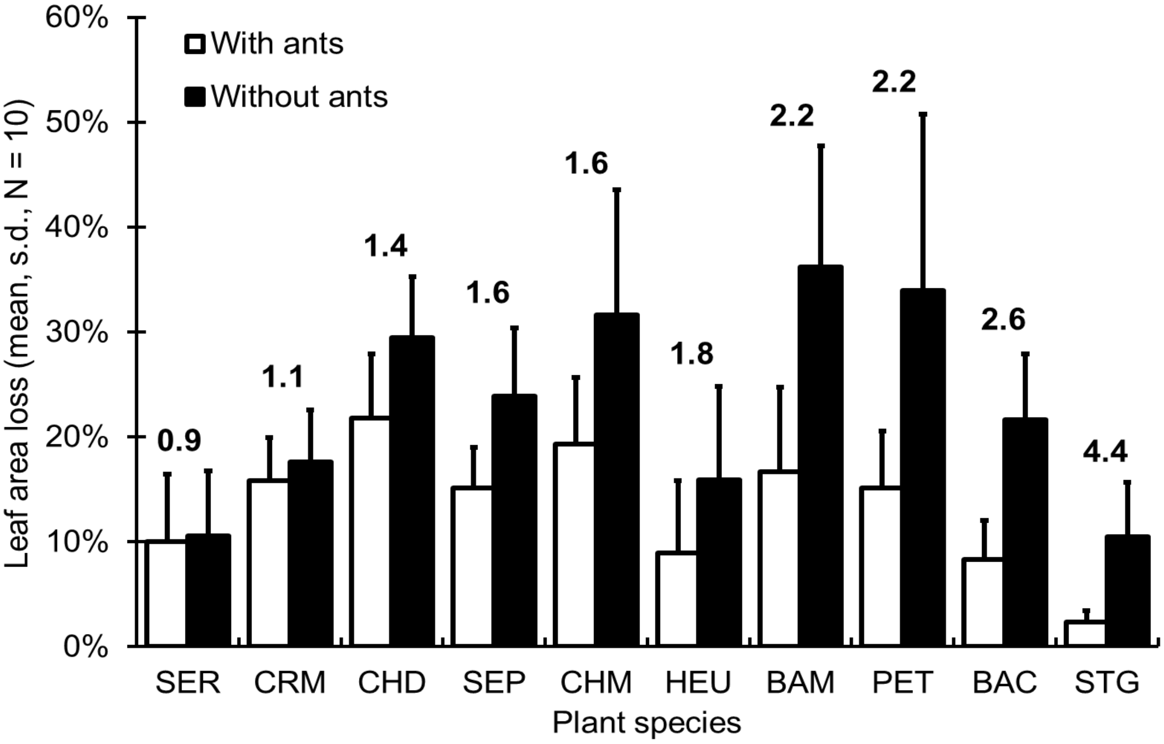
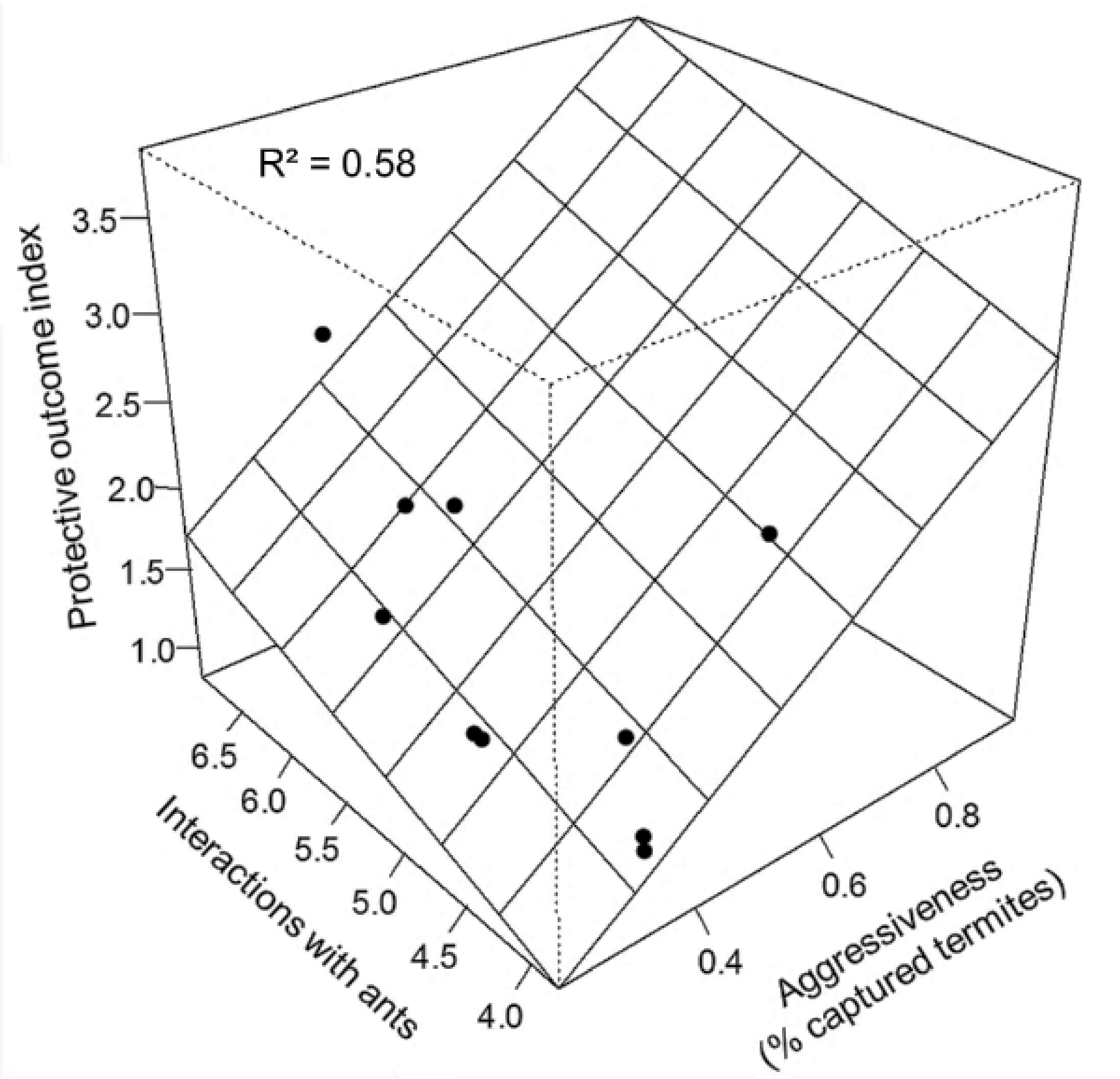

R² = 0.73
Aggressiviness index
(% captured termites; mean ± s.e.)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-2.0
-1.0
0.0
1.0
2.0
Resource index (PCA Axis 1:
NEFs: 0.35 ; Nectar volume: 0.39; Sugar concentration: 0.48)
STG
PET
SER
CRM
CHD
CHM
HEU
BAC
SEP
BAM

Figure 4



60%
50%
40%
30%
20%
10%
0%
Leaf area loss (mean, s.d., N = 10)
☐ With ants
■ Without ants
0.9
1.1
1.4
1.6
1.6
1.8
2.2
2.2
2.6
4.4
SER
CRM
CHD
SEP
CHM
HEU
BAM
PET
BAC
STG
Plant species

Figure 5


R² = 0.58
Protective outcome index
3.5
3.0
2.5
2.0
1.5
1.0
Interactions with ants
6.5
6.0
5.5
5.0
4.5
4.0
Aggressiveness
(% captured termites)
0.4
0.6
0.8

Capítulo 3

Qualidade do néctar determina hierarquia interespecífica de dominância em mutualismos formiga-planta

Resource quality determines interspecific dominance hierarchy in ant-plant mutualisms

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Running title: *Dominance hierarchies in ant-plant interactions*

Resumo:

Interações entre formigas e plantas com nectários extraflorais (NEFs) são mutualismos generalizados baseados na oferta de recursos (néctar), em troca de proteção contra herbívoros. O consumo de néctar aumenta o fitness das formigas, que muitas vezes competem por este recurso. Os resultados dessas relações pode influenciar a estabilidade e a evolução das interações formiga-planta. Este estudo teve como objetivo avaliar a relação de dominância entre as formigas visitantes de plantas portadoras de EFNs em uma área de Campo rupestre (Brasil). Nós testamos a hipótese de que formigas em plantas têm alta sobreposição no uso de néctar o que gera interações agressivas de disputa pelo recurso. Esta agressividade cria hierarquias de dominância, que por sua vez, explicariam as diferenças na frequência de interação entre espécies e plantas de formigas. Avaliou-se a rede de interações entre 32 espécies de formigas e 10 espécies de plantas por dois anos (2011-2013). Além disso, foi aplicado um experimento comportamental com encontros pareados entre as espécies de formigas que mais interagiram com plantas, replicado em todas as espécies de plantas. Observou-se que as espécies de formigas numericamente e agressivamente dominantes foram as que mais interagiram com plantas (interativamente dominantes). Fomos capazes de demonstrar que essas formigas interagiram de forma agressiva uns com os outros criando hierarquias de dominância. Mais importante, nós mostramos que essas hierarquias são dinâmicas e dependentes da qualidade do recurso. Ou seja, tanto a estrutura como o nível assumido pelas espécies podem ser diferentes, dependendo da qualidade do néctar. Conclui-se que as relações dinâmicas de dominância e partição de mutualismos podem ser importantes mecanismos de convivência formigas.

Palavras-chave: coexistência de espécies, mutualismo trofobiótico, redes formiga-planta, competição por interferência, ecologia comportamental, Campos rupestres.

Abstract*:*

Interactions between ants and plants bearing extrafloral nectaries (EFNs) are generalized mutualistic relationships based in offer of food resource (nectar) in exchange of protection against herbivores. Nectar consumption increases the fitness of the ant, which often compete for this resource. Thus, interspecific aggressiveness towards nectar may regulate access to the plants and therefore, the establishment of interactions. This study aimed to evaluate the dominance relationship between ant visitors of EFN-bearing plants in an area of Rocky Montane Savannah (Brazil). We tested the hypothesis that ants on plants have high overlap in nectar usage what generates aggressive interactions and then creates dominance hierarchies. This aggressiveness may explain differences in interaction frequency between species of ants and plants. We evaluated the network of interactions between 32 species of ant and 10 species of plants for 2 years (2011-2013). In addition, we applied a behavioural experiment with paired encounters between the most interacted ant species, replicated in every plant species. We observed that the numerically and behaviourally dominant ant species were the most interacted with plants (interactively dominant). We were able to demonstrate that these ants aggressively interacted with each other creating dominance hierarchies. More importantly, we show that these hierarchies are dynamic and context-dependent. That is, both the structure and the level assumed by the species may differ depending on the quality of the nectar. We conclude that the dynamic relationships of dominance and the partitioning of interactions may be important mechanisms of ant coexistence in highly diverse mutualisms.

Key words: species coexistence, trophobiotic mutualism, ant-plant networks, interference competition, behavioural ecology, Rupestrian Fields.

Introduction

Plants bearing extrafloral nectaries (EFNs) secrete nectar that attracts several ant species, which protect them against herbivores (Del-Claro et al. 1996) and often increase plant reproductive success (Nascimento & Del Claro 2010). On the other hand, nectar collected by ants is a nutritionally and energetic advantageous resource (Heil & McKey 2003) that improves the fitness of the ant colony (Byk & Del-Claro 2011). Therefore, although it is a mutualistic relationship, these ants-plant interactions can be treated as trophic relationships between consumers and resources. Based on this assumption, the formation of these interactions at community level should respond to ecological mechanisms of trophic relationships such as intra- and interspecific competition (Fellers 1987), niche partitioning (Schoener 1974), and resource-consumer dependency (Chamberlain & Holland 2009). For example, asymmetries in the frequency of interaction between ant species interacting with plants in the coastal of Mexico is attributed to differences in the capability of numerically dominate resources in the environment (Dáttilo et al. 2014a). Thus, highly interacted ants would be aggressive and territorial species that dominate most of the resource (i.e. plants) whereas lowly interacted ants would be submissive species tolerated by dominants on few plants (Dáttilo et al. 2014a). However, the existence of resource dominance interactions in ant-plant mutualisms remains unclear due to lack of experimental and behavioural studies directly involving the interacted ants and plants in the field, rather than using interactions outcomes on artificial baits.

Mutualism between ants and plants bearing EFNs are facultative, so many ant species can interact with the same plant species (Oliveira et al. 1999; Rico-Gray & Oliveira 2007). This high overlap in resource usage, high number of shared interaction, can lead to fights and mutual repellence between ant species on plants, and thus regulate the frequency of interactions between ants and plants. These relationships suggest the foundation of interference competition, in which the species hinder access to resources (i.e. nectar on EFNs) through territories (Fellers 1987; Davidson et al. 2004). Aggression and territoriality are energetically costly behaviours and can injury or even kill competitors (Fellers 1987; Davidson et al. 2004). Therefore, it is common to find a balance between aggression and interspecific tolerance to save energy (Axelrod & Hamilton 1981; Fellers 1987). By the definition of competition, this balance should be regulated mainly by the availability and quality of limiting resources (Robinson 1985; Blüthgen et al. 2004). Therefore, high competition and interspecific aggressiveness may occur when the resource is highly energetic and nutritional as much as limited. In fact, nectar itself is a limited and advantageous resource to ants (Byk and Del-Claro 2011) and can generate competitive pressures (Heil & MacKey 2003). Additionally, there is great interspecific (Rudgers & Gardener 2004) and intraespecific (Calixto et al. 2015) variation in nectar offered by plants. This variation may be related to different periods of the day (Dáttilo et al. 2015), seasonality (Lange et al. 2013, Vilela et al. 2014), soil type (Kersch & Fonseca 2005) and herbivory pressure (Heill et al. 2001; Korndorfer and Del-Claro 2006). Therefore, the competitive dynamics on ant-plant interaction can interfere with the probability and frequency of interactions between species (i.e. context-dependent mechanism, Chamberlain & Holland 2009).

Ants competition for food resources often create dominance hierarchies in which more aggressive species use the majority of resources while less aggressive species collect what is left or what is allowed by the dominant (Davidson et al. 2003; Blüthgen and Fiedler 2004; Blüthgen et al. 2000). Superiority on the hierarchy are determined by better adaptation to the resource usage (Davidson et al. 2004) and aggressive behaviours (Fellers 1987). However, dominant behaviours may be also a response to environmental factors (Palmer 2003; Cerdá et al. 1998), the species in the top of the hierarchy can be replaced under different contexts (Tanner & Addler 2009). As a hierarchy consequence, the dominant species may be more abundant and interacted with plants at the expense of submissive species (Blüthgen & Fiedler 2004; Blüthgen & Stork 2007), because limiting access to nectar favours population growth (Byk & Del-Claro 2011). In addition, there may be a spatial segregation between dominant species in mutually exclusive territories (Dejean et al. 2003), a pattern known as Ant Mosaic (Blüthgen & Stork 2007), which can be explained by competition for nectar and honeydew (Blüthgen et al. 2004; Davidson et al. 2003). Moreover, regarding the present study, those hierarchies of resource dominance can shape the interactions between plants and ants when dominant species are those that interact with most plants and consequently access greatest amount of nectar while submissive species interacts conditionally to the outcomes of dominant interactions (Blüthgen & Fiedler 2004; Dáttilo et al. 2014a).

In the present study, we evaluated the dominance hierarchy in the usage of EFNs-bearing plants between ant species as a mechanism of resource partition that structures the interactions between ants and plants. Ants species highly interacted with plants are competitive dominants (Blüthgen et al. 2004; Dáttilo et al. 2014a). In this context, we tested the main hypothesis that the highly interacted ant species partition the interactions between them by a mechanism of hierarchical dominance. For this, we questioned: (1) How many and which ant species are dominant and submissive? (2) Does the dominant species and hierarchy structure differ between plant species? In addition, we tested the hypothesis that the hierarchy would be stronger in plant species with high quality nectar (higher volume and sugar concentration), and we also asked the following questions: (3) Does the quality of nectar offered by the plants explain the structure and interaction patterns of dominance hierarchies (context-dependency)? Finally, we hypothesized that the hierarchy between ant species determines the pattern of interaction with plant species. To test this, we asked: (4) Does dominant species interact with more plants than submissive species? (5) Does ant species that collect nectar from the same plants fight each other more frequently?

Methods

Study area

We conducted the study in the Itacolomi State Park (PEIT) in Ouro Preto, Minas Gerais, Brazil. The park preserves a 7 000 hectares of Tropical Atlantic Forest and Cerrado savannah. We conducted the study in an area of Rupestrian Field (rocky montane savannah), which predominates in 60% of the area. Rupestrian Field is an ecosystem belonging to Cerrado (Brazilian savannah), composed by shrubs and small trees scattered in grass fields or aggregated into large patches of vegetation interspersed with rocky outcrops (Alves et al. 2014). The study area is located between 1 100 and 1 300m altitude and the climate is temperate humid (Cwb, Koeppen). The average annual temperature is 21° C and the average rainfall is 2 018mm.

Sampling ant-plant interactions

We performed the study between July 2011 and July 2013. In a 4 ha area, we selected 10 plant species with EFNs: (code: BAC) *Banisteriopsis campestris* (a. Juss.) Little (Malpighiaceae), (BAM) *Banisteriopsis malifolia* (Nees & Mart.) B. Gates (Malpighiaceae), (CHD) *Chamaecrista desvauxii* (Collad.) Killip. (Caesalpinioideae), (CHM) *Chamaecrista mucronata* (Spreng.) H.s. Irwin & Barneby (Caesalpinoideae), (CRM) *Crotalaria micans* Link (Fabaceae), (HEU) *Heteropterys umbellata* A. Juss. (Malpighiaceae), (PET) *Peixotoa tomentosa* a. Juss. (Mapighiaceae), (SEP) *Senna pendula* (Willd.) Irwin & Barneby (Fabaceae), (SER) *Senna reniformis* (g. Don) H.S.Irwin & Barneby (Fabaceae) and (STG) *Stachytarpheta glabra* Cham. (Verbenaceae). Initially, we selected 20 shrubs of each plant species (200 in total). We visited these shrubs three times a day (morning, afternoon and evening) to count the number of workers of each ant species feeding on EFNs. We repeated these observations four times weekly. Then, we waited three months, and then repeated this procedure with 20 new plants for each species. This sequence was carried for 24 months, totalling eight monthly collections, 24 days of observation and 72 observational events. We collected only few specimens of each ant species for confirmation of field identifications. The identification of the species is according to specimens of the Zoological Collection of Biodiversity, Evolution and Environment Department (DEBIO) of Federal University of Ouro Preto (Ouro Preto, Brazil). We deposited vouchers specimens in this collection.

Evaluating hierarchy of resource dominance

We evaluated the dominance hierarchy through the results of dyadic encounters between pairs of species (dyadic encounters). The development of this procedure to a large number of species is very laborious due to the large number of pairings needed and the difficulty in finding sufficient numbers of replicates to rare species. Thus, we applied the experiment only to the most abundant ant species, which together have summed 95% of the total abundance of ants observed in the plants. The species were: (code: BRH) *Brachymyrmex heeri* Forel 1974 (Formicinae), (CAC) *Camponotus crassus* Mayr, 1862 (Formicinae), (CAN) *Camponotus novogranadensis* Mayr, 1870 (Formicinae), (CAR) *Camponotus rufipes* Mayr 1775 (Formicinae), (CAS) *Camponotus senex* Smith 1858 (Formicinae), (DOB*) Dorymyrmex brunneus* Mayr 1908 (Dolichoderinae), (PSG) *Pseudomyrmex gracilis* Fabricius 1804 (Pseudomyrmicinae), (PSP) *Pseudomyrmex pallidus* Smith 1855 (Pseudomyrmicinae), (PST) *Pseudomyrmex termitarius* Smith 1855 (Pseudomyrmicinae).

We confronted each of the 10 ant species with the other nine. We replicated each combination 10 times, that is 10 workers of each species confronted with 10 workers of each other nine species. Therefore, we performed 450 dyadic encounters in 45 combinations. We repeated this procedure for ants interacting with each of the ten plant species in order to determine the structure of the hierarchy present in each ant-plant interaction. Therefore, we performed 4500 dyadic encounters in total. The encounters was conducted in the field, immediately after removing the ants from their interacting plants. In each encounter, we removed an ant from a shrub and placed in a sterile pot (5 mL) together with an ant belonging to another species, which was removed from a different shrub (a least 10m away from the first) but from the same plant species. We closed the pots and evaluate the dominance relationship for an hour. We considered the species as dominant when it attacked and killed the other worker and submissive when it was attacked and killed.

Addtitionally, we observed interspecific interactions in the field (field encounters) to gather complementary information about dominance hierarchy. We selected 20 shrubs from each plant species. We observed each plant for 30 minutes. In each observation, we count the number of aggressive interactions between ant species. We repeated the observation two weeks later. We randomized observation order. We considered a species as dominant when the worker attacked other workers with bites, amazed with aggressive behaviours (open mandible, threat with bites, release of formic gas and swarming) or massive recruitment, or killed another ant. We considered a species as submissive when the worker avoid contact with other workers, jump from the shrub, runaway from aggressors, or died in conflicts.

Measuring resource quality

To measure the nectar quality in each plant species, we used the same bushes visited to quantify the ant-plant interactions. Once every three months between July 2011 and July 2013, we measured the volume of nectar produced by EFNs and the sugar concentration. In every shrub, we covered ten nectaries with voile bags for 24h, to allow accumulation of measurable amount of nectar and avoid ant consumption. Then, we collected all the nectar and measured the volume with microcapillary tube graduated in microliters scale. Immediately after collection, we measured the concentration of sugar (BRIX) in the nectar through a refractometer. The BRIX value was converted to concentration in mg/μL according to the conversion equation: $y = 0.0023 + (0.0094x) + (0.0001x^2)$ ", where" y "is the sugar concentration and" x "is the BRIX value (Dafni et al. 2005).

Data analysis

Dominance hierarchy

We represented the hierarchies of dominance using graphs of aggressive interaction networks produced by Pajek v.4.01a software. We believed that the network approach is more effective to represent interspecific dominance hierarchies than the linear approach commonly used in social and individual approach because a species rarely assaults another without counterattacks and a dominant species normally attacks several submissive species rather than only the direct submissive one in the hierarchies (Felles 1987; Savolainen & Vepsalainen 1988; LeBrun 2005; Dáttilo et al 2014a). We build ten matrices of dominance interaction for ants from each plant species, in which vertices “*Aij*” represents the number of times that the ant species “*i*” dominated the ant species “*j*”, while “*Aji*” is otherwise. We also build one matrix considering all data together for dyadic encounters and one matrix for field encounter. To establish the levels of hierarchy, we compared the percentage of dominated species among the 10 ant species using χ^2 with sequential correction (according to Fellers 1987). Thus, the ant specie that dominated the largest number of other species occupied the top of the hierarchy, while species that were completely submissive, occupied the base. When species did not differed in the number of dominated species, we compared the percentage of encounters in which the specie was dominant (number of dominated ant workers) to determine the intermediate levels of the hierarchy. We used these levels as network partitions and plotted the network using the Y-Layer function.

To find patterns between the hierarchies, we classified the hierarchy matrices using cluster analysis and then correlated the hierarchy matrices of each plant species using Mantel test to confirm the classification (Legendre and Legendre 1988). We considered as structurally similar the hierarchies approximated by cluster analysis and significantly correlated in the Mantel test. For each hierarchy, we also identified the follow structural parameters: identity of the dominant species; number of dominant and submissive species; and frequency of interspecific aggressions (percentage of wins in fights).

We used the quality of the nectar to explain the variation in hierarchy structure using general linear models (GLM). The average volume of nectar and sugar concentrations were dependent variable and species of plants independent cases (N = 10). Then, we tested the relationship between the qualities of nectar (independent variable) and the parameters of the hierarchy structure (dependent variable). At last, we built a response matrix, where vertices "*Aij*" presenting in vertex "*A*" the Mantel correlation coefficient between the hierarchy "*i*" and "*j*" (used as similarity index). As a predictor, we created a matrix of similarity (Bray-Curtis index) among the ten plant species according to the volume and the concentration of sugar in the nectar. Then, we tested the correlation between the two matrices with Mantel test (Legendre and Legendre 1998).

Species dominance structuring ant-plant interactions

We build a network of interactions between ants and plants using software Pajek v.4.01a. We built a matrix of ant-plant interaction where the vertices "Aij" presents the number of shrubs of the plant species "i" occupied by the ant species "j" (Bascompte and Jordano 2007). So we built the graph of the network using the Energy function (Kamada-Kawai: Separate Components) to highlight the species of the generalist core and those of the network periphery. Then, the average number of interactions and recruitment per plant species was compared among the ten species of ants in the hierarchy through mixed GLM. The ant species were a fixed factor (N = 10), while plant species were a random factor (N = 10). Then, we evaluated the relationship between competitive dominance and ant-plant interactions through another GLM. We tested the relationship between dominance and aggressiveness of each ant species against the number of interactions with plants and the recruitment effort presented by the same ant species. Finally, we tested the dominance hierarchy structure as an explanation for the pattern of interactions observed in ant-plant network. For this, we tested the correlation between the matrix of dominance interactions against a similarity matrix of ant-plant interactions where vertices "*Aij*" represents the number of plant interactions shared by ant species "*i*" and "*j*". We tested the correlation between those two matrices using the Mantel test, since we expected that ant species interacting with the same plants (niche overlap) would attack each other more frequently creating dominance hierarchies.

All dependent variables were linearized by logarithmic function to attend assumptions of linear models and ANOVA. Univariate analysis were performed in Action (Equipe Estatcamp, 2014), a free statistical software based on R platform (R Core Team, 2014). Multivariate analysis were performed on PAST free statistical software.

Results

Dominance hierarchy

Considering the data resulted from paired interspecific interactions, we observed that ants associated with plants bearing EFNs interacted aggressively with each other in dominance hierarchies (Figure 1). An example of interspecific aggressive interaction in dyadic encounters is in Appendix 1. According to the differences between species in the results of paired encounters (Table 1; Figure 1), we observed that *Camponotus crassus* was the most dominant species because it attacked the largest proportion of ants (85%, $X^2_1 = 7.3$, $p < 0.001$). *Cephalotes pusillus* (59%) and *C. novogranadensis* (57%) presented the second largest proportion of attacks ($X^2_1 = 0.3$, $p = 0.8$) and occupied the second level in the hierarchy ($X^2_1 = 3.1$, $p = 0.002$). *Camponotus rufipes* (34%), *Camponotus senex* (29%) and *Pseudomyrmex gracillis* (24%) attacked a similar proportion of ants ($X^2_1 = 0.4$, $p = 0.7$), and occupied the third level of the hierarchy ($X^2_1 = 2.44$, $p = 0.02$). At last, *Pseudomyrmex termitarius* (6%), *Pseudomyrmex pallidus* (2%), *Brachymyrmex heeri* (2%) and *Dorymyrmex brunneus* (2%) were positioned in the fifth and last level of the hierarchy because they were submissive in almost all encounters ($X^2_1 = 4.1$, $p < 0.01$). The hierarchy resulted from dyadic encounters did not differed from the hierarchy resulted from field observation (Mantel test: Correlation coefficient = 0.93, p <

0.001). Additionally, ant species highly aggressive in dyadic encounters were also the most aggressive in field encounters (Pearson: r = 0.98, $p < 0.001$).

Considering the structure of the hierarchies for each plant species, we observed two main hierarchical patterns (Mantel correlation: $p < 0.01$) (Table 2; Figure 2). (1) Hierarchies dominated by *C. crassus* in *B. malifolia*, *C. mucronata*, *S. reniformis,* S. *pendula* and *S. glabra*. (Mantel correlation: $p < 0.05$). (2) Hierarchies dominated by *C. novogranadensis* in *B. campestris*, *C. desvauxii*, *C. micans* and *P. tomentosa*. Two other sub-groups distinguished within the hierarchies dominated by *C. novogranadensis*. (2.1) Hierarquies dominated by *C. novograndensis* as the main aggressor in *B. campestris*, *C. desvauxii* and *P. tomentosa* (Corr. coeff. = 0.62). (2.2) hierarchies dominated by *C. novogranadensis* and other co-dominant ants (*C. crassus* or *C. pusillus*) in *C. micans and H. umbellata* (Corr. coeff. = 0.68) (Figure 2; Table 2).

Context-dependence by resource quality

We observed that the plant species similarities in nectar quality (volume and concentration of sugar) explained the plant similarities in hierarchies structures (Mantel test: Corr. Coeff.: = 0.26, p = 0.03). Therefore, hierarchies on plants that produced nectar with similar volume and sugar content presented similar pattern of dominance interactions between ants, i.e. the same dominant species and similar frequency of interspecific aggressions (Table 3). Additionally, plants with sugar-rich nectar interacted with more species (MLR: $R^2 =0.66$, $\beta = 0.9$, $F_{1,7} = 13.4$, p = 0.01; Figure 3) and these ants were more aggressive, i.e. killed each other more frequently (MLR: $R^2 = 0.64$, $\beta = 0.9$, $F_{1,7} = 11.9$, p = 0.01; Figure 3). Nectar volume did not affected the number of dominant species ($\beta = -0.3$, $F_{1,7} = 1.3$, p = 0.3) or aggressiveness ($\beta = -0.4$, $F_{1,7} = 3.1$, p = 0.12).

Interspecific dominance in ant-plant interactions

We found 147 interactions (46% of the possible number of interactions) between 32 ant species (13 888 individual ants observed) and 10 species of plants bearing EFNs (1 600 observed shrubs; Figure 4). The ten most common species, those used in the behavioural experiments, totalized more than 95% of the total abundance of ants observed collecting nectar. Of these 10 species, eight interacted with all plant species (Figure 4, Table 4). We found no difference between plant species regarding the total number of interactions with ant species (12-16 interactions) (Table 3). On the other hand, ant species did not interacted with plants similarly, and the dominance behaviour was a key factor (Figure 4; Table 4). *C. crassus* and *C.*

novogranadensis interacted with most of the plants and recruited greatest number of workers, *C. pusillus* and *P. gracillis* presented intermediate interaction frequency and recruitment, and the remaining six species showed low interaction frequency and recruitment (ANOVA: Recruitment: $F_{9,\,81}$ = 16.1, p = 0.001; Interactions: $F_{9,\,81}$ = 18.9, p <0.001) (Table 4).

The species aggressiveness (number of workers killed) and dominance (number of species aggressed) explained the variation between ant species on the number of shrubs occupied (MLR: R^2 = 0.64, $F_{2,\,7}$ = 10.1 p =0.01) and the number of workers recruited to collect nectar (MLR: R^2 = 0.83, $F_{2,\,7}$ = 17.2, p = 0.002). Therefore, the most aggressively dominant ant species (recruitment: β = 1.8, interactions: β =1.4) were able to forage on plants more intensively and access high proportion of the shrubs available in the environment (Table 1). Moreover, we observed that the ant species that collected nectar from the same plants (larger niche overlap) fought each other more often (high aggressiveness) than with species that interacted with different plants (Mantel test: Corr. coeff. = 0.79; p = 0.002; Figure 2; Table 5). For example, the dominant species *C. crassus* and *C. novogranadensis* were the two species that more frequently co-occur in the same plant (295 shrubs), and they were the species that most fought each other (73 fights) while *C. crassus* and *P. termitarius* co-occurred in 21 plants and fought only 23 times (Table 5).

Discussion

Our main hypothesis, that dominance hierarchy in the usage of EFNs-bearing plants between ant species act as a mechanism of resource partition that structures the interactions between ants and plants was confirmed. We also confirmed the hypothesis that ant-plant relationships are competitive mechanisms based on resource-consumer relationship filtering ant-plant mutualistic interactions and shed light on the behavioural relationship between ant species interacted with EFN-bearing plants. Moreover, we showed that ant species interacted aggressively with each other creating competitive dominance hierarchies. Ants commonly compete for resources in such dominance hierarchies (Fellers 1987; Andersen & Patel 1994; LeBrun 2005), and this is stronger in vegetation habitat where sugar resources, nest site and pathway limitations dictate ant assembly and interactions (Blüthgen et al. 2004; Blüthgen & Fiedler 2004; Par & Gibb 2010). Studies about ant hierarchies commonly access interspecific interaction on baits to determinate competitive relationships (Fellers 1987; Savolainen & Vepsalainen 1988; Andersen & Patel 1994). We corroborated these studies with additional behavioural evidences that ants may compete for mutualisms, as previous predicted (Cushman 1991; Cushman and Addicott 1991; Cushman and Beattie 1991).

In general, ant-plant mutualisms are facultative and generalist interactions since many ants act as opportunists and have low host fidelity (Hölldobler & Wilson 1990; Vilela et al. 2014). However, recent studies predicts that highly interacted species would be dominant species that outcompete other species and restrict their access to a few species of plants in a hierarchical structure of interactions known as nested pattern of interactions (Dáttilo et al. 2014a, 2014c). Specifically, as showed by Dáttilo et al. (2014a), highly interacted species are numerically dominants and act aggressively against each other creating hierarchies that defines its interaction frequency. However, our results showed that few highly interacted and numerical dominant species were truly behavioural dominant. These species vary in dominance behaviour and half of it was completely submissive. For example, *C. crassus* and *C. novogranasensis* were the two dominant species on the hierarchy. Both were interactive dominants since it interacts with all plants species, numerically dominants since it maintained large and persistent recruitment on plants, and behaviourally dominant because it frequently engaged in aggressive interactions and won its fights. These dominant species fought heavily to detain access to the plants, especially between each other, partitioning the interactions with plants. On the hand, the other four species from the core (*C. pusillus*, *C. rufipes*, *C. senex* and *P. gracillis*) were numerically and interactively dominant, but low aggressive. These species may be co-dominants, acting as dominants in specific situations (Dejean et al. 2003). The last four species (*B. heeri*, *D. brunneus*, *P. termitarius* and *P. pallidus*) were completely submissive and restricted to few shrubs but interacted with all plant species. The dominance of *C. crassus* is expected because this species is very common in ant-plant (Oliveira & Leitão-Filho 1987; Del-Claro 2004; Lange et al. 2013), ant-hemipteran (Del-Claro 2004; Fagundes et al 2013) and ant-butterfly interactions (Sendoya et al 2009; Bachtold et al 2014) in Brazilian Cerrado (Rico-Gray and Oliveira 2007). *C. crassus* is a highly aggressive species removing herbivores and other intruders from the plant and even repelling pollinators (Del-Claro & Oliveira 2000; Nahas et al 2012; Assunção et al 2014).

According to our results, not only dominance but also submissive strategies may contribute to stable interactions between ants and plants. More interesting, we observed that ant species switched levels in the hierarchy according to the interacted plant species and the quality of the nectar. Therefore, we confirmed our hypothesis that dominance hierarchies are context-dependent. Three of our results explain this scenario. First, some submissive species were locally dominant, that is numerically dominant on few shrubs of each plant species. In some cases, such as *C. pusillus* and *C. senex*, this local dominance may be linked to the fact that these species build its nests on trees (Byk & Del-Claro 2010; Santos & Del-Claro 2009), as we also observed. Territoriality around nests is very high and can confer a competitive advantage on the host plants (Blüthgen et al. 2004). Second, submissive species acted as opportunistic accessing the plants where the behaviourally dominant specie recruited less workers or in the period of low activity. For example, *C. rufipes* is mainly nocturnal (Dáttilo et al. 2014b) and may act as submissive during daytime. Additionally, some species forage stealthily such as species of *Pseudomyrmex* (Amador-Vargas 2012), which would prevent frequent encounters with the dominant ant and allows nectar consumption. Third, small submissive species did not reacted aggressively to the dominant ants and might be tolerated (Dejean et al. 2003; Tanner & Adler 2009). For example, *B. heeri* and *D. brunneus* were very common on plants but rarely aggressive. The result of interspecific encounters between ants on plants largely depends on the results of previous interactions between species, and non-aggressive species in past encounters tend to be less attacked in future encounters (Tanner & Adler 2009).

Generally, the numerical and behavioural dominance of ants are stronger in plants that provide high-quality nectar (Koptur 2005). We observed a strong dependency between the hierarchy structure and the quality of the resource offered to the ants. Plants with high-sugar nectar, as *S. glabra* and *C. mucronata*, showed a large amount of aggressive ants climbing on them, which confronted each other in clear hierarchies with one dominant, few co-dominant and several submissive species. On the other hand, ants less disputed plants that produced low-sugar nectar, such as *C. desvauxii* and *P. tomentosa*. In these plants, hierarchies presented a centralized structure with one low-aggressive dominant species that subjugated the remaining species. The selection of dominant ants in a clear hierarchy may generates positive consequences for plants (Rosumek et al. 2009). For example, plants that interact with more aggressive species might be better protected against herbivores (Heill & McKey 2003; Katayama & Suzuki 2004), and possibly produce more seeds (Nascimento & Del-Claro 2010). In addition, plant species with high-quality nectar had higher percentage of its population protected by ants. Therefore, the ant-plant interaction itself may not define the mutualism outcomes, but the identity and effectiveness of the protective ants and the proportion of plant population actually protected. Further studies should investigate the effectiveness of each ant species, especially the dominants, as plant protectors.

We observed that behavioural dominance of an ant species in the hierarchy correlates with high recruitment (numerical dominance) and frequency of interaction (interactive dominance). The aggressiveness needed to ensure greater access to resources requires massive recruitment to hinder the access of competitors (Fellers 1987; Parr & Gibb 2010). For example, *C. crassus* and *C. novogranadensis* co-occurred in many shrub, which indicates high overlap in the use of resources. This high niche overlap associated to the high value of the resources are basic mechanisms of competition and hierarchical patterns of interspecific interaction (Albrecht & Gotelli 2001; Blüthgen et al. 2004). In fact, *C. crassus* and *C. novogranadensis* were the species that most fought each other, as well as with other species, assuming a dominant role in the hierarchy. More interesting, despite the high co-occurrence on shrubs, both species alternated in dominance of plant species. In fact, ant species that collected nectar from the same shrubs were antagonists in the hierarchy and occupied different hierarchical levels depending on the plant species. Thus, although several species occurred in the same shrubs, depending on the plant species, an ant species will be submissive or dominant, but both may live in constant dispute for nectar. This dynamic hierarchy may be an important mechanism structuring ant plant interactions with two main consequences. Firstly, it may create temporal and spatial variation in ant-plant interactions, since abiotic factors (Cerdá et al. 1998), habitat heterogeneity (Palmer 2003), environment disturbances (Andersen 1995), parasitoids (LeBrun 2005) and dispersion abilities (Bernstein & Gobbel 1979) may change dominance relationships. Secondly, it may increase local ant diversity because several species may coexist as dominant in different plant species or live as submissive species ("hidden dominants") tolerated by the dominant specie (Andersen 1995; Cerdá et al. 1998). Each dominant species has a different set of submissive species (Blüthgen & Stork 2007), so a dynamic mosaic of dominant species can contribute to the diversity of species. Therefore, species coexistence with constraints in resource usage may be more common and stable than previously predicted.

The coexistence of many species in highly diverse environments is a matter quite discussed in ecology (Palmer 2003; Sarty et al. 2006; Andersen 2008). The fact that we found so many ants in a same location interacted with virtually the same ten plant species indicates a high niche overlap that, in theory, could not occur without a mechanism of resource partition (Schoener 1974; Fellers 1987; Albrecht & Gotelli 2001). Our results pointed that dominance hierarchies of nectar among ant species are competitive mechanisms of resource partitioning among the most aggressive and most abundant species. More interesting, we found that these species could coexist by dominating different interactions and switching between dominance and submission role according with the host plant. Moreover, several submissive species were capable of access all plant species and coexist with the dominant at low abundance. These may also be a result of hierarchical partitioning of the resource and may promote accumulation of species diversity. Taking together, these results are empirical evidences that dominance hierarchies occur in ant communities and act as important mechanisms of species coexistence. Moreover, we provide important insights about the role of interspecific aggressiveness and interference competition as mechanisms regulating ant-plant mutualistic interactions.

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Tables

Table 1. The most frequent ant species on EFN-bearing plants (95% of total ant abundance) used as models in interspecific encounters to study hierarchies of nectar dominance. Species position in the hierarchy was defined by X^2-test with sequential correction. Ants are sorted in descending order according to the number of aggressive interactions.

	Dyadic encounters				**Field encounters**				**Interactions**	
Ant specie	**Species aggressed**	**Aggressor species**	**Ants killed**	**Deaths**	**Species aggressed**	**Aggressor species**	**Ants repelled**	**Repellences**	**Shrubs occupied**	**Recruitment on plants**
Camponotus crassus	9	7	287	50	9	4	60	14	809	7 805
Cephalotes pusillus	9	5	75	53	7	3	19	18	269	1 261
Camponotus novogranadensis	9	7	124	93	6	5	16	17	494	2 313
Pseudomyrmex gracillis	6	3	21	40	3	3	9	13	261	672
Camponotus rufipes	4	5	19	46	4	4	8	19	59	320
Camponotus senex	6	5	32	102	1	1	1	5	90	298
Pseudomyrmex termitarius	1	3	2	34	0	3	0	6	47	100
Brachymyrmex heeri	1	1	1	34	0	1	0	4	44	123
Dorymyrmex brunneus	1	4	1	48	0	3	0	10	55	177
Pseudomyrmex pallidus	1	5	1	63	0	3	0	7	113	170

Table 2. Differences in quality of nectar offered by the 10 plant species studied in Rupestrian Fields (Ouro Preto, Brazil). Values represent average per shrub and standard error (N = 160 shrubs per specie). Plants are sorted in descending order according to the concentration of sugar in the nectar.

Plant species	**Sugar concentration (mg/µL)**	**Nectar volume (µ/EFN/24h)**	**Dominant ant species**	**Hierarchy levels**	**Ant aggressive encounters**	**Aggressive species**	**Number of interactions**	**Number of ants recruited**
Stachytarpheta glabra (Verbenaceae)	1.04 ± 0.03	2.16 ± 0.83	CAC	5	125	8	15	4 498
Chamaecrista mucronata (Leguminosae)	0.61 ± 0.06	1.15 ± 0.45	CAC	4	110	5	15	2 877
Banisteriopsis campestris (Malpighiaceae)	0.52 ± 0.03	2.04 ± 0.64	CAN	4	53	4	15	721
Heteropteris umbellata (Malpighiaceae)	0.47 ± 0.03	0.69 ± 0.42	CAN	4	58	7	15	1 065
Crotalaria micans (Fabaceae)	0.22 ± 0.02	1.75 ± 1.9	CAN	3	29	3	16	1 386
Senna renniformis (Leguminosae)	0.25 ± 0.05	0.07 ± 0.02	CAC	3	50	5	12	531
Banisteriopsis malifolia (Malpighiaceae)	0.15 ± 0.03	1.33 ± 0.31	CAC	3	53	3	16	822
Peixotoa tomentosa (Malpighiaceae)	0.18 ± 0.02	0.12 ± 0.02	CAN	3	40	3	15	590
Senna pendula (Leguminosae)	0.11 ± 0.03	0.09 ± 0.03	CAC	3	46	5	12	1 025
Chamaecrista desvauxii (Leguminosae)	0.05 ± 0.01	0.05 ± 0.02	CAN	3	17	5	16	385

Table 3. Results from Mantel test correlating the structures of hierarchies (matrices with vertices “A_{ij}” presenting the number of aggressive interactions between ant species “i” and “j”). On the top, the correlation coefficient between two hierarchies. On the bottom, p-value from the Mantel tests.

		Correlation coefficient									
		BAC	BAM	CHD	CHM	CRM	HEU	PET	SEP	SER	STG
p-value (Mantel test)	BAC		0.41	0.65	0.17	0.03	0.46	0.61	0.36	0.01	0.33
	BAM	0.11		0.34	0.74	0.46	0.44	0.04	0.63	0.59	0.70
	CHD	>0.01	0.09		0.35	0.72	0.64	0.78	0.42	0.12	0.25
	CHM	0.15	>0.01	0.10		0.44	0.56	0.01	0.60	0.66	0.80
	CRM	0.38	0.05	>0.01	0.02		0.67	0.42	0.38	0.35	0.47
	HEU	0.02	0.03	>0.01	>0.01	0.01		0.41	0.46	0.30	0.34
	PET	0.03	0.45	>0.01	0.26	0.05	0.04		0.07	0.06	0.07
	SEP	0.07	>0.01	0.02	>0.01	0.05	0.02	0.32		0.34	0.39
	SER	0.27	0.05	0.23	0.04	0.08	0.12	0.45	0.06		0.80
	STG	0.10	0.02	0.13	0.01	0.01	0.07	0.29	0.03	>0.01	

Table 4. List of ant species observed collecting nectar from 1600 shrubs belong to 10 species of plants bearing extrafloral nectaries. Plants were observed quarterly between July of 2011 and July of 2013. Codes depict abbreviations used in the text and figures. Recruitment presents the total number of ants observed on plants. Occupied shrubs presents the number of shrubs visited by the ant species. Interacted species presents the number of plant species visited by the ant species.

Ant specie	**Code**	**Recruitment**	**Foraged shrubs**	**Interactions**
Family Formicidae				
Subfamily Dolichoderinae				
Dorymyrmex brunneus Mayr 1908	DOB	177	59	9
Linepithema micans Forel 1908	LIM	10	5	2
Linepithema sp. 1	LI1	3	1	1
Linepithema sp. 2	LI2	2	1	1
Subfamily Formicinae				
Brachymyrmex heeri Forel 1974	BRH	123	55	9
Brachymyrmex pictus Mayr 1887	BRP	10	6	4
Camponotus atriceps Smith 1858	CAA	38	11	1
Camponotus crassus Mayr 1862	CAC	7805	809	10
Camponotus melanoticus Emery 1894	CAM	171	38	4
Camponotus novogranadensis Mayr 1870	CAN	2313	494	10
Camponotus rufipes Mayr 1775	CAR	320	113	7
Camponotus senex Smith 1858	CAS	298	90	10
Myrmelachista catharinae Mayr 1887	MYC	7	2	1
Tapinoma sp1	TAM	1	1	1
Subfamily Ectatomminae				
Gnamptogenys sulcata Smith 1858	GNS	10	4	2
Subfamily Myrmicinae				
Cephalotes pusillus Klug 1824	CEP	1261	269	10
Crematogaster sp. 1	CR1	103	29	8
Crematogaster sp. 2	CR2	121	33	8
Crematogaster sp. 3	CR3	2	1	1
Pheidole sp. 1	PH1	77	17	10
Pheidole sp. 2	PH2	30	7	4

Pheidole sp. 3	PH3	6	2	2
Pheidole sp. 4	PH4	1	1	1
Wasmannia sp. 1	WA1	32	8	2
Wasmannia sp. 2	WA2	1	1	1
Wasmannia sp. 3	WA3	9	3	1
Wasmannia sp. 4	WA4	2	1	1
Subfamily Ponerinae				
Pachycondyla sp. 1	PA1	4	1	2
Pachycondyla sp. 2	PA2	9	3	3
Subfamily Pseudomyrmicinae				
Pseudomyrmex gracilis Fabricius 1804	PSG	672	261	10
Pseudomyrmex pallidus Smith 1855	PSP	170	47	10
Pseudomyrmex termitarius Smith 1855	PST	100	44	10

Table 5. Similarity matrices used to correlate ant dominance hierarchy and ant-plant interactions. On the top, a matrix ant dominance interactions where the vertex “*Aij*’ presents the number of fights between species “*i*” and “*j*”. On the bottom, a matrix of niche overlap in plant interaction where the vertex “*Aij*” presents the number of plants shared by species “*i*” and “*j*”.

		Number of fights (interspecific aggressiveness)									
		BRH	CAC	CAN	CAR	CAS	CEP	DOB	PSG	PSP	PST
Number of shared plants (niche overlap)	BRH		26	8	0	0	1	0	0	0	0
	CAC	25		73	31	36	44	25	44	35	23
	CAN	17	295		18	10	22	17	43	15	11
	CAR	3	40	21		2	6	0	2	2	0
	CAS	4	68	39	10		7	0	10	0	0
	CEP	9	156	91	12	13		4	31	11	2
	DOB	2	36	40	2	2	13		3	0	0
	PSG	9	169	120	20	24	85	22		1	0
	PSP	4	64	40	5	6	39	6	30		0
	PST	2	21	24	3	7	6	3	15	5	

Figures

Figure 1. Hierarchy of dominance between ant species using EFNs bearing plants, based on paired interspecific encounters in dyadic encounters (top) and field observations (bottom). Different hierarchical levels are based in differences in the proportions of ants attacked and killed in encounters (X^2_1: $p < 0.05$). Arrows indicates aggressive encounters and arrow direction indicates dominants attacking submissive species

Figure 2. Interspecific hierarchies of nectar dominance observed in each of the ten species bearing EFNs. Hierarchical levels are defined based on differences in the proportions of species attacked and in encounters (X^2_1: $p < 0.05$). Lines indicates aggressive encounters and arrow direction indicates a dominant species attacking a submissive one.

Figure 3. Relation between nectar quality and hierarchy structure. Sugar concentration of nectar offered by the 10 plant species bearing EFNs explained differences in linearity of the hierarchies (number of levels; $p = 0.008$), dominance degree (number of aggressive species; $p = 0.01$) and ant aggressive interactions (number of ants killed; $p = 0.01$).

Figure 4. Network of interactions between ants (13 888 workers, 32 species) and plants (1 600 shrubs, 10 species) observed between Jul/2011 and Jul/2013 in Rupestrian Fields (Ouro Preto/Brazil). Vertices closed to the network core represent highly interacted species. Black circles depict plants and white circles depict ant species. Lines width refers to relative frequency of interactions (shrubs occupied by an ant species). Codes for plant species are in Methods section and for ant in the Table 4.

Figures

Figure 1

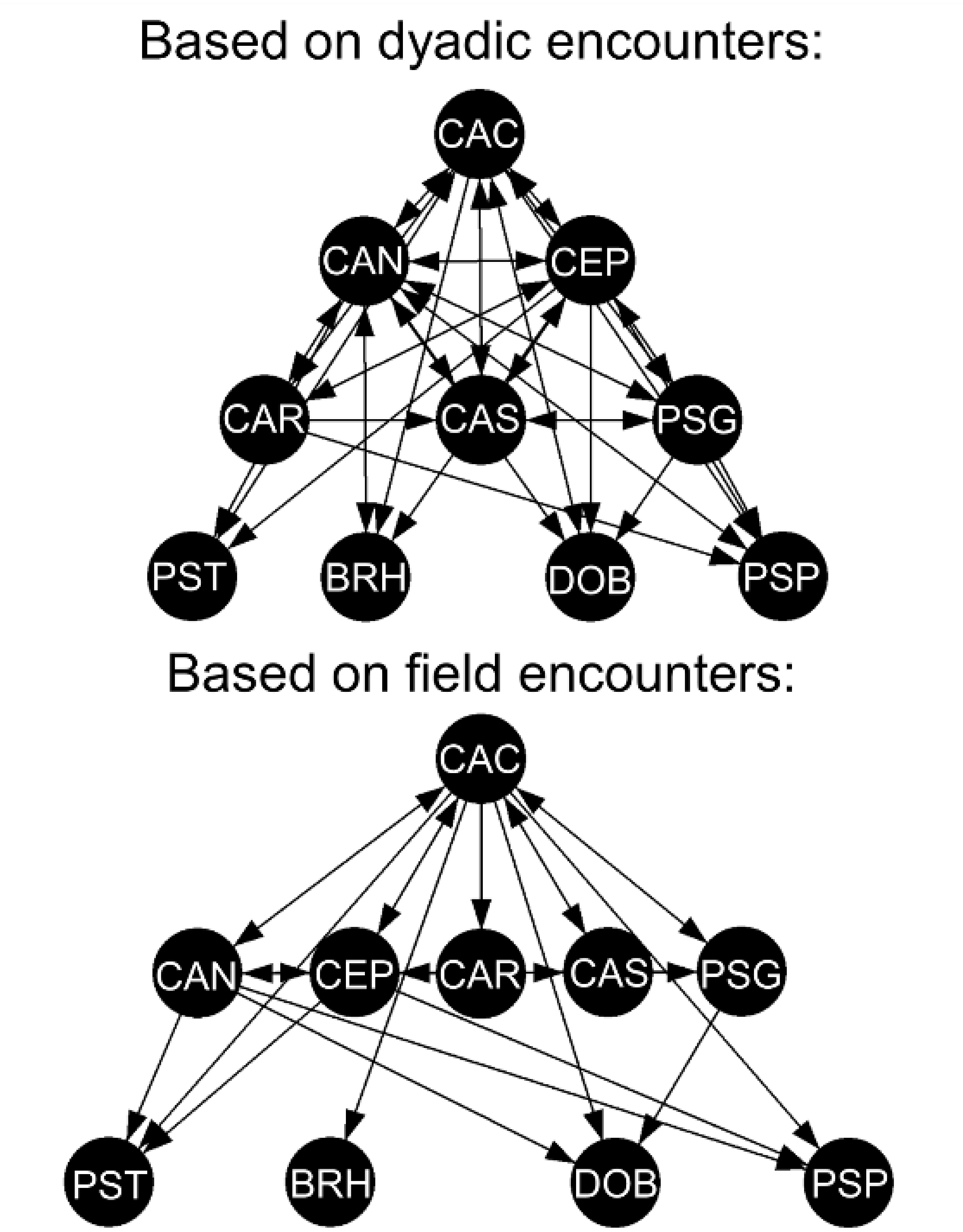
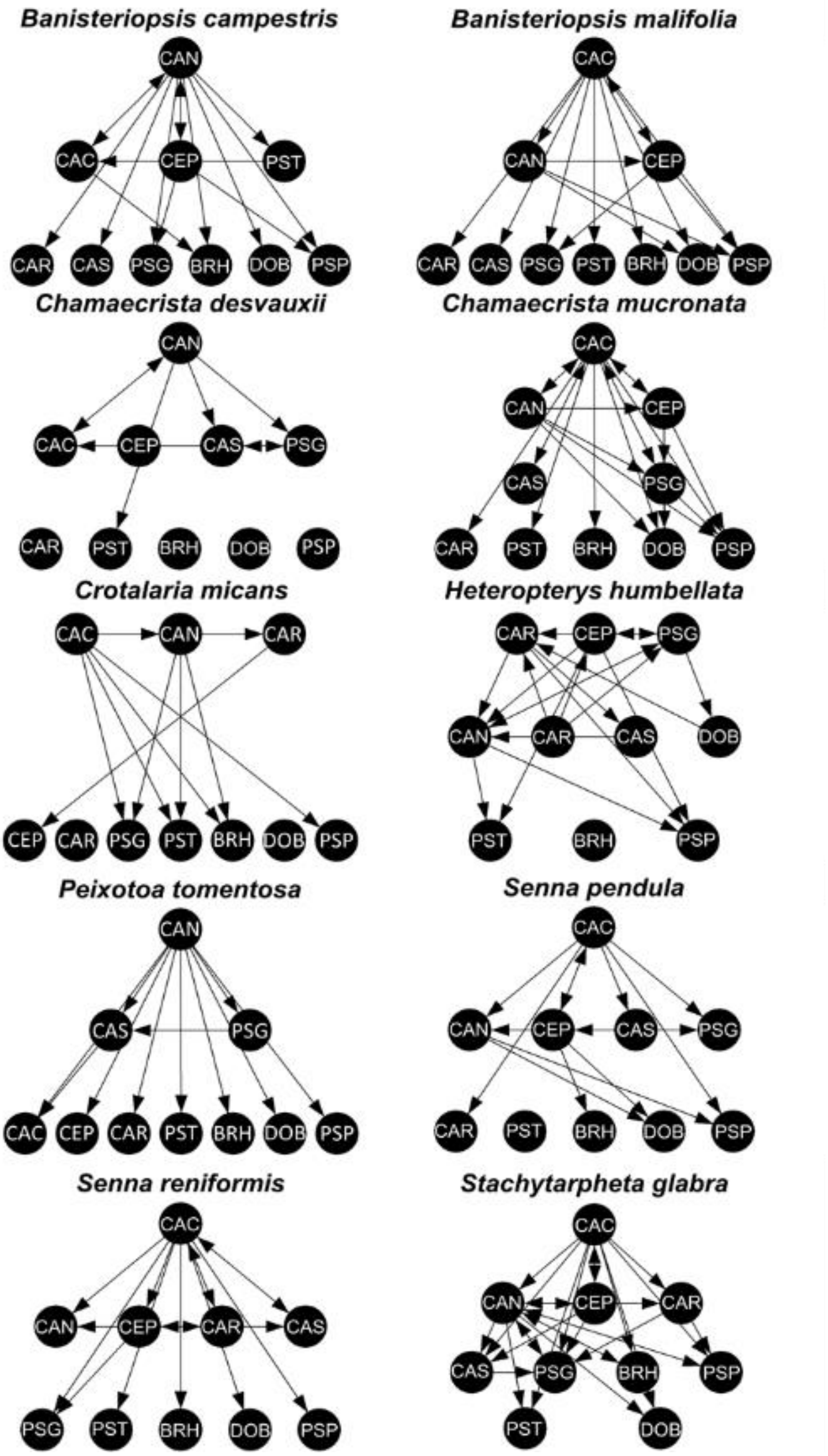


Figure 2



Banisteriopsis campestris

Banisteriopsis malifolia

Chamaecrista desvauxii

Chamaecrista mucronata

Crotalaria micans

Heteropterys humbellata

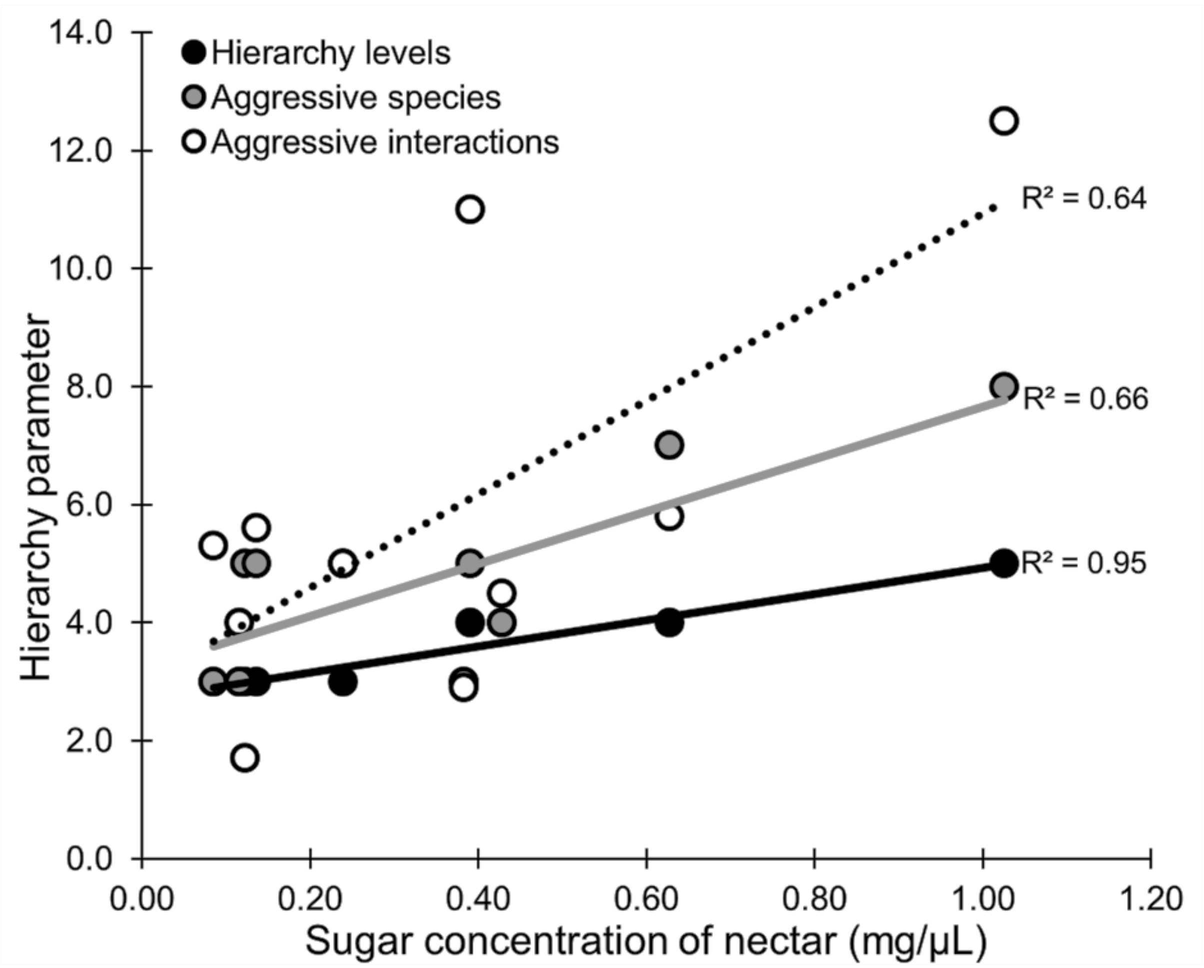
Peixotoa tomentosa

Senna pendula

Senna reniformis

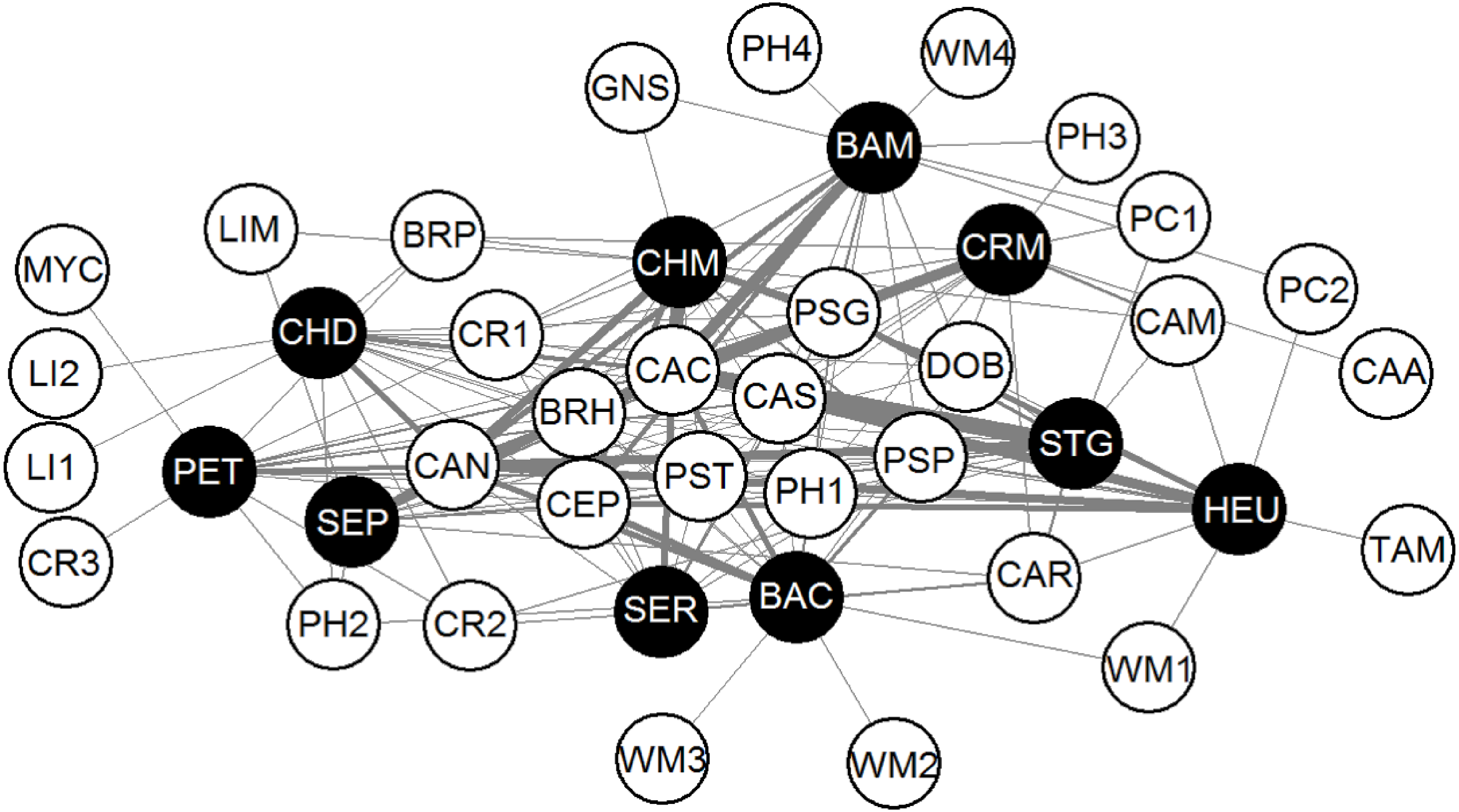
Stachytarpheta glabra

Figure 3



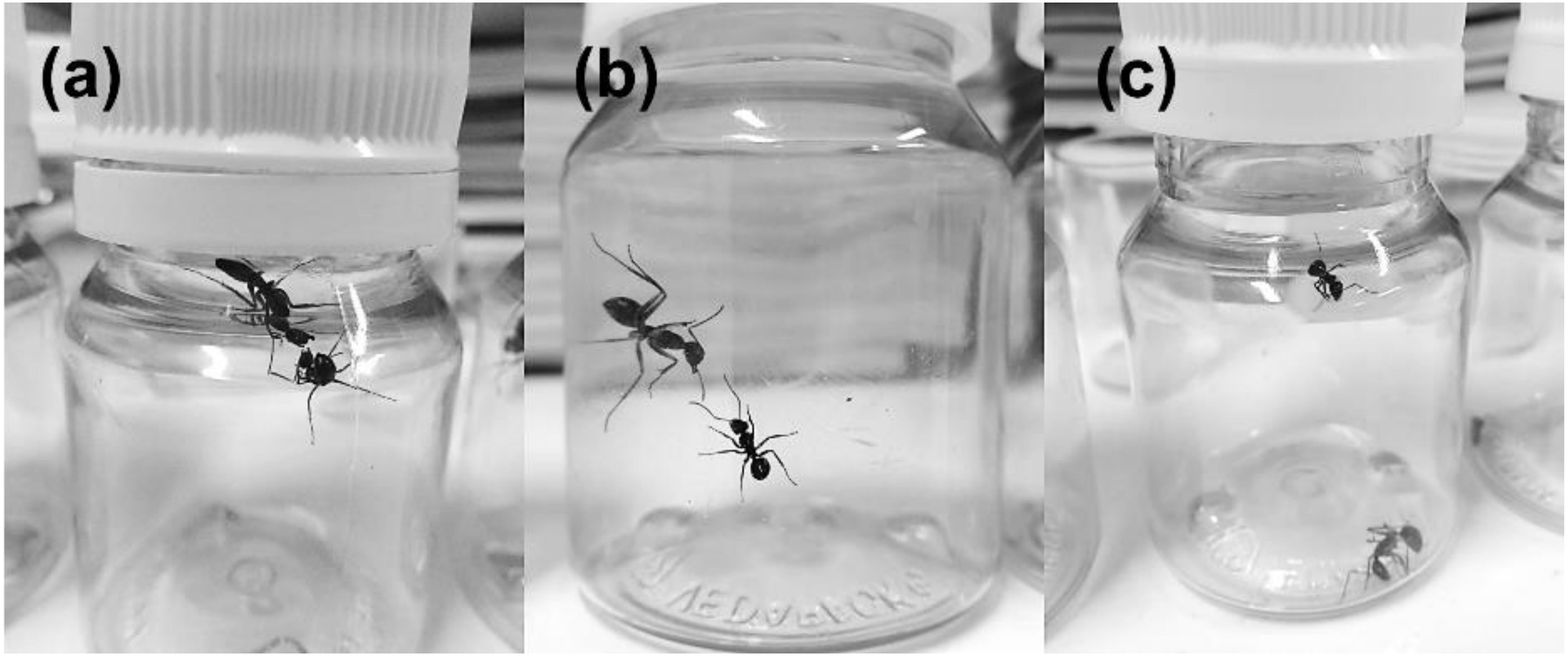
Hierarchy levels
Aggressive species
Aggressive interactions
R² = 0.64
R² = 0.66
R² = 0.95
Hierarchy parameter
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Sugar concentration of nectar (mg/μL)

Figure 4


PH4
WM4
GNS
BAM
PH3
PC1
CRM
LIM
BRP
CHM
MYC
PC2
CHD
CR1
PSG
CAM
CAA
LI2
CAC
DOB
CAS
BRH
STG
LI1
PET
CAN
PST
PSP
HEU
SEP
CEP
PH1
TAM
CR3
CAR
SER
BAC
PH2
CR2
WM1
WM3
WM2

Appendices

Appendix 1. Example of dyadic encounter between two species: *Camponotus crassus* and *Camponotus melanoticus*. (a) *C. crassus* attacking *C. melanoticus* with formic gas. Note the position of the ant body: Open mandible and antennas, gaster curved to opponent direction. (b) *C. melanoticus* attacking *C. crassus* with bites. (c) Result of the contest: *C. meloticus* (submissive) killed by *C. crassus* (dominant).



Capítulo 4

Muitas formigas, poucos mutualistas: identificando protetores e exploradores em redes de interação formiga-planta

Many ants, few mutualists: identifying protectors and exploiters in ant-plant interaction networks

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For submission in Oikos

Running title: *Context dependency in ant-plant mutualisms*

Resumo

Interações entre formigas e plantas com nectários extraflorais são modelos comuns no estudo das interações mutualistas. A maioria dos estudos parte do pressuposto de que todas as espécies de formigas que se alimentam de néctar protegem suas plantas hospedeiras de danos por herbivoria. No entanto, estudos recentes que tratam de diferentes sistemas de mutualismo têm mostrado que, por vezes, as plantas não recebem benefícios decorrentes da interação com alguns dos seus parceiros (ou seja, exploração ou trapaça). Nesse estudo, avaliamos o grau exploração em redes de interação formiga-planta através da técnica de paisagem de efetividade de proteção com base no comportamento de defesa das formigas. Usando experimentos clássicos de herbívoros simulados e observação adicional de comportamentos agressivos exibidos por cada espécie formiga, nós discriminamos espécies em verdadeiros protetores e espécies exploradoras (trapaceiras). A rede de interação entre formigas (26 espécies) e plantas (10 espécies) apresentou 40% de espécies exploradoras (não-defendem), 50% de espécies oportunistas (defendem pouco), e 10% de espécies verdadeiramente protetoras. Além disso, estas espécies de formigas protetoras interagiram com quase todas espécies de plantas, o que pode assegurar a estabilidade das redes mesmo em alto grau de exploração. Além disso, nossos resultados indicam que a eficácia de proteção de formigas, e o grau de exploração, é dependente do contexto, uma vez que as pequenas plantas com néctar rico em açúcar foram melhor protegidas. Isso indica que a evolução do melhor néctar poderia impedir a exploração e aumentar a especialização com protetores verdadeiros. A exploração de mutualismos pode desestabilizar interações formiga-planta através da competição por néctar entre espécies exploradoras e mutualistas e, assim, impedir o desenvolvimento de interações de proteção mais especializado.

Palavras-chave: proteção de plantas, contexto-dependência, néctar extrafloral, cerrado brasileiro

Abstract

Interactions between ants and plants bearing extrafloral nectaries are common models in the study of mutualistic interactions. Most studies start from the assumption that all ant species feeding on nectar protect their host plants from herbivore damage. However, dealing with different mutualism systems, recent studies have shown that mutual benefits are not so common and the plants can receive no benefits arising from the interaction with some of their ant partners (i.e., exploitation or cheating). This behavior could destabilize ant-plant mutualisms through resource competition between exploitative and mutualistic species and thus hinder the development of more specialized and protective interactions. We evaluated exploitation in ant-plant interaction networks through a framework of protection effectiveness landscape based on ant behavior. Using classical experiments of simulated herbivores and additional observation of defensive behavior displayed by each ant species, we discriminated between true protectors, opportunistic protectors, and exploiter species. Our results show that ant-plant networks had 40% of exploiter species, and plant species were effectively defended by only 10% of the 26 associated ant species. However, these protector species are part of the central core of highly interactive species, which ensures the stability of this ecological network even at high degree of exploitation. Furthermore, our results indicate that the protection effectiveness of ants, and the degree of exploitation, is context dependent, once small plants with sugar-rich nectar were better protected. We presented empirical evidence for the evolutionary context-dependent mechanisms in the stability of mutualisms involving free-living species.

Key-Words: plant protection, context-dependency, extrafloral nectar, Brazilian savanna

Introduction

In protective mutualisms involving ants and plants with extrafloral nectaries (EFNs), nectar attracts ants that can repel or predate potential herbivores, reduce plant pathogens and remove encroaching vegetation (Rosumek et al. 2010). Thus, plants interacting with ants suffer less herbivory (Rosumek et al. 2010), and in some cases produce more seeds (Nascimento and Del-Claro 2010). However, interactions involving ants and EFN-bearing plants are particularly diffuse mutualisms because many ant species have different adaptations to collect and feed of nectar while others species collect nectar opportunistically (Davidson et al. 2004). Therefore, it is possible to expect that within a highly diverse environment some ant species are mutualists (protectors) while others collect nectar but do not protect their plant hosts against herbivores (exploiters, parasites or cheaters; Bronstein 2001). However, despite a well-known framework (Jordano et al. 2003; Bascompte e Jordano 2007) where mutualistic and exploiter partners can generate ant-plant interactions, the evolutionary stability of ant-plant interactions at the community level remain unclear for evolutionary biology (Bronstein 2001).

What is an effective ant mutualistic partner in protective mutualisms involving ants and plants? We could say that effective ants are those that quickly find and attack a large proportion of herbivores and recruit a large number of workers (Oliveira and Pie 1998; Rosumek et al. 2009). In this sense, ant benefits may vary among ant species in terms of taxonomic identity and abundance on plants (Rudgers and Strauss 2004; Rosumek et al. 2009), frequency of using EFNs (Apple and Feener-Jr 2008), recruitment (Fiala et al. 1989; Agrawal 1998) and effectiveness in capturing herbivores (Fiala et al. 1989), leading to different effects on fitness between plant species (Lange and Del-Claro 2014). In fact, ant-plant mutualisms present great variation in the benefits provided by different partner species (Lange and Del-Claro 2014) probably due to differences in the quality and quantity of their interactions (Rosumek et al. 2009). These variations in mutualism benefits have also been observed in other types of mutualistic interactions such as pollination (Morris 2003; Rodríguez-Rodríguez et al. 2013) and seed dispersion systems (Ness et al. 2004; Schupp et al. 2010). However, unlike other mutualisms involving free-living species, EFN-bearing plants are often visited by many ant species, but only a few species can monopolize the resource (i.e. plants with EFNs) and the benefits (plant protection) may be higher when these dominant species are highly frequent (Blüthgen et al. 2000; Rosumek et al. 2010).

Ants commonly forage on vegetation, but the presence of a source of highly energetic and predictable resource is a key-mechanism that multiplies the recruitment (Del-Claro 2004;

Fagundes et al. 2013) and increases aggressiveness (Dreisig 1988; Grover et al. 2007), thus creating the ant benefits for their host plants (Blüthgen et al. 2000; 2004). Recruitment and aggressiveness is higher near the nests (Tanner and Adler 2009) or surrounding food sources (Oliveira et al. 1999) because defensive territories are considered one of the main mechanisms structuring local ant assemblages (Blüthgen et al. 2004). In fact, plants that produce highly nutritious rewards are better protected against herbivores (Chamberlain and Holland 2009a; Grover et al. 2007). However, nectar production is related to nutrient availability and light conditions (Kersh and Fonseca 2005), herbivory pressure (Dicke 1999), plant shape and ontogeny (Chamberlain and Holland 2009b). Consequently, the benefits of ant-plant mutualisms can also vary through space and time (Rico-Gray and Oliveira 2007; Lange and Del-Claro 2014). Such variation in the outcomes of interactions caused by biotic and abiotic context is common in nature and described as context dependency (Chamberlain et al. 2014, and references therein).

The causes and consequences of context dependency are important for mutualisms because its stability and evolution relates to conservative benefits among interacted species (Bronstein 1998). Depending on the environmental context, interactions between ants and plants may lead to not only mutualism, but also commensalism and parasitism (Bornstein 2001). The variation in the interaction outcome, either due to quality or identity of the interacting species is ecologically plausible and easily observed, as shown for plant pollinator systems (Genini et al. 2010). However, studies with interactions between ants and EFN-bearing plants still assumes that all interactions are equally positive even when the literature has stressed that the benefits of plants are highly variable (Rico-Gray and Oliveira 2007; Chamberlain and Holland 2009a; Rosumek et al. 2009; Lange and Del-Claro 2014).

In the present study, we evaluated the frequency of exploitative interactions between ants and EFN-bearing plants, that is, ant species that collect the nectar but do not provide plant protection (Bronstein 2001), and propose practical methods for assessing the protection effectiveness of associated ants. We tested the hypothesis that exploitation is more common than protection in ant-plant interactions because nectar is easy to access (Davidson et al. 2004) and ant-plant interactions tend to be highly generalized (Chamberlain and Holland 2009a). Some studies show that the results of an ant-plant interaction may be context-dependent, i.e., could change according to the interacting species and habitat features (Chamberlain and Holland 2009a; Trager et al. 2010). Thus, we tested the hypothesis that ant species may have different degree of protection effectiveness depending on the partner plant species, and this would be determined by context dependency factors (Chamberlain and Holland 2009a), that is, plant morphology (foraging area) and nectar offer (resource quality).

Methods

Study area

We conducted the fieldwork at Parque Estadual do Itacolomi (PEIT), an area of biodiversity conservation near to city of Ouro Preto, Minas Gerais State, Brazil. PEIT covers an area of approximately 7,000 ha of Atlantic Forest and Cerrado, at an elevation of 700 to 1,770m. The climate in the region is subtropical humid, with a mean annual temperature of 21°C and mean annual precipitation of 2,018mm (Alves et al. 2014). We conducted the experiments in an area of approximately 8ha covered by a rocky montane savana (known as *Campo Rupestre*; Alves et al. 2014) surrounded by Atlantic Forest. *Campo Rupestre* is an ecosystem belonging to the Cerrado, consisting of quatizitic or iron rock outcrops surrounded by continuous grassland with small, tortuous shrubs and trees, and woody vegetation both in rock crevices or nested within large vegetation patches (Alves et al. 2014).

Ant-plant interactions

We sampled ant-plant interactions in the early rainy season (November-December 2013) when flowering and resprouting increases EFN activity and thus ant interactions (Lange et al. 2013). We searched and found 10 ENF-bearing plant species in the study area. We standardized the sampling by selecting 20 shrubs of each species avoiding shrubs less than 10 m apart to reduce overlapping between foraging areas of ant nests. In each shrub, we identified the ant species collecting nectar from EFNs (interactions). We also quantified the number of workers of each ant species as a measure of recruitment (investment in foraging) on these plants. We repeated this observation three times per day: 8:00 to 11:00h, 12:00 to 15:00h and 16:00 to 19:00h. We repeated this procedure four times, once every week, altering the sequence of sampling plants.

Based on all ant-plant interactions recorded in our study area we constructed an adjacency matrix ***A*****,** in which $a_{ij} = 1$ if the consumption of EFN from a plant species *j* by an ant species *i* was recorded, and zero otherwise. Subsequently, we plotted all interactions recorded as an ecological network using the bipartite package of ‘R’ software, and defined ant and plant species as a central core of highly interacting species (*i.e.*, those with the most interactions) or as peripheral (*i.e.*, those with few interactions) components of our ant-plant network. We highlighted in the network graph the ant species that collected nectar and protected the plant.

Protection effectiveness landscape

After defining how were structured all interactions between ants and plants within our study area, we experimentally quantified the protection effectiveness of ant species on each plant species using a simple bioassay conducted in the field using simulated herbivores. For this, we placed worker termites (*Syntermes* sp.) on plants to simulate the presence of an enemy (*sensu* Oliveira 1997). The use of termites as herbivores simulators allows the standardized assessment of the defense capability of several species of ants in a short period of time. In addition, one can apply a statistically reasonable number of samples (ant-termite encounters) for each ant species in different contexts (different species of plants).

The experiment was applied in the middle of the rainy season (January-February 2014), shortly after the sampling of ant-plant interactions (see above), and using the same selected s of plant species (10 plant species and 20 shrubs per species). Firstly, we excluded ants from the shrubs by manual removal followed by application of a nontoxic resin (Tanglefoot, The Tanglefoot Co. ®) to block ant access to the plants. Otherwise, we could have overestimated the protective capacity for ant species already present on plants. After 24h of blockage, we began observations. We observed one shrub of each ant species per day, totaling 10 shrubs. We randomly selected five different apical branches of each shrub to introduce termites, one termite in each branch, totaling five termites. We observed ant behavior for 15 minutes. After that, we remove the ants, reapply the blockage, and then proceeded to another shrub until we accessed all 10 shrubs in sequence. At the end, we returned to the first shrub and restarted the experiment with five new termites for each shrub. We repeated the experiment four times, totaling 20 termites per shrub. At the end of the day, we blocked 10 new bushes, one for each plant species, to reapply the experiment two days later. At each day, we randomized the sequence of observation of plant species. This procedure continued until the experiment was applied to all 200 shrubs of the 10 plant species. We finished having sampled 4,000 encounters between ants and termites and proximately 6,000 hours of observation distributes in a period of 40 days.

Unlike the classic experiment, termites were not glued because some species are too small to detach the termite (e.g. *Brachymyrmex* spp.) and others repel enemies without physical contact by swarming or releasing formic acids (i. e. *Camponotus* spp.). We also kept the termites alive because it is expected that herbivores respond to the attack of ants in natural conditions. In each observation, we recorded the time between ant climbing on the plant and finding the termite (measure of discovery time), limiting the observation time to 15 minutes. We considered as a success when ants captured or repelled the termite and failure when ants found but ignored the termites, or when ants did not found the termite (measure of protective efficiency). Assessed in approximately 50 hours of observation (5 hours per plant species).

For identification of defender and exploiter ant species in each ant-plant interaction, we used a model of mutualistic effectiveness landscape previously proposed in studies involving pollination and seed dispersal (Schupp et al. 2010; Rodríguez-Rodríguez et al. 2013). In our case, we defined Protection Effectiveness (*PE*) of ants based on qualitative and quantitative components. Qualitative component ($QLC = t^{-1} \times a$) was based on two parameters: the encounter time (t), which is the time spent from the moment the ant climbs the shrub to the moment that it encounters the termite or leaves the shrub without finding the termite, and the removal rate of herbivores (a), which is the percentage of termites found that were removed. The quantitative component (*QNC*) was the number of workers an ant species recruits to a plant species. Ant species were considered mutualistic when they had both high values of *QNC* and *QLC* on the contrary, they were considered exploiters. Thus, true mutualistic ant species were those that recruited many workers, quickly discovered, and attacked a large amount of herbivores on a plant.

After calculating *QNC* and *QLC*, we estimated the protection effectiveness for each plant species using the formula: $PE = QNC \times QLC$. In order to know which was the main factor responsible for a better effectiveness protection landscape, we also calculated the relative contribution for each of three subcomponents: average number of workers recruited (*QLC*), removal rate of herbivores (*QNC*), and the encounter time of herbivores (*QNC*). For this, we prepared a multiple linear regression model using the square root (sqrt) transformed numbers of PE as the response variable and the three *PE* subcomponents as predictor variables. Owing to non-normal distribution of data, we used simple linear regression (*SLR*) to test whether the number of workers recruited, removal rate of herbivores and encounter time of herbivores are greater in ant species of the central core than in peripheral ant species. We used the number of interactions as independent factor, i.e. species with more interactions are more close to the network core than species with fewer interactions. For plants, we used the same *SLR* to test whether plant species of the central core were better protected than peripheral species. The protection of each plant species was defined as the sum of the values of *PE* of all ant species interacting with these plants. All Protection Effectiveness Landscape and *SLR* analyses were carried out with ‘R’ software version 2.13.0 (R Development Core Team 2011).

Factors of protection effectiveness landscape

We evaluated four factors that could affect protection effectiveness of ants: (1) the volume of and the (2) sugar concentration present in the nectar as a measure of quality of nectar, (3) the number of branches and (4) the size of plants as measure of space to forage and space to protect. For nectar quality, we first counted the number of EFNs present in every shrub. Then, we randomly selected 10 young leaves of each of the 200 shrubs. We covered the leaves with white voile cloth bags for 24 h to accumulate nectar sufficient to measure volume in

graduated microcapillary and sugar concentration in light refractometer (*sensu* Radhika et al 2008). We extracted the mean volume of nectar produced per nectary and multiplied by the number of nectaries present in the shrub to estimate the volume of nectar produced per shrub. We calculated the mass of sugar present in the nectar using the equation: "$y = 0.00226 + (0.00937x) + (0.0000585\ x^2)$", where "y" represent sugar content and "x" represent the brix value acquired with refractometer (*sensu* Dafni et al 2015). We measured plant height from the ground to the end of the highest branch and the diameter of the crown using the north-south direction as arbitrary measure. Then, we estimated the volume of the plant by calculating the volume of a cone. Finally, we estimated the number of branches of the plant by counting the number of apical meristems of the plant.

To calculate the effect of predictive factors on protection effectiveness presented by ants on each plant species (response variable), we first calculated the average of the predictive variables for each species (20 samples per species, 10 species). Thus so, the predictive variables were (1) nectar volume produced per plant (uL/plant/24h), (2) concentration of sugar in the nectar (mg / uL), (3) volume of the plant (cm^3) and (4) number of branches (branches / plant). We then evaluated the association between the predictor variables and the response variables using one multiple linear regression model for each dependent variable. The predictor variables were linearized by logarithmic transformation (base 10). The response variables did not require transformation. The analysis was performed using 'R' V.2.13.0 (R Development Core Team 2011).

Results

Ant-plant interactions

We recorded 81 interactions involving the 10 EFN-bearing plant species and 2, 270 ants from 23 ant species (Table 1 and 2; Figure 1; Appendix 1). Only two plant species, *Chamaecrista desvauxii* (10 interactions) and *Crotalaria micans* (10), and three ant species, *Camponotus crassus* (10), *C. novogranadensis* (10) and *Pseudomyrmex gracilis* (8) were found as part of the central core of highly interacting species (Figure 1). All other ant and plant species had significant lower number of interactions in relation to other species, and therefore, were considered as species constituting the periphery of our ant-plant network. In all plant species, *C. crassus* recruited the largest number of ant workers to collect the nectar (1, 067; 47% of the observed ants), followed by *C. novogranadensis* (403; 18%), *P. gracillis* (230; 11 %), *Cephalotes pusillus* (227; 11%) and *Camponotus rufipes* (126; 6%) (Table 1).

Protection effectiveness landscape

We observed significant differences in the relative contribution of each of the subcomponents for PE. Number of workers recruited, removal rate of herbivores and encounter time of herbivores explained 36%, 35% and 4% of all variation in PE respectively. We observed that ants closest to the generalist core recruited more workers per plant (Mean ±SD: 3.31 ± 3.09) as compared to peripheral ant species (0.143 ± 0.273) (SLR: $\beta = 0.7$; $t = 4.1$; $p = 0.0006$). Moreover, ants closest to the generalist core also attacked ten times more termites (23.61 ± 20.17 %) as compared to peripheral ant species (2.81 ± 4.92 %) (SLR: beta = 0.75, $t = 5.2$, $p = 0.0001$). However, the time to find termites on plants was not different between the ant species of the generalist core (253.01 ± 73.97 seconds) and periphery (425.71 ± 172.37 seconds) (SLR: beta = 0.32, $t = 1.6$, $p = 0.14$).

Dealing with the ants' effectiveness in protect their host plants, only nine of the 23 ant species show some effectiveness in protect the plant against herbivores. We found that ants closest to generalist core showed the highest values of protection effectiveness (PE = 1.33 ± 1.74) than peripheral ant species (PE = 0.01 ± 0.03) (RLS: beta = 0.76, $t = 5.4$, $p = 0.0001$) (Figure 2). Ant species with significant highest value of PE were the species from the network core: *C. crassus* (PE = 3.34), *C. novogranadensis* (PE = 0.51), and *P. gracilis* (PE = 0.14) (Figure 2). *Camponotus crassus* was the most effective ant protecting all ten plant species presenting high values of PE because it recruited large numbers of workers, found the herbivores quickly and removed most of the herbivores found (Figure 2). On the other hand, *C. novogranadensis* showed intermediate PE in eight species of plants due to high recruitment, although it was not very efficient in removing herbivores (Figure 2). While P. *gracillis* showed high PE in five plant species due to high efficiency in herbivores removal, despite not recruited large numbers of workers (Figure 2).

Predictive factors of protection effectiveness landscape

Adding the values of PE of all ant species found on a given plant species, plant species with the most protective ants were: *Stachytarpheta glabra* (PE= 9.21) and *Banisteriopsis* *campestris* (PE= 7.35) (Table 2; Figure 3). Plant species least protected were *Peixotoa tomentosa* (PE= 0.77) and *Senna pendula* (PE= 1.08) (Table 2; Figure 3). Despite the large variation in the PE received by plants, we did not find higher ant protection in plant species closest to the generalist core (0.123 ± 0.05*)* than those closest to the periphery (0.231 ± 0.15) (RLS: β= 0.13, t = 0.4, p = 0.7). Additionally, we found that plant morphology and nectar quality and quantity were important factors explaining the variance in ants' protection effectiveness among plant species (GLM: $r^2 = 0.88$; $F_{4,5} = 9.4$; p= 0.01) (Figure 4). Ants were more effective protectors in plant species that produced lower volume of nectar (β = -0.64, t = 2.44, p = 0.05) but with high sugar concentration (β = 1.32, t = 5.33, p = 0.01), and these factors explained most of the variance in protection effectiveness (Figure 4). Ant species were also more effective in protecting smaller plants (β = -0.04, t = 0.26, p = 0.8) with high number of branches (β = 0.14, t = 0.83, p = 0.4), although this factors did not explained much of the variance in protection effectiveness (Figure 4).

Discussion

How many mutualist species truly occur in a network of interactions between ants and EFN-bearing plants? According to our results, nine of the 23 ant species (40%) showed some potential to offer effective protection against herbivores. Among these, only the three species from the network core (highly interacted) showed significant protection: *C. crassus*, *C. novogranadensis* and *P. gracillis*. Mutualistic species defended its host plant by different mechanisms. Some plants were better defended due to high recruitment (*C. novogranadensis*), while other interacted with ants that quickly attack many herbivores but presents low recruitment (*P. gracillis*), but only the true protector (*C. crassus*) presented high recruitment and quickly captured most of the herbivores. Based on these results, we could classify ant species in four functional groups: (1) true mutualists, being species that forage intensively and capture almost all herbivores, as *C. crassus*. (2) opportunistic mutualists, being species that forage intensively, finds many herbivores but capture few, as *C. novogranadensis* and *C. pusillus*; (3) occasional mutualists, being aggressive ants with low recruitment that find few herbivores but capture all at every opportunity, as *P. gracilis and C. rufipes*; and (4) exploiters, being species that collect nectar but provide no defense to the host plant.

Many herbivores are specialists and in avoid ant predation (Pereira and Trigo 2013; Alves-Silva et al. 2014). Therefore, interaction with various species with different forage strategies may complement the defense by the main mutualist. These species would be ecological equivalents, which could replace each other in different contexts or complement each other to enhance the effectiveness of plant protection. Ecological equivalents guarantee a backup protection for the plant and reduces the fragility in ant-plant mutualisms (Brown et al. 1979; Zamora 2000). For example, *Pseudomyrmex* spp. can capture endophytic larvae and trips that lives inside meristems and flowers (Del-Claro 2004). *C. crassus* reduces its forage at night and are replaced by the nocturnal *C. novogranadensis, C. rufipes and Camponotus melanoticus* (Dáttilo et al. 2014b), which could assume the protective role. However, *C. crassus* was the most effective mutualist, presenting six times more protections than the second most protector *C. novogranadensis*, therefore plants the successfully interacts with many *C. crassus* may have better protection and higher benefits from the mutualisms. Therefore, plants need to interact with protector species, but increase its attractiveness to the true protectors, which can guarantee minimal protection in most cases, while opportunistic and occasional protectors may be effective only in specific cases.

Most ant species observed collecting nectar from EFN-plants had low values of PE, confirming our hypothesis that interactions between ants and EFN-plants are diffuse mutualisms with high degree of exploitation. Exploitation is common in generalized mutualistic interaction including polinization, seed dispersal and microbial living in plant roots (Schupp et al. 2010; Rodríguez-Rodríguez et al. 2013; Orona-Tamayo and Heill 2013). However, we believe that even with few truly mutualistic species, the ant-plant network remains stable. The benefits of a mutualism have a point of stasis when the protection of the plant does not increase with the accumulation of ant species because few species would already be enough to ensure maximum protection (Holland et al. 2005). Therefore, as we observed, true mutualists ensure protection for all plant species because they are highly interactive species of the network core. This results highlight the importance of the network core for the evolution of the mutualistic networks because it provide the ecological service properly and also distribute the benefit to all network.

The ant recruitment and aggressiveness, but not the time of discovery, was important factors for the effectiveness of protection because those ants that quickly found herbivores not necessarily removed them, or otherwise. True protector species should recruit several workers, which increase probability to find herbivores, and be very aggressive, which increase success in herbivore removal. This numerical and behavioral dominance might be possible only because the mutualist acquire enough energy being a competitive dominant species and consuming nectar from all plants (Davidson et al. 1998; 2005). The big issue about exploiter species is that it can destabilize mutualisms since they compete for resources with protector species and do not provide benefits for host plant (Herre et al. 1999; Bronstein 2001; Raine et al. 2004). Therefore, to perform an energetically advantageous forage, the true mutualists need to protect the EFNs (i.e. the reward source) to increase its gains by dominating the food source (Orona-Tamayo and Heil 2013). This territoriality is energetically expensive and may be advantageous only on plants that predictably produce a high energetic nectar (Davidson et al. 2004).

Our results shown evidences that the quality of the reward directly affects the protection effectiveness of ant mutualists. The relative importance of recruitment and predation for the effectiveness of the true mutualist is context dependent and vary with the nectar availability and quality. In this case, plants that produce large amounts of nectar with low sugar content tend to have many exploitative interactions. On the other hand, plants that produce nectar with high sugar content have fewer exploitative species but high number of true mutualists, and consequently more protection. However, the most protective species, *C. crassus*, interacted with all plant species but was more effective in protect plants offering sugar-rich (PE: 9.2 in *S. glabra*), than sugar-poor nectar (PE: 0.4 in *P. tomentosa*). Thus, not only the number of interactions but also the quality of the reward may affect the protective outcome. However, in cases where *C. crassus* was low effective, another mutualistic species was the main protector, confirming the hypothesis of ecological equivalents mutualists. For example, *C. novogranadensis* (PE: 0.7) in *S. pendula* (*C. crassus*' PE: 0.1), *P. gracillis* (PE: 0.6) in *C. deavauxii* (*C. crassus*' PE: 0.3). Therefore, three aspects are imperative to the success of the mutualism: (1) the quality of the nectar, (2) the number of interactions with protector species and (3) the effectiveness of the protector species, whereas the last two are the result of the first factor.

In summary, our results highlight the importance of assessing, even indirectly, the possible outcomes of interactions between ants and plants to reach a more accurate conclusion about the structure of the interaction networks. This is the first study that empirically demonstrated the degree of exploitation in ant-plant networks, although this interaction is one of the most studied mutualism. Our study provides evidence that networks based on observation of ants collecting nectar without evaluating the benefit of the ants could be overestimating interactions. We show that most of ant fauna collecting extrafloral nectar does not provide protection for interacting plants. Thus, our results shed an important issue against preconception in considering all interactions between ants and plants as mutualistic, and urges caution when interpreting the results on these networks. We also show that mutualism may vary according to the identity of the plant species and the quality of nectar (reward) offered. We reaffirm the context-dependency of these ant-plant interactions (Chamberlain and Holland 2009), and the importance of the specific biology and behavior of interacted species. Therefore, empirical studies on the natural history of pairs of interacting species are very significant for understanding the network as a whole and the ecological and evolutionary processes behind the structure of interactions.

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Tables

Table 1. List of interactions between ant species and plant species observed in an area of Rupestrian Fields (Ouro Preto, Brazil). Numbers represents total number of ants observed collecting nectar in 20 shrubs of each plant species. Ants and plants are classified in decreasing order of total number of interactions. Protection effectiveness (PE) depict the mean of protection effectiveness presented by each ant species observed.

	Plant species										
	Chamaecrista desvauxii	*Crotalaria micans*	*Senna reniformis*	*Heteropterys umbellata*	*Peixotoa tomentosa*	*Senna pendula*	*Stachytarpheta glabra*	*Banisteriopsis malifolia*	*Chamaecrista mucronata*	*Banisteriopsis campestris*	
Ant species	Recruitment										PE
Camponotus crassus	17	133	51	122	43	35	265	164	187	50	3.34
Camponotus novogranadensis	37	85	2	27	17	41	44	49	31	70	0.52
Pseudomyrmex gracillis	24	60	2	8	1	66	0	6	63	0	0.15
Cephalotes pusillus	31	15	11	8	28	25	0	0	102	7	0.12
Pseudomyrmex termitarius	3	2	7	3	0	5	0	1	0	0	0.002
Brachymyrmex heeri	1	2	0	0	0	0	5	0	2	0	0
Pseudomyrmex pallidus	0	1	0	2	2	0	0	0	20	5	0
Crematogaster sp3	1	0	3	2	0	8	0	0	0	2	0.02
Camponotus rufipes	0	60	11	0	0	0	0	55	0	0	0.08
Crematogaster sp.1	0	0	5	2	1	0	0	6	0	0	0.006
Pheidole sp.1	0	0	0	0	3	0	0	0	0	4	0.04
Camponotus melanoticus	1	50	0	0	0	0	8	0	0	0	0
Camponotus senex	0	0	8	4	0	0	0	0	20	0	0
Brachymyrmex pictus	0	0	0	0	0	0	1	0	0	0	0
Linepithema micans	0	0	0	0	0	7	0	0	0	0	0
Dorymyrmex brunneus	4	0	0	0	0	0	0	0	0	0	0
Wasmannia sp.1	0	0	0	0	0	0	0	4	0	0	0
Crematogaster sp.2	0	0	3	0	0	0	0	0	0	0	0
Pheidole sp.2	0	0	0	0	3	0	0	0	0	0	0
Camponotus fastigatus	0	0	0	0	0	0	2	0	0	0	0
Gnamptogenys sulcata	0	0	0	0	2	0	0	0	0	0	0
Camponotus atriceps	0	1	0	0	0	0	0	0	0	0	0
Linepithema sp.1	1	0	0	0	0	0	0	0	0	0	0

Table 2. Parameters of plant morphology (plant volume and braches per plant) and resource quality (nectar volume and sugar concentration) as predictive factor of the protection effectiveness of the interacted ant species. All variables, except protection effectiveness, depict mean values and stardard deviation (N = 20 shrubs per plant species). Protection effectiveness depict the sum of protection effectiveness presented by all ant species observed protecting each plant species.

Plant specie	**Plant volume (m^3)**	**Number of branches per plant**	**Sugar concentration (mg/µL)**	**Nectar volume (µL/plant/24h)**	**Protection effectiveness**
Banisteriopsis campestris	0.23 ± 0.23	13.8 ± 8.2	0.43 ± 0.12	0.02 ± 0.01	6.21
Banisteriopsis malifolia	0.19 ± 0.19	82.2 ± 67.26	0.08 ± 0.04	0.10 ± 0.06	3.88
Chamaecrista desvauxii	0.10 ± 0.05	28.9 ± 17.78	0.12 ± 0.06	0.15 ± 0.1	0.38
Chamaecrista mucronata	0.14 ± 0.09	73.15 ± 43.04	0.39 ± 0.2	0.08 ± 0.07	3.05
Crotalaria micans	0.09 ± 0.07	42 ± 43.43	0.38 ± 0.1	0.77 ± 0.69	2.46
Heteropterys umbelatta	0.14 ± 0.12	33.4 ± 16.37	0.63 ± 0.23	0.40 ± 0.42	4.93
Peixotoa tomentosa	0.54 ± 0.39	84.25 ± 58.87	0.12 ± 0.05	0.06 ± 0.02	0.77
Senna pendula	0.10 ± 0.08	56.3 ± 38.35	0.14 ± 0.08	0.06 ± 0.03	0.12
Senna reniformis	0.52 ± 0.34	41.2 ± 30.61	0.24 ± 0.09	0.08 ± 0.03	2.41
Stachytarpheta glabra	0.07 ± 0.07	57 ± 67.56	1.03 ± 0.05	0.29 ± 0.19	9.20

Figure 1. Ant–plant interaction networks highlighting the protection effectiveness (PE) of all ant species recorded on each of the 10 plant species studied between January and February 2014 at the Parque Estadual do Itacolomi, Minas Gerais State, Brazil. The nodes above represent ant species and the nodes below correspond to plant species. Rectangle length is proportional to the number of interactions of each species. Line width represents the values of protection effectiveness of each ant species for each plant species. Asterisks (*) denote ant species that was effective in plant protections. Two asterisks (**) comprise the central core of highly generalized species. Note that the values of PE are represented as (PE × 10) +1, because some ant species had values of PE = 0.

Figure 2. Protection effectiveness (PE) of all ant species recorded on each of the 10 plant species studied in January and February 2014 at the Parque Estadual do Itacolomi, Minas Gerais State, Brazil. Open and closed triangles represent peripheral and core ant species respectively (CAC: *Camponotus crassus*, CAN: *Camponotus novogranadensis*, PSG: *Pseudomyrmex gracillis*). Isoclines connect all values of quantity (QNC) and quality (QLC) components with the same values of PE. Note that, QLC = t-1 × a, where, a = removal rate of herbivores, and t = the encounter time of herbivores. QNC = the average number of workers recruited.

Figure 3. Protection effectiveness landscape for all 10 plant species highlighting the more effective ant species: *Camponotus crassus*. Each point represents an ant species on its host plant. Isoclines connect all values of quantity (*QNC*) and quality (*QLC*) components with the same values of PE.

Figure 4. Relation between resource availability (right; mean nectar volume and sugar concentration) and foraging area (left; mean plant volume and number of ramifications per shrub; $n = 20$ shrubs per plant species), and the protection effectiveness observed for ants interacting with each plant species. Only sugar concentration ($\beta = 1.32$, $p = 0.03$) and nectar volume ($\beta = -0.63$, $p = 0.05$) significantly explained variation on protection effectiveness.

Figures

Figure 1

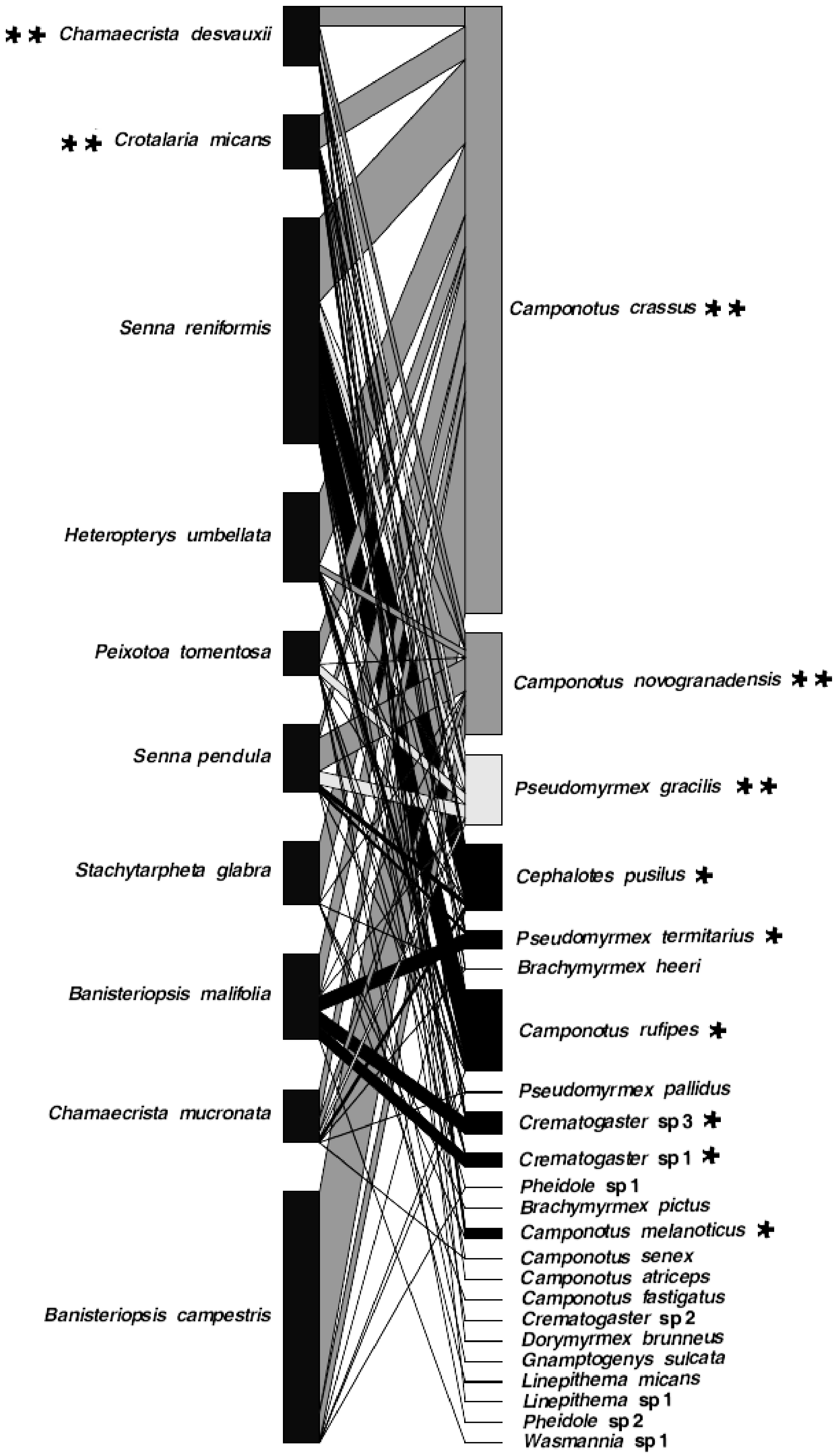


Figure 2

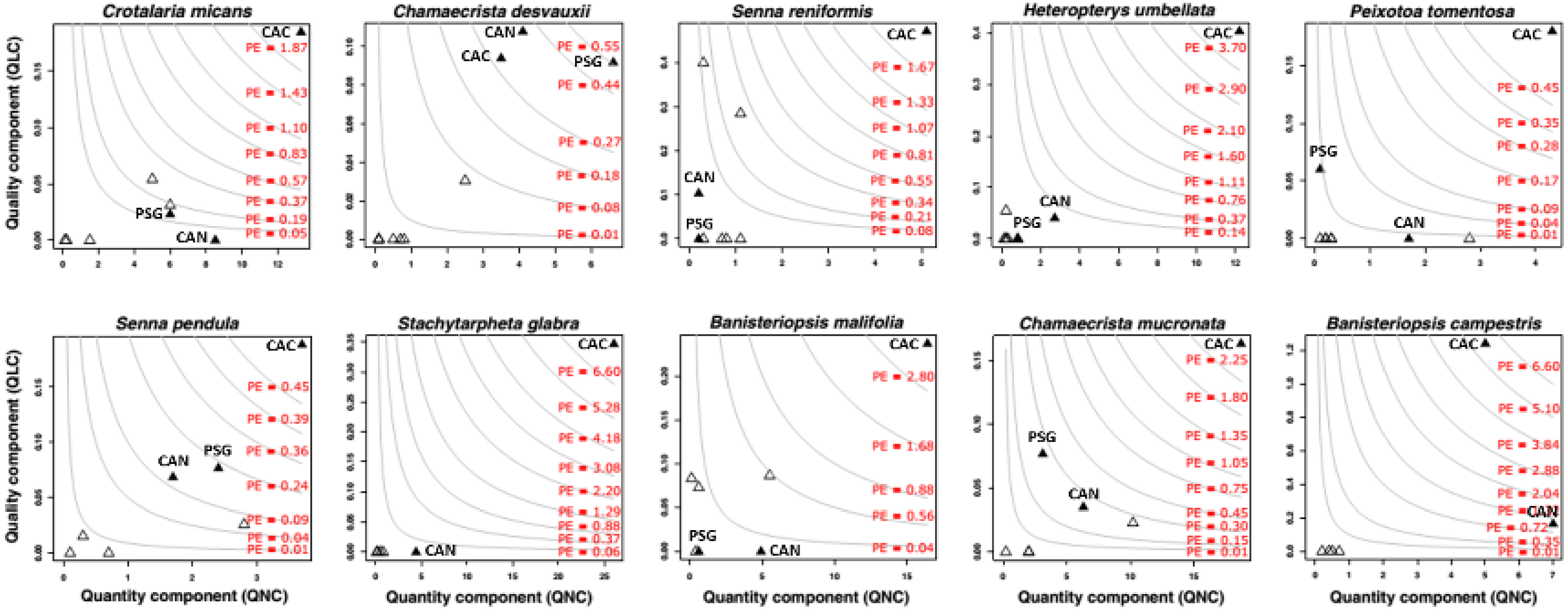
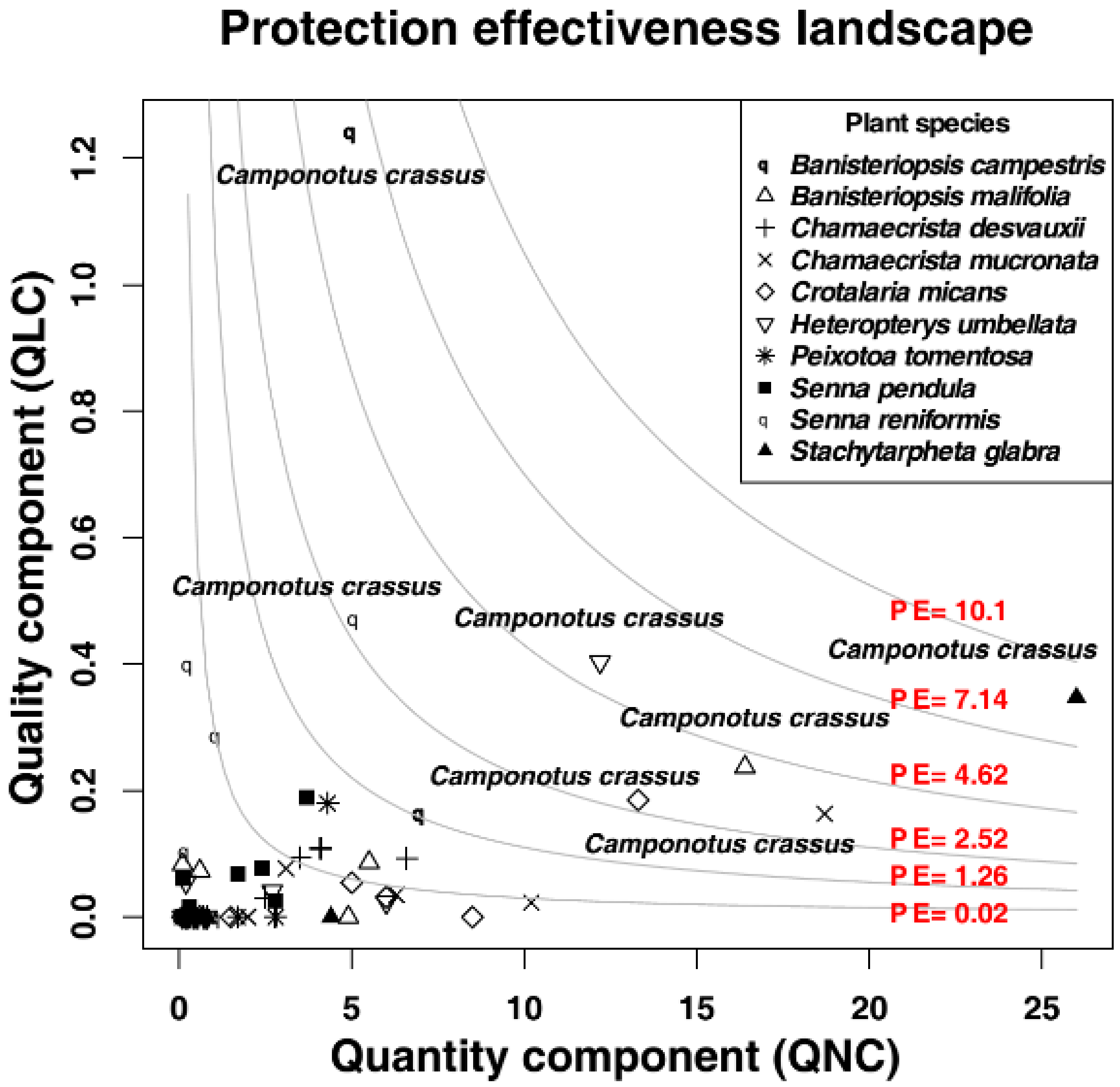
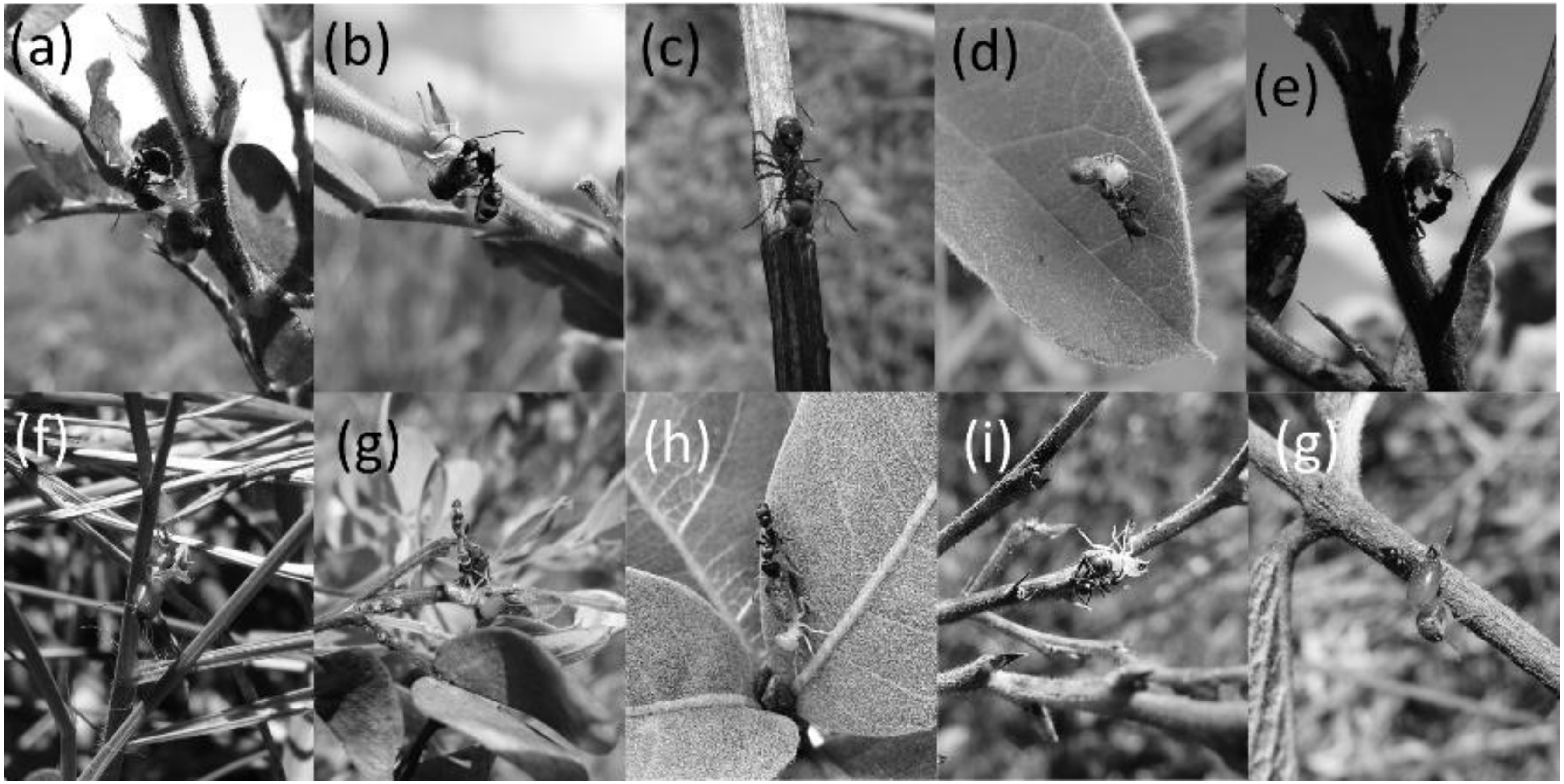

Crotalaria micans
CAC
PE = 1.87
PE = 1.43
PE = 1.10
PE = 0.83
PE = 0.57
PE = 0.37
PE = 0.19
PE = 0.05
PSG
CAN
Chamaecrista desvauxii
CAN
CAC
PSG
PE = 0.55
PE = 0.44
PE = 0.27
PE = 0.18
PE = 0.08
PE = 0.01
Senna reniformis
CAC
PE = 1.67
PE = 1.33
PE = 1.07
PE = 0.81
PE = 0.55
PE = 0.34
PE = 0.21
PE = 0.08
CAN
PSG
Heteropterys umbellata
CAC
PE = 3.70
PE = 2.90
PE = 2.10
PE = 1.60
PE = 1.11
PE = 0.76
PE = 0.37
PE = 0.14
CAN
PSG
Peixotoa tomentosa
CAC
PE = 0.45
PE = 0.35
PE = 0.28
PE = 0.17
PE = 0.09
PE = 0.04
PE = 0.01
PSG
CAN
Senna pendula
CAC
PE = 0.45
PE = 0.39
PE = 0.36
PE = 0.24
PE = 0.09
PE = 0.04
PE = 0.01
CAN
PSG
Stachytarpheta glabra
CAC
PE = 6.60
PE = 5.28
PE = 4.18
PE = 3.08
PE = 2.20
PE = 1.29
PE = 0.88
PE = 0.37
PE = 0.06
CAN
Banisteriopsis malifolia
CAC
PE = 2.80
PE = 1.68
PE = 0.88
PE = 0.56
PE = 0.04
PSG
CAN
Chamaecrista mucronata
CAC
PE = 2.25
PE = 1.80
PE = 1.35
PE = 1.05
PE = 0.75
PE = 0.45
PE = 0.30
PE = 0.15
PE = 0.01
PSG
CAN
Banisteriopsis campestris
CAC
PE = 6.60
PE = 5.10
PE = 3.84
PE = 2.88
PE = 2.04
PE = 1.36
CAN
PE = 0.72
PE = 0.35
PE = 0.01
Quality component (QLC)
Quantity component (QNC)

Figure 3


Protection effectiveness landscape
Quality component (QLC)
Quantity component (QNC)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
5
10
15
20
25
Plant species
Banisteriopsis campestris
Banisteriopsis malifolia
Chamaecrista desvauxii
Chamaecrista mucronata
Crotalaria micans
Heteropterys umbellata
Peixotoa tomentosa
Senna pendula
Senna reniformis
Stachytarpheta glabra
Camponotus crassus
Camponotus crassus
Camponotus crassus
Camponotus crassus
Camponotus crassus
Camponotus crassus
Camponotus crassus
P E= 10.1
P E= 7.14
P E= 4.62
P E= 2.52
P E= 1.26
P E= 0.02

Appendices

Appendix 1. Ant behaviours against plant intruders used to evaluate protective effectiveness of different ant species. Termites was used as herbivore simulators. (a) *Camponotus crassus* bitten the prey, curved the gaster in prey direction and released formic gas. (b) *Camponotus novogranadensis* bitten the prey and consumed the inner fluids. Note the gaster expanded by food storage. (c) *Camponotus rufipes* released formic gas and carried the dizzy prey back to the nest. (d) *Cephalotes pusillus* bitten the prey and throw it out of the plant from the leaf border. *Pseudomyrmex gracillis* (e), *P. pallidus* (f), *P termitarius* (g) and *Gnamptogenys sulcata* (h) stung the prey and carried to the nest. *Dorymyrmex brunneus (i)* and *Crematograster* sp. (j) grab only slow and unaggressive termites and carried them alive to the nest



Capítulo 5

Redes de interação baseadas em indivíduos: variação da estrutura ao longo do dia e relação espécie-área

Individual-based ant-plant networks: structure throughout the day and species-area relationship

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Resumo:

Neste estudo, utilizando amostragem tanto diurna quanto noturna no Cerrado brasileiro, avaliou-se pela primeira vez a estrutura topológica ao longo do dia e a relação espécie-área em redes formiga-plantas baseado em indivíduos com base em para duas espécies de plantas. Em geral, observou-se que as redes diurnas formiga-planta apresentam o mesmo padrão de interações quando comparado com as redes noturnas: um padrão aninhado e não-modular de interações e um nível médio de especialização. Apesar da alta semelhança na composição das espécies de formigas entre os dois períodos de coleta, as espécies presentes no núcleo generalista (ou seja, aquelas com o maior número de ligações) muda totalmente entre a amostragem diurna e noturna para todas as espécies de plantas estudadas. Em outras palavras, este "noturno" indica que diferentes processos e funções ecológicas estão ocorrendo em pequena escala espacial, no entanto, temporalmente dividiram (dia e noite). Assim, é possível que em alguns casos, processos de formação de redes formiga-planta podem ser negligenciados quando usado apenas amostragem diurna. Além disso, não observamos qualquer efeito do tamanho da planta sobre a riqueza de formigas (i.e., maior grau dentro de uma rede aninhada) forrageando em tais plantas. Nossa hipótese é que as formigas competitivamente superiores poderiam dominar este recurso e permitir a coexistência de apenas algumas outras espécies de formigas, no entanto, outras hipóteses alternativas também são discutidas. Em suma, nosso estudo contribui para uma melhor compreensão sobre a importância do período de amostragem e de relacionamento estruturantes redes formiga-planta espécies-área.

Palavras-chave: redes formiga-planta; redes intrapopulacionais; dinâmica temporal; amostragem diurno e noturno; modelo espécie-área; ligações proibidas.

Abstract

In this study, using both diurnal and nocturnal sampling in the Brazilian Neotropical savanna, we evaluated for the first time the topological structure throughout the day and species-area relationships in individual-based ant-plant networks for three plant species. In general, we observed that diurnal ant-plant networks exhibit the same pattern of interactions when compared with nocturnal networks: a nested and non-modular pattern of ant-plant interactions and an average level of network specialization. Despite the high similarity in the ants' composition between the two collection periods, the ants' composition present in the generalist core (*i.e.*, those with the most links) totally changes between diurnal and nocturnal sampling for all plant species studied. In other words, this "night-shift" indicates that different processes and ecological functions are occurring at a small spatial scale, however, temporally partitioned (day and night). Thus, it is possible that in some cases processes shaping mutualistic networks formed by protective ants and plants may be being overlooked only in diurnal sampling. Moreover, we did not observe any effect of plant size on ant richness (*i.e.*, greater degree within a nested network) foraging on such plants in any of the studied ant-plant network. We hypothesized that competitively superior ants could dominate this resource and allow the coexistence of only a few other ant species, however, other alternative hypotheses are also discussed. In short, our study contributes to a better understanding about the importance of the sampling period and species-area relationship structuring ant-plant networks.

Keywords: ant-plant networks; intrapopulation networks; temporal structure; diurnal and nocturnal sampling; species-area model; forbidden links.

Introduction

Ants and plants can interact in different ways, from facultative to highly specialized relationships (Rico-Gray & Oliveira 2007). For example, extrafloral nectar-mediated ant–plant mutualisms are among the most remarkable ecological interactions in terrestrial ecosystems (Rico-Gray & Oliveira 2007). In this type of ant-plant interaction, plants with extrafloral nectaries (EFN-bearing plants) produce a liquid rich in carbohydrates and amino acids, which attract different ant species (Koptur et al. 1998). In exchange for food, ants protect the host plant against potential herbivores (Del-Claro et al. 1996; Rico-Gray et al. 2007). At the community level, different ant and plant species can interact with each other and generate complex ecological networks of ant-plant interactions (Guimarães et al. 2006; Chamberlin et al. 2010; Dáttilo et al. 2013a). In these interaction networks, plant and ant species are depicted as nodes and their interactions are depicted by links describing the use of plants by ants (Guimarães et al. 2006; Chamberlin et al. 2010; Dáttilo et al. 2013a).

Focused on the structure of ant-plant ecological networks, some studies have found some non-random patterns of interactions around the world, as for example the nested pattern (Guimarães et al. 2006; Dáttilo et al. 2012; Lange et al. 2013). This pattern indicates that within an ant-plant network there is a core of generalist species (those with the most interactions) interacting among themselves, and specialists species (those with fewer interactions) interacting also with the generalist species in cohesive subgroups (Bascompte et al. 2003). Another characteristic of these facultative ant-plant networks is that they do not present a modular pattern of interactions, since there are no groups of ants specialized in feeding on a given group of plants (Díaz-Castelazo et al 2013; Dáttilo et al. 2014a), as previously demonstrated in symbiotic networks involving ants and myrmecophytes (Dáttilo et al. 2013b). However, despite the importance and increase knowledge of ant-plant networks at the community level, no study has evaluated how intrapopulation variation in plants can contribute to organization of the ant species associated to individual EFN-bearing plants. As a single EFN-bearing plant can be associated to several ant species in a predictable way, we also can use a network approach, to evaluate the structure of such individual-based ant-plant networks.

We already know that within an EFN-bearing plant population the ant richness associated differs among plants (De la Fuente & Marquis 1999; Oliveira et al. 1999; Rios et al. 2008). One of the main factors explaining this intrapopulation variation is based on the difference in the reward offered (quantity and quality of nectar), in which individuals with better rewards would be most visited by ants (Baker-Méio & Marquis 2012; Alves-Silva & Del-Claro 2013). On the other hand, nectar quantity and quality can vary along of day in the same plant (Schupp & Feener 1991; Heil 2000; Falcão et al. 2014), which means that one plant may be considered a good resource over the day but not at night. Therefore, this variation in nectar features can influence the rate of ants' foraging throughout the day (Falcão et al. 2014). In addition, foraging of some ant species, including those that forage on EFN-bearing plants, can be strictly diurnal, nocturnal, or both (Hölldobler & Wilson 1990; Díaz-Castelazo et al. 2004). Therefore, there are good reasons to expect temporal turnover of ants' composition and intrapopulation variation in ant-plant networks.

An individual EFN-bearing plant can be viewed an "island of resources" for ants and compared with the classical model of species-area relationship proposed by MacArthur and Wilson (1967). In ant-plant interactions, it is possible to expect that during plant ontogeny there is an increase in the availability of food resources for ants, and consequently, in the richness of associated ants (Blüthgen et al. 2000; Davidson et al. 2003; Campos et al. 2006). This possibly occurs because large plant individuals allow a greater spatial segregation in the use of their food resources, which increase the coexistence of ants (Campos et al. 2006). Thus, the difference in the ants' presence on plants from the same population is linked not only by the features of the food resource available, but also by the foraging time of each ant species and species-area relationships. This intrapopulation variation can directly affect the fitness of each individual plant (Gómez & Perfectti 2012), since it is expected that the most visited individuals can be more protected against herbivores (Heil & McKey 2003).

Here, using both diurnal and nocturnal sampling, we evaluated for the first time the topological structure throughout the day and species-area relationships in individual-based ant-plant networks. Specifically, due nectar features and ant foraging shift between day and night, we hypothesize that there would be changes in the composition and in the specific positions of ants within the network when compared diurnal and nocturnal networks. Moreover, based on species-area relationship, we also postulate that larger plant individuals have greater richness of associated ants (*i.e*, greater degree within a nested network). In order to test our hypotheses, we conducted diurnal and nocturnal sampling of ants interacting with three plant species in different regions in the Brazilian Neotropical savanna.

Material and Methods

Study area and species studied

We sampled individual-based ant-plant networks in three different regions inserted in the Brazilian Neotropical savanna (Cerrado biome). In each region we collect the interactions between individuals of an EFN-bearing plant species and their associated ants. As the plants' composition changes geographically, is not logistically possible to access the same species in all sites, we choose the most common EFN-bearing plant species in each site. The plant species sampled were: *Chamaecrista mucronata* (Leguminosae-Caesalpinioideae), *Stachytarpheta glabra* (Verbenaceae), and *Qualea grandiflora* (Vochysiaceae).

We quantify the interactions between ants and *S. glabra* in July of 2011 and *C. mucronata* in September 2011 at Parque Estadual do Itacolomi (PEIT/IEF) (20º26'S and 43º30'W, elev. 1290 m, for *S. glabra* and 20°24'S and 43°30'O, elev. 1150 m, for *C. mucronata*), located in Ouro Preto, in the center of State of Minas Gerais, Brazil. The climate is subtropical humid (Cwa), with mean temperature 19-22 °C and mean precipitation between 1600-1800 mm. The park cover an area of 7000 ha of semideciduous forests (56 %) and *Campos Rupestres* (44 %) (Fujaco et al. 2010). *S. glabra* is an endemic species of the ecosystem "*Campos rupestres ferruginosos*" (Outcrops ironstones or *Cangas*) while *C. mucronata* is endemic of "*Campos rupestres quartizíticos*" (Rupestrian Fields). The *Canga* is a vegetation of shrub and tree species in patches separated by large rock outcrops covered with lichens and grasses. The *Rupestrian Field* is a vegetation of large extensions of grasses with patches of shrubs above quartizitic rock outcrop (Jacobi & Carmo 2012). *S. glabra* is the most common specie of *Canga*, with mean height of 1.5 to 2 m with very small extrafloral nectaries (0.1-0.4 mm in diameter) spreading on the upper leaf surface (15 ± 9 per leaf , n=1200 leaves) (Antonini et al. 2005) . *C. mucronata* is one of the most common species of Rupestrian Fields. *C. mucronata* is a high branched shrub with average height of 0.8-1 m, presenting compound leaves with 6-8 leaflets with the nectaries in the leaf petiole (one per leaf) (Dutra et al. 2008).

We sampled the interactions between ants and individuals of *Q. grandiflora* in November 2013 at Estação Ecológica Serra das Araras (EESA) (15°38′S and 57°12′W, elev. 217 m), located in the municipality of Porto Estrela, state of Mato Grosso, Brazil. According to the Köppen classification, the climate is Tropical savanna (Aw) with an average annual temperature of 28 °C, humidity 70 %, and 1.400 mm of precipitation. It has two well-defined seasons, a rainy season between November and April and dry season between May and October. The reserve area covers 28.700 ha of continuous forest with different physiognomies. The terrain is undulating with altitudinal variation of 500 m between the plateaus, riverside terrains, and mountains (Falcão et al. 2014). *Q. grandiflora* is a typical and abundant woody tree of Brazilian Neotropical savanna which reaches up to 7 m tall, and that bears paired extrafloral nectaries on the stem next to the insertion of the leaves, and on the bud pedicels (Costa et al. 1992).

Sampling

We sampled the interactions between ants and plants along one transect of 300 x 10 m for *C. mucronata* and *S. glabra* and 500 m x 10 m for *Q. grandiflora*. At each transect, we checked fortnightly all individuals of those three species and recorded all ants collecting nectar from EFNs. For all three-plant species studied, all individuals sampled over day were marked to be reviewed at night. Ant-plant interactions were sampled in two time intervals: 1) Diurnal sampling: 08:00-12:00, and 1) Nocturnal sampling: 20:00-23:00. However, the number of plants sampled could vary among samplings, since not all plants sampled over day had ants over night, and *vice versa*. Moreover, no plants with homopterous or any other visible liquid-resource sources were included in our sampling. We also recorded the maximum height of each individual (from the ground to canopy) at the time of each collection to use as a covariate of ant richness.

Ant specimens were identified at the lowest possible taxonomic level with the assistance of identification keys available in the literature and by morphological comparisons of ant species deposited in our reference collections. Ant vouchers were deposited in the: Coleção Entomológica do Departamento de Biodiversidade Evolução e Meio Ambiente da Universidade Federal de Ouro Preto for ants associated to *C. mucronata* and *S. glabra,* and Coleção Entomológica of Universidade Federal de Mato Grosso (CEMT) for ants associated to *Q. grandiflora*.

Network analysis and statistics

In this study, we use each plant species and associated, as independent ant-plant networks. Each individual-based ant-plant network was defined by an adjacency matrix $\boldsymbol{A}$, where a_{ij} = number of interaction from an individual plant j by the ant species i, and zero otherwise (Bascompte et al. 2003). For each plant species (*C. mucronata, S. glabra,* and *Q. grandiflora*), we built interaction ant-plant networks according to the sampling period (diurnal, nocturnal, or both together), totaling nine networks.

Initially, we evaluated the specialization for each of the nine ant-plant networks using an index extremely robust to changes in sampling intensity and the number of interacting species called: H_2' (Blüthgen *et al.* 2006, 2007). In this index, extreme generalization of an ecological network is H_2'= 0 and extreme specialization is H_2'= 1. After, we performed a second approach that involves the search for non-random patterns of interactions commonly found in ant-plant networks studied at the community level. Specifically, we evaluated if selective ant species would visit only a subset of plant individuals visited by the generalist ant species (*i.e*, nested pattern of ant-plant interactions). For this, we estimated nestedness using the NODF-metric (Almeida-Neto et al., 2008) in the ANINHADO (Guimarães and Guimarães, 2006). The values of this metric range from 0 (non-nested) to 100 (perfectly nested). NODF-values are less prone to Type I statistical error when compared to other nestedness indices (Almeida-Neto et al. 2008). Moreover, we tested whether within each ant-plant network there were groups of ant species strongly associated with a particular set of individuals plants, as expected in a modular network. For this we used the modularity index (*M*) based on Simulated Annealing (*SA*) (range 0-1) (Guimerà et al. 2004; Guimerà & Amaral 2005) using the software MODULAR (Marquitti et al. 2014). This index ranges from 0 no subgroups, to 1 totally separated subgroups (Olesen et al., 2007). We estimated the significance of nestedness and modularity in our observed matrices with the Null Model II (CE) (n= 1000 randomizations for each network). In this null model, the probability of an interaction occurring is proportional to the number of interactions of both ant species and plant individuals (Bascompte et al., 2003). We used these network descriptors and null model because they provide a way to characterize the organization of these networks in a way that allows direct comparison with previous works on ant-plant networks. We used a recent formula proposed by Dáttilo et al. (2013a) to describe ant species as peripheral (selective species, those with fewer interactions) or generalist core (generalist species, those with the most interactions) components of the networks: $Gc = \left(\frac{k_i - k_{mean}}{z} \right)$, where k_i = mean number of links for a given ant species, k_{mean} = mean number of links for all ant species in the network, and z = standard deviation of the number of links for ant species. $Gc > 1$ are ant species of the generalist core, and $Gc < 1$ are peripheral ant species. This categorization enables us to evaluate the temporal turnover in the specific positions of ants within each network (*e.g.*, shifting from peripheral to generalist core between diurnal and nocturnal networks). Moreover, we computed for each network the Jaccard's similarity index (*JSI*) in order to explore the turnover in the all ants' composition between diurnal and nocturnal networks (Díaz-Castelazo et al. 2013). Jaccard's similarity index between *D* (diurnal) and *N* (nocturnal) network was computed as follows: $\mathrm{JSI}_{(DN)} = A/(A + B + C)$, where *A* is the number of ant species shared between the two sampling time periods, *B* is the number of ant species present only in the first period, and *C* is the number of ant species present only in the last period.

Finally, to test our hypothesis that plant height could increase the richness of associated ants (*i.e.*, greater degree within a nested network), for each individual-based ant-plant network studied, we used Simple Linear Regressions (SLR) with the plant height as predictor variable and ant richness as a dependent variable. However, before of performed SLR analysis, we used a Student's *t*-test for one sample to evaluate the statistical variation of our predictor variable (plant height) around their own mean for all plant species (all p-values < 0.05). We did SLR analysis and Student's *t*-tests using the *vegan* package (Oksanen et al. 2007) in R–software version 2.1.3.1 (R Core Team 2012).

Results

In this study, we recorded 32 ant species representing 13 genera and six subfamilies (Appendix 1) interacting with the three studied plant species. Specifically in individual-based networks of *C. mucronata*, we collected 11 ant species in both diurnal and nocturnal sampling, and these ants were feeding on nectaries of 24 and 26 individuals respectively (Figure 1A). Examples of ant species foraging at different periods of the day can be found in Appendix 2. The total ant richness considering the two periods of sampling together was 13 species, and only one ant species was exclusively collected at each sampling period. The mean of ant richness per individual was similar in the diurnal (Mean ± SD: 2.12 ± 1.03) and nocturnal (2.03 ± 1.08) networks. However, when we combined the two periods of data sampling, there was an increase in the mean of ant richness per individual (3.14 ± 1.61). In addition, despite the high similarity in the ants' composition between diurnal and nocturnal networks (Jaccard similarity index= 0.866), the ants' composition present in the generalist core (i.e., those with the most links) totally changed between the two sampling periods. The ant species *Camponotus crassus* (Formicinae) and *Pseudomyrmex gracillis* (Pseudomyrmecinae) were part of the generalist core in the diurnal networks (18.1 % of all possible ant species), while only the species *Camponotus melanoticus* (Formicinae) was part of the generalist core in the nocturnal networks (9.09 % of all possible ant species). Three ant species were part of the generalist core including both diurnal and nocturnal sampling: *C. crassus*, *C. melanoticus*, and *P. gracillis* (23 % of all possible ant species). In addition, we observed an average level of network specialization in both diurnal and nocturnal networks (Diurnal: H_2'= 0.519. Nocturnal: H_2'= 0.499). However, the level of network specialization decreases when we included both diurnal and nocturnal data (H_2'= 0.409) (Table 1).

For *S. glabra*, we collected nine ant species (one exclusive species) in the diurnal network and 13 species (five exclusive species) in the nocturnal network (Figure 1B). These ant species were interacting with 41 individuals of *S. glabra*, in both diurnal and nocturnal sampling. Total richness considering both sampling periods was 13 ant species. The mean of ant richness per individual plant was greater when we consider networks of both sampling periods together (3.02 ± 1.35) when compared with diurnal (1.85 ± 0.89) or nocturnal (2.21 ± 1.51) networks. The Jaccard similarity index revealed high similarity between the diurnal and nocturnal networks (0.722), and the number of ant species found in the generalist core did not change between these two networks (just two species). This number of ant species represents 22.2 % and 15.3% of the total richness in diurnal and nocturnal network respectively. On the other hands, the identities of ant species constituting the generalist core changed between the two sampling periods: Diurnal network: *C. crassus* and *Pseudomyrmex pallidus* (Pseudomyrmecinae). Nocturnal network: *C. melanoticus* and *Camponotus novogranadensis* (Formicinae). Incluing both sampling periods, the ant species of the generalist core were: *C. crassus*, *C. melanoticus*, and *C. novogranadensis*, in which represent 23% of the total ant richness. The network specialization was similar between diurnal and nocturnal sampling (Diurnal: H_2'= 0.494. Nocturnal: H_2'= 0.518). However, networks tend to be more generalized when we combined our two sampling periods (H_2'= 0.401) (Table 1).

In the diurnal network of *Q. grandiflora*, we recorded 13 ant species (no exclusive) foraging on nectaries of 31 individuals, and 15 ant species (two exclusive) on 30 individuals in the nocturnal network (Figure 1C). Considering the two sampling periods together, we found 15 ant species. The mean of ant richness per plant individual was 1.87 ± 0.84 (Mean ± SD) in the diurnal network, 2.22 ± 1.11 in the nocturnal network, and 2.54 ± 0.99 for both sampling periods together. Although ant composition was relatively constant through time of data collection (Jaccard similarity index: 0.882), the relative importance of component species in the generalist core varied greatly. In the diurnal network, ant species categorized as generalist core components were: *C. crassus* and *P. pallidus* (15.3% of the total ant species sampled). Nevertheless, only the giant tropical ant *Paraponera clavata* (Ponerinae) was a generalist core component in the nocturnal network (6.6% of the total ant species sampled). Including the two periods of sampling, the species components of the generalist core were: *C. crassus* and *P. clavata* (13.3% of the total ant species sampled). Additionally, as we observed for the other two plant species studied, network specialization also was higher in diurnal (H_2'= 0.509) and nocturnal (H_2'= 0.499) networks when compared to ant-plant networks including both sampling periods (H_2'= 0.424) (Table 1).

Evaluating non-random patterns of ant-plant interaction within each network, we observed that all individual-based networks for the three plant species studied, exhibited a significantly nested network topology (NODF values ranging from 27.84 to 58.62. All *p*-values < 0.05). This indicates that the interactions recorded in individuals less visited by ants are a cohesive subset of the interactions found on the most visited individuals. In addition, no network was significantly modular when compared with the neutral patterns of ant-plant interactions (null models) (*M* values ranging from 0.293 to 0.421. all *p*-values > 0.05) (Table 1), and therefore, there is not a group of ant species that feed specifically on a particular group of plant individuals. Finally, when we tested the hypothesis of species-area relationship, we observed that increasing the individual height values does not cause an increase in ant richness (i.e. number of links) foraging on such individuals in any of the studied networks (Simple Linear Regression: all *p*-values > 0.05).

Discussion

Using an intrapopulation approach involving three plant species, we evaluated for the first time the structure of individual-based ant-plant networks by both diurnal and nocturnal sampling in the Brazilian Neotropical savanna. In general, we observed that diurnal ant-plant networks exhibit the same pattern of interactions when compared with nocturnal networks. However, despite the high similarity in the ants' composition between the two collection periods, the ants' composition present in the generalist core (*i.e.*, those with the most links) totally changes between diurnal and nocturnal sampling for all plant species studied. Moreover, we did not observe any effect of plant size on ant richness (*i.e.*, greater degree within a nested network) foraging on such plants in any of the studied ant-plant network.

Studies dealing with ecological interactions networks at the community level are growing steadily in the literature (Ings et al. 2009; Vázquez et al. 2009; Hagen et al. 2012). However, only few studies address intrapopulation variations within ecological networks (Araújo et al., 2010; Cantor et al., 2013; Dáttilo et al. 2014). Moreover, there is a discussion about the standardization in sampling effort (Nielsen & Bascompte 2011), and in some cases, additional nocturnal sampling (Devoto et al. 2011). In this study, we show that individual-based ant-plant networks exhibit the same patterns of interactions found in networks involving ants and EFN-bearing plants studied at the ecological community level: a nested and non-modular pattern of ant-plant interactions and an average level of network specialization (Guimarães et al. 2006; Blüthgen et al. 2007; Dáttilo 2012; Díaz-Castelazo et al. 2013; Lange et al. 2012). Interestingly, these non-random patterns were stable in all our intrapopulation ant-plant networks regardless of the sampling period (diurnal, nocturnal, or including both sampling periods together).

At the community level, we know that different mechanisms can structure both nested and non-modular patterns into ant-plant networks, as for example: temperature, precipitation, soil pH, canopy openness, nectar features and phenology, abundance, body size, and possibly dominance hierarchy among ants (Chamberlin & Holland 2009; Chamberlin et al. 2010; Rico-Gray et al. 2012; Dáttilo et al. 2013c; Lange et al. 2013; Dáttilo et al. 2014a,c). So, why individual-based ant-plant networks exhibit similar patterns of ant-plant interactions when compared to ant-plant networks evaluated at the community level? We observed that species of the genus *Camponotus* were the most extreme generalist ant species in our networks, followed by *Pseudomyrmex* and *Paraponera*. Excepting *Paraponera*, all other genus are particularly abundant in the Brazilian Neotropical savanna (Silvestre & Brandão 2001). Moreover, *Camponotus*, *Pseudomyrmex* and *Paraponera* are strongly associated with EFN-bearing plants (Oliveira & Brandão, 1991; Davidson et al. 2004). Such tree-dwelling genus are highly aggressive, occupy large territories, have mutually exclusive patterns of distribution in a environment, and use diversified strategies to monopolize this highly nutritive resource (Oliveira & Brandão, 1991; Del-Claro & Oliveira, 1999; Livingston & Philpott 2010; Schoereder et al., 2010). Therefore, based on our knowledge of natural history in the field and literature (see information above), we hypothesize that dominance hierarchy could really be the main mechanism that structures the nested pattern in our ant-plant networks. Competitive dominance can occur by: 1) numerical dominance, as observed for *Camponotus* (Rico-Gray & Oliveira 2007); or 2) aggressive displacement of competitors by solitary foragers, as in *Paraponera clavata* and *Camponotus crassus* (pers. obs.). In both cases, dominant ant species would visit most plants in an environment (*i.e.*, generalist core components) while submissive and subordinate ant species co-occur with dominant species only in a few plants. Thus, this pattern of ant-plant interactions would generate the cohesive subgroups expected in nested networks. Additionally, the lack of a modular pattern in our ant-plant networks possibly can be explained because the resource offered by EFN-bearing plants is seasonal over space-time, ants do not have "fidelity" of foraging on the same group of plant individuals (Díaz-Castelazo et al. 2004; Schoereder et al., 2010; Dáttilo et al. 2012). Therefore, when a plant does not secrete nectar, the ants can use other resources available on foliage, and therefore the interactions tend to be more generalized (Schoereder et al., 2010).

For all plant species, when we included data of both diurnal and nocturnal sampling together, we observed a decrease in network specialization. This was possibly due to the increase in the mean of ant richness per individual. Using both diurnal and nocturnal sampling (greater sampling effort), it is expected an increase in the number of species and interactions (Nielsen & Bascompte 2011), and consequently, an increase in the probability to find unique interactions and ant species that foraging in only particular period (forbidden links) (Devoto et al. 2011; Olesen et al. 2011). The record of these forbidden links can exert a strong effect on the structure of an ecological network (Jordano et al. 2003; Vázquez 2005), mainly because it reduces potential sampling bias, as for example, the record only of spatially abundant species. We know that ants foraging can be strictly diurnal, nocturnal, or both (Hölldobler & Wilson 1990), including ants that feed on EFN-bearing plants (Díaz-Castelazo et al. 2004; Chamberlin & Holland 2009). Therefore, when recording ant-plant interactions in different sampling periods, we can increase our understanding of the true role of ant species within an ecosystem.

In our study, some ant species changed their role in network structure, shifting from peripheral to generalist core between diurnal and nocturnal sampling. In other words, this “night-shift” indicates that different processes and ecological functions are occurring at a small spatial scale, however, temporally partitioned (day and night). For example, in sites with high temperature and low humidity during the day (eg. Brazilian Neotropical savanna and deserts), most herbivores display strong nocturnal activity (Strauss et al. 2009; Byk & Del-Claro 2010) and some EFN-bearing plants tend to secrete higher amount of nectar at night to attract their protective ants (Holland et al. 2010; Falcão et al. 2014). Thus, in some cases, processes shaping mutualistic networks formed by protective ants and plants may overlooked by diurnal sampling alone. We believe that the generalist core of nocturnal ant species could drives more strongly the ecological dynamic of the entire system in these highly seasonal environments.

Here, despite a significant variation in the height of plant individuals, our hypothesis that larger plant individuals have greater richness of associated ants was not supported. At least two alternative hypotheses that are not mutually exclusive could explain this pattern. First, due to extrafloral nectar be a highly nutritive and predictable food resource, competitively superior ants could dominate this resource and allow the coexistence of only a few other ant species (Blüthgen & Fiedler 2004ab). In this case, ant richness would not increase during plant ontogeny (plant size), because competitively superior and territorial ant species could limit the access to the EFN-bearing plants by submissive ant species (Heil & McKey 2003; Rico-Gray & Oliveira 2007). This pattern can be stronger in environments where plants are relatively low, since the territory of an ant species would not only the plant, but also the soil below it. Therefore, most of EFN-bearing plants in these environments could support only a few ant species. Second, due to the limited number of workers in an ant colony, it is possible that the dominant species can monopolize just low trees and with low canopy connectivity (*i.e.*, resource access) (Powell et al. 2011). In fact, in the Brazilian Neotropical savanna plants are relatively low (Silva-Junior 2005), and in our study the largest tree had 7.43 m. However, in environments where the tree height can reach up to 50 meters (*eg.*, some tropical rainforests) (Milliken 1998), it is expected that different ant species can coexist on the same individual (Campos et al. 2006). This is because plant growth leads to gradual increase in the number of microhabitats for nesting sites (Djieto-Lordon et al. 2004), and in the abundance of food resources: liquids secretions (nectaries and honeydew-producing hemipterans) (Blüthgen et al. 2000; Davidson et al. 2003) and herbivore insects (important resource for arboreal predatory ants) (Floren et al. 2002). Therefore still remains uncertain about the true role of species-area relationships into ecological ant-plant networks, and future studies should evaluate what is the effect of plant height on ant-plant networks in structurally more complex environments.

In summary, we demonstrated that our individual-based ant-plant networks exhibit similar patterns of interactions when compared with ant-plant networks studied at the community level. Our data suggest that ant-plant interactions are much more dynamic than expected a priori, since, ant species can change their role in network structure between diurnal and nocturnal period. Finally, we observed that increasing the plant height does not cause an increase in ant richness foraging on such plants. Clearly, our study contributes to a better understanding about the importance of the sampling period and species-area relationship structuring ant-plant networks. However, there is still much to do to acquire a better knowledge of patterns and processes related to intrapopulation ant-plant networks, mainly the true role of ant dominance hierarchy and availability of food resources in such networks.

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Tables

Table 1. Networks descriptors for individual-based ant-plant networks involving three plant species: Chamaecrista mucronata (Leguminosae-Caesalpinioideae), Stachytarpheta glabra (Verbenaceae), and Qualea grandiflora (Vochysiaceae). Sampling of ant-plant interactions were performed in the Brazilian Neotropical Savanna (see text for more information).

	Day	**Night**	**Whole-Day**
Chamaecrista mucronata			
Plant individuals sampled	24	26	26
Mean height	2.41 ± 0.78	2.32 ± 0.82	2.32 ± 0.82
Ant richness	11	11	13
Exclusive ant species	1	1	-
Mean of ant richness per individual	2.12 ± 1.03	2.03 ± 1.08	3.14 ± 1.61
Ant species in the generalist core	2	1	3
Network specialization	0.519	0.499	0.409
Nestedness[a]	46.93	46.29	48.42
Modularity[b]	0.388	0.359	0.293
Stachytarpheta glabra			
Plant individuals sampled	41	41	41
Mean height	2.71 ± 1.73	2.82 ± 1.71	2.82 ± 1.71
Ant richness	9	13	13
Exclusive ant species	1	5	-
Mean of ant richness per individual	1.85 ± 0.89	2.21 ± 1.51	3.02 ± 1.35
Ant species in the generalist core	2	2	3
Network specialization	0.494	0.518	0.401
Nestedness[a]	58.62	43.29	54.75
Modularity[b]	0.319	0.394	0.301
Qualea grandiflora			
Plant individuals sampled	31	30	31
Mean height	1.64 ± 0.66	1.60 ± 0.38	1.64 ± 0.66
Ant richness	13	15	15
Exclusive ant species	0	2	-
Mean of ant richness per individual	1.87 ± 0.84	2.22 ± 1.11	2.54 ± 0.99
Ant species in the generalist core	2	1	2
Network specialization	0.509	0.499	0.424
Nestedness[a]	58.62	27.84	30.16
Modularity[b]	0.434	0.421	0.391

[a] All ant-plant networks were significantly nested ($P < 0.05$)

[b] No ant-plant networks showed a modular pattern of interaction ($P > 0.05$).

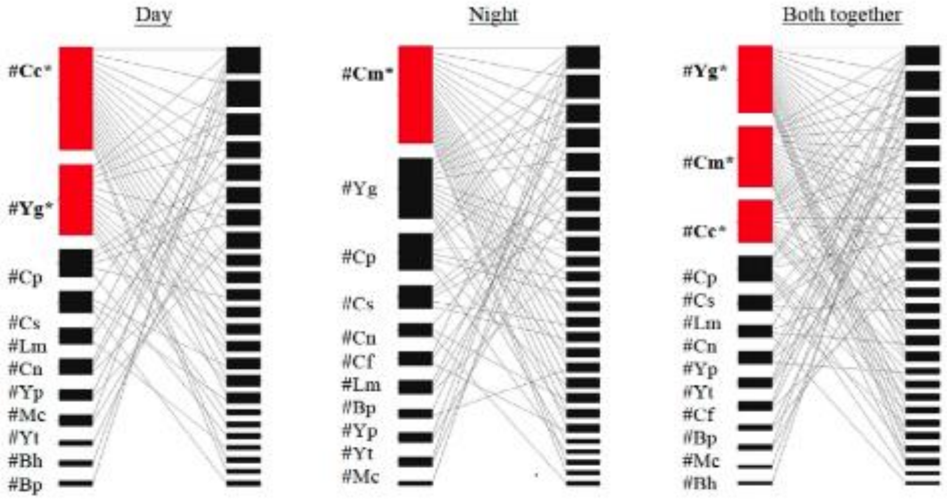
List of Figures

Figure 1. Individual ant-plant networks sampled in the Brazilian Neotropical Savanna involving for three plant species: (A) Chamaecrista mucronata (Leguminosae-Caesalpinioideae), (B) Stachytarpheta glabra (Verbenaceae), and (C) Qualea grandiflora (Vochysiaceae). For each plant species we built ant-plant networks using diurnal, nocturnal, and both together sampling. Within each network, node represents one ant species (left) or plant individual (right), and lines represent ant-plant interactions. The nodes are arranged according their position in the nestedness ranking. (*) Indicates those ant species that were present in the generalist core. Species codes are in Appendix 1.

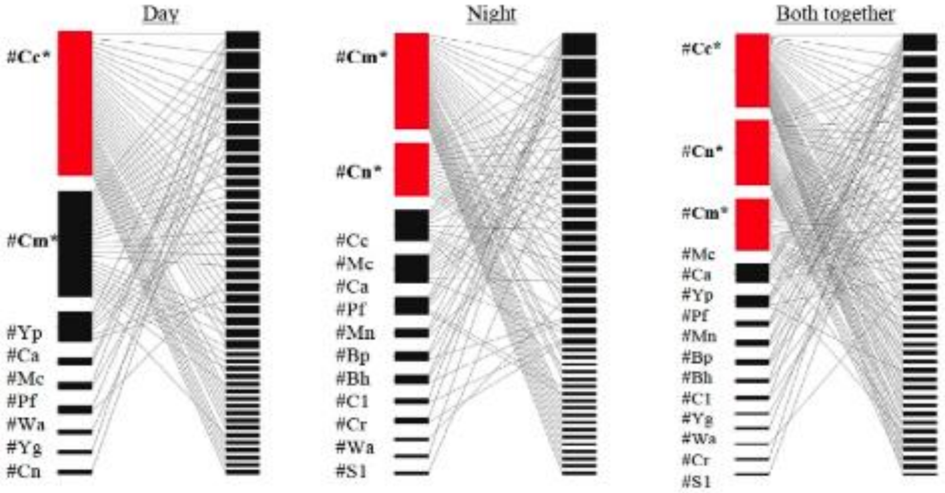
Figures

Figure 1

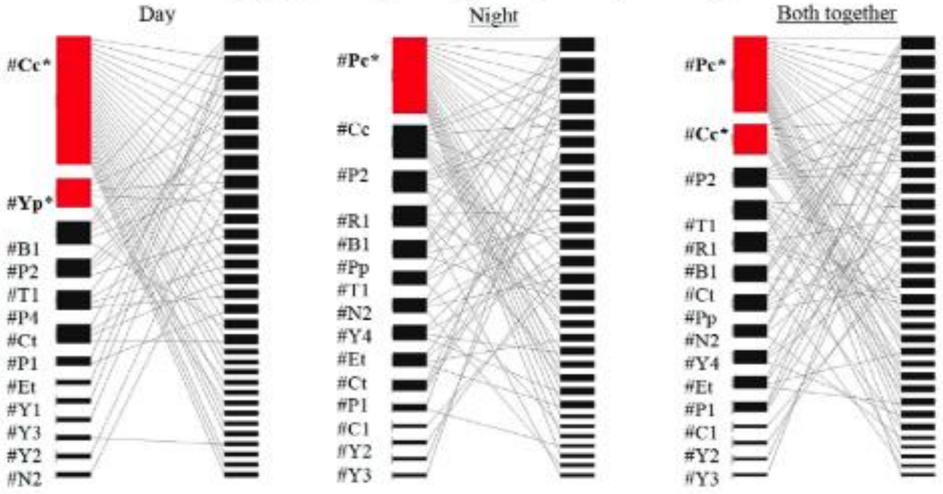
(A) *Chamaecrista mucronata* (Leguminosae)



(B) *Stachytarpheta glabra* (Verbenaceae)



(C) *Qualea grandiflora* (Vochysiaceae)

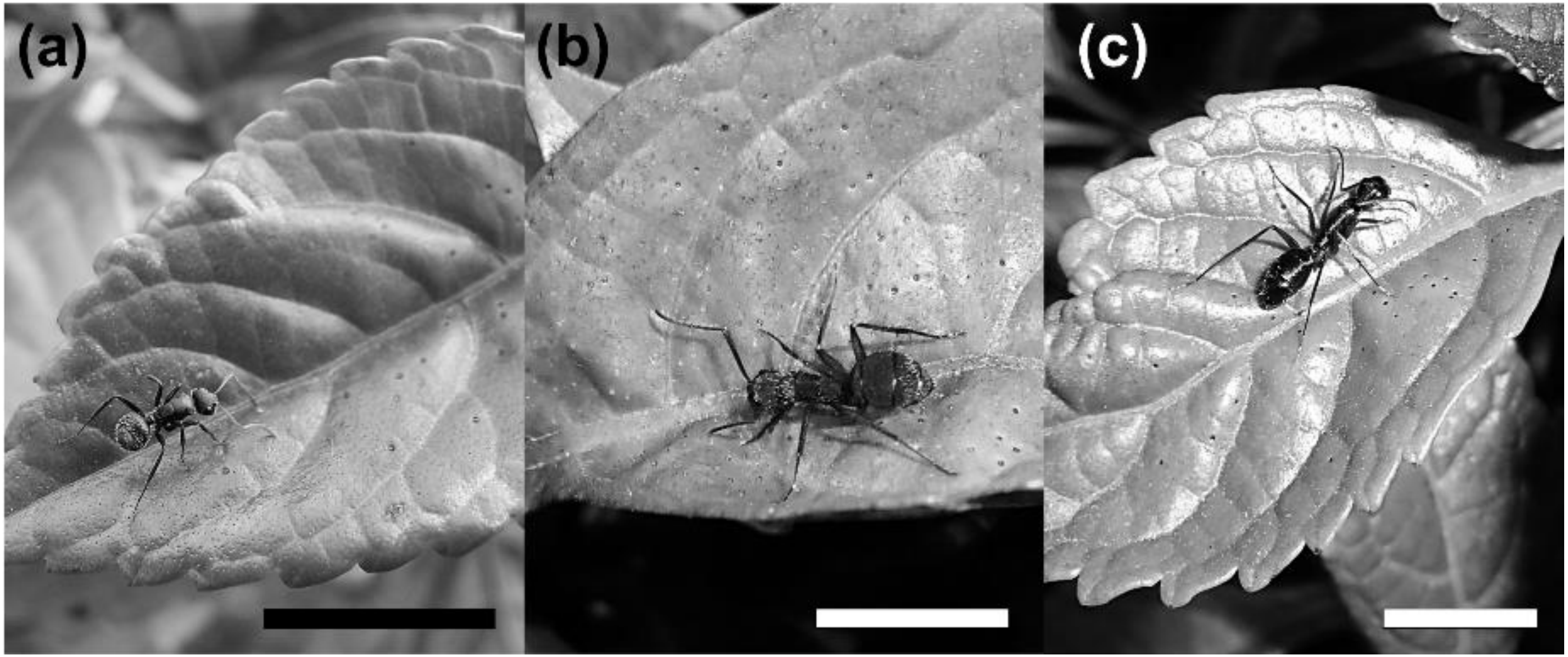


Appendices

Appendix 1. Species codes of ants recorded forraging on individuals of Chamaecrista mucronata (Leguminosae-Caesalpinioideae), Stachytarpheta glabra (Verbenaceae), and Qualea grandiflora (Vochysiaceae) in the Brazilian Neotropical Savanna. Please see Figure 1 and text for more information.

Subfamily	Ant species	Species code
Formicinae	*Brachymyrmex* sp1	B1
Formicinae	*Brachymyrmex heeri*	Bh
Formicinae	*Brachymyrmex pictus*	Bp
Formicinae	*Camponotus atriceps*	Ca
Formicinae	*Camponotus crassus*	Cc
Formicinae	*Camponotus melanoticus*	Cm
Formicinae	*Camponotus novogranadensis*	Cn
Formicinae	*Camponotus renggeri*	Cr
Formicinae	*Camponotus rufipes*	Cf
Formicinae	*Camponotus senex*	Cs
Formicinae	*Camponotus* sp1	C1
Myrmicinae	*Cephalotes atratus*	Ct
Myrmicinae	*Cephalotes pusillus*	Cp
Myrmicinae	*Cephalotes* sp1	C1
Myrmicinae	*Crematogaster* sp1	R1
Ectatomminae	*Ectatomma tuberculatum*	Et
Dolichoderinae	*Linepithema micans*	Lm
Formicinae	*Myrmelachista catarinae*	Mc
Formicinae	*Myrmelachista nodigera*	Mn
Paraponerinae	*Paraponera clavata*	Pc
Myrmicinae	*Pheidole fallax*	Pf
Myrmicinae	*Pheidole* sp1	P1
Myrmicinae	*Pheidole* sp2	P2
Pseudomyrmecinae	*Pseudomyrmex gracillis*	Yg
Pseudomyrmecinae	*Pseudomyrmex pallidus*	Yp
Pseudomyrmecinae	*Pseudomyrmex* sp1	Y1
Pseudomyrmecinae	*Pseudomyrmex* sp2	Y2
Pseudomyrmecinae	*Pseudomyrmex* sp3	Y3
Pseudomyrmecinae	*Pseudomyrmex* sp4	Y4
Pseudomyrmecinae	*Pseudomyrmex termitarius*	Yt
Myrmicinae	*Solenopsis* sp1	S1
Myrmicinae	*Wasmannia auropunctata*	Wa

Appendix 2. Ants foraging nectar in Stachytarpheta glabra at both daily periods. (a) Camponotus crassus foraging at day. (b) Camponotus rufipes foraging at night. (c) Camponotus melanoticus foraging at night. Scale bar: 1cm. Images: Fagundes R (2014).



Conclusão Geral

Conclusão geral

O estudo da ecologia de interações recebeu grande atenção nos últimos anos com o advento da aplicação teoria de redes complexas para descrever padrões de interações ao nível de comunidade (Bascompte et al. 2003; Bascompte and Jordano 2007)). Nesse contexto, descreveu-se padrões de interação em mutualismo (Bascompte et al. 2003), parasitismo (Lima et al. 2012), simbiose (Dáttilo et al 2013) e predação (Dunne et al. 2002). Por exemplo, sabe-se que redes interação formiga-planta são geralmente formadas por muitas espécies, que interagem generalizadamente, de forma assimétrica e com padrão de interação aninhado (Bascompte et al. 2003). Esse padrão foi demonstrado para diversos ecossistemas e nosso estudo contribui com mais uma evidência ao apresentar a rede de interações entre formigas e plantas produtoras de néctar extrafloral em Campo Rupestre (Capítulo 1). Mais ainda, nós demonstramos que a rede de interação entre formigas e hemípteros produtores de *honeydew* (excreção açucarada) não segue o padrão esperado para mutualismos (Capítulo 1), apesar dessa interação constituir um equivalente ecológico às interações formiga-planta pois ambos se baseiam em relações recurso-consumidor (Del-Claro et al. 2004). Mostramos que a rede formiga-planta é também estruturada pela oferta do recurso (Capítulo 1 e 2), pois espécies de planta com maior quantidade de nectários apresentaram mais interações. Porém, hemípteros com grandes agregações não acumularam muitas interações com formigas, pelo contrário, uma só espécie dominou os hemípteros e reduziu o número de interações à uma ou duas espécies (Capítulo 1). Portanto, recursos mais limitados e mais energéticos, logo mais vantajosos, reduziram a co-ocorrência das espécies (Capítulo 1) possivelmente por aumentarem a disputa pelo recurso como mostrado no Capítulo 3. Esses resultados juntos sugerem que as interações formiga-planta baseado em troca de alimento por proteção poderiam ser, ecologicamente falando, relações recurso-consumidor e portanto seguiriam os mecanismos que regem essa relação: disponibilidade do alimento, competição por alimento e partição de alimento entre espécies consumidoras.

Até então pouco se sabe sobre os mecanismos que estruturam as redes e regem sua dinâmica espacial e temporal (Bascompte et al. 2003; Chamberlain and Holland 2009). Nós seguimos os mecanismos de relação recurso-consumidor como hipótese e mostramos que a disponibilidade e qualidade do recurso determinam o padrão de interação apresentado pelas plantas (Capítulo 2). Ou seja, plantas que produzem maior quantidade de néctar e, principalmente, cujo néctar é altamente concentrado em açucares, apresentam maior número de interações com formigas (Capítulo 2). Essa é uma importante evidência de que adaptações à produção de néctar mais atrativo podem ser importantes para o sucesso e a estabilidade das interações formiga-planta. Essa suposição foi comprovada quando mostramos que plantas que produzem néctar de melhor qualidade, e consequentemente interagem mais, apresentam menos perda de área foliar comparado a plantas com poucas interações (Capítulo 2). Assim, ao nível trófico das plantas, a quantidade de interações é determinada pela qualidade do recurso (Capítulo 2), logo, comprovando parcialmente o mecanismo baseado em relação recurso-consumidor.

Nosso objetivo principal foi finalmente alcançado através dos resultados apresentados no Capítulo 3, quando demonstramos que as interações realizadas pelas formigas eram determinadas por hierarquias de dominância do recurso. Encontramos relações de agressividade entre as espécies de formigas em interação com plantas que definiram a frequência das interações entre. Esses resultados são evidências empíricas de que mecanismos competitivos podem também estruturar redes formiga-planta. Considerando o padrão aninhado previsto para esse tipo de mutualismo (Bascompte et al 2003), esse mecanismo é plausível e já vem sendo destacado na literatura, mas até então não foi testado empiricamente. A dominância de secreções açucaradas de plantas é comum em vários ecossistemas (Blüthgen and Stork 2007), incluindo o Cerrado (Del-Claro 2004), e desempenha um importante papel na estruturação da assembleia de formigas através de territorialidade e repelência interespecífica (Bluthgen et al 2000). Portanto, nossos resultados trazem importantes evidências que suportam a teoria dos mecanismos competitivos estruturando mutualismos baseados no consumo de alimento, ou seja, suporta a teoria de competição por mutualismos (Cushman and Whitham 1991).

Nós demonstramos ainda no Capítulo 3 que as hierarquias de dominância são dinâmicas e dependentes da qualidade do recurso ofertado, ou seja, da espécie de planta interagida. Esses são indícios de que mutualismos são contexto-dependentes (Chamberlain and Holland 2009) A hierarquia se tornou mais definida, com mais agressividade interespecífica e níveis de dominância, em plantas que produziram néctar mais concentrado em açucares. Esse resultado confirma a hipótese que a competição deriva da qualidade do recurso, ou seja, o quão ele é vantajoso energeticamente. Essa competitividade cria um filtro que determina quantas interações cada espécie de formiga fará e consequentemente quantas interações cada planta realizará. Esse filtro explicaria ligações proibidas em redes de interação formiga planta quando a espécie compartilha o mesmo ambiente no tempo e espaço mas não interage com a mesma espécie parceira. Assim podemos afirmar, conjuntamente ao resultado do Capítulo 2 que mostra o benefício do mutualismo condicionado a agressividade da formiga interagida, que uma hierarquia bem definida é determinante para a estabilidade do mutualismo, mais do que o somatório das espécies interagentes. Assim, plantas com melhor néctar geram maior competição por recurso que determinam quais espécies de formigas poderão coletar néctar (interações) e selecionam as espécies mais agressivas que conferem maior proteção (mutualismos). Diferimos portanto os conceitos de interação interespecífica e mutualismo propriamente dito.

Considerado em conjunto, nossos resultados mostram que a disputa por recursos tem papel fundamental na formação das interações. As formigas apresentaram alta sobreposição de interações, ou seja, sobreposição de nicho no que tange o uso de diferentes tipos de néctar. Mas, surpreendentemente houve alta coexistência, mesmo muitas delas especializadas no consumo dos mesmos líquidos açucarados (Davidson et al. 2004). Isso se deu por partição espacial e temporal do recurso. Partição espacial, porque apesar da coexistência cada uma acessou diferente quantidade de NEFs dependendo da espécie de planta (Capítulos 2 e 3) por subir em diferentes arbustos ou por agir submissamente em alguns arbustos e dominantemente em outros (Capítulos 1 e 3). Partição temporal, porque espécies altamente interagentes (núcleo da rede; Capítulos 2 e 3) e comportamental e numericamente dominantes (Capítulo 3) se alternaram nas interações entre dia e noite sem causar perturbação na estrutura da rede (Capítulo 5). Esses mecanismos, associados à fenologia das plantas e formigas (Lange et al. 2013; Santos et al. 2014), podem ser importantes mecanismos geradores da alta coexistência de formigas na vegetação e consequentemente da alta generalização e sobreposição de interações na rede. Além disso, os resultados do Capítulo 5 chamam a atenção sobre a importância dos estudos de interações noturnas para reduzir a sub-amostragem das interações e esclarecer ligações dadas como proibidas. Além disso, o Capítulo 5 apresenta uma abordagem de interações a nível intraespecífico, o que é relevante do ponto de vista evolutivo porque processos de co-evolução e especialização acontecem a nível de indivíduo. Portanto, padrões observados a nível de comunidades podem ser explicados por mecanismos a nível populacional.

Apesar da importância da agressividade para o sucesso do mutualismo formiga-planta, nós mostramos também que nem todas as formigas são agressivas e mesmo assim coletam néctar por acessar a planta quando a espécie dominante está em baixo número ou quando são toleradas por elas (Capítulo 3). Essa alta quantidade de interações com espécies submissas que ocorrem em alguns indivíduos de cada espécie de planta, ou em períodos específicos, mas sempre em forrageio reduzido, pode ser a explicação do alto generalismo encontrado em interações formiga-planta. Quando espécies de formigas ocorrem de forma esporádica em diferentes arbustos dentro das populações de cada espécie de planta, ao somar as interações de cada espécie de planta resulta-se em uma ou poucas espécies de formigas muito interagentes (as dominantes) e muitas espécies raramente interagentes (as submissas). Mostramos que apesar das espécies dominantes serem altamente interagentes o contrário não ocorre, algumas espécies conseguem se posicionar no núcleo generalista da rede ao se comportar submissamente ou dominar poucos arbustos de cada espécie de planta (Capítulo 3). O mesmo é verdade para plantas que interagem pouco mas sofrem baixa herbivoria (Capítulo 2), e portanto devem depender de outros mecanismos de defesa que não mirmecofílicas. Assim, outros mecanismos ao nível de espécies podem ser importantes para determinar a estrutura da rede de interações e futuros estudos comportamentais e de história natural a nível específico de ambos os níveis tróficos podem ajudar a esclarecer esses mecanismos.

As consequências das interações generalizadas e repletas de espécies pouco agressivas, logo pouco protetoras, foi explorada no Capítulo 4. Reforçamos a ideia de que as espécies mais agressivas são as espécies verdadeiramente protetoras (Capítulo 3). Mais importante, demonstramos que os mutualismos formiga-planta possuem alto grau de exploração, ou seja, formigas que coletam néctar mas não defendem (Capítulo 4). Essa exploração causa desestabilidade do mutualismo, pois espécies exploradoras roubam néctar de espécies protetoras e reduzem a frequências dessas nas plantas podendo prejudicar os benefícios do mutualismo (Bronstein 2001). Porém, fomos capazes de demonstrar que o grau de exploração nesses mutualismos é reduzido quando a planta produz néctar de melhor qualidade. Portanto, em conjunto com resultados dos Capítulos 2 e 3, podemos inferir que a competição por recurso de alta qualidade leva a seleção de espécies dominantes altamente agressivas que excluem às espécies exploradoras das plantas por territorialidade e aumentam a estabilidade do sistema. Nós observamos que, para cada espécie de planta, a espécie de formiga mais protetora (Capítulo 4) é a espécie mais agressiva (Capítulo 2), que mais recruta e ocupa arbustos da planta (Capítulo 2 e 5) e é a espécie de topo na hierarquia de dominância (Capítulo 3). Como essas espécies competitivamente dominantes são as verdadeiras protetoras e interagem com todas as espécies de plantas (núcleo da rede), elas garantem a proteção das plantas e consequentemente a estabilidade da rede. É importante notar que essa proteção depende da espécie de planta e da qualidade do recurso ofertado, sendo que plantas que produzem néctar de baixa qualidade interagem basicamente com espécies de baixa eficiência de proteção. Esses resultados trazem importantes contribuições para o entendimento da importância das formigas como mecanismo defesa da planta e ajuda a explicar a alta variação espacial e temporal nos benefícios recebidos pela planta, e mais importante, são evidências de um mecanismo ecológico-evolutivo para interações mutualistas.

Em suma, esse estudo trouxe evidências empíricas que contribuem para entendimento da ecologia das interações mutualísticas entre formigas e plantas portadoras de NEFs. Não só descrevemos as interações e testamos o poder de predição dos padrões descritos na literatura, como também testamos mecanismos ecológicos e comportamentais que podem ajudar a explicar como as relações entre as espécies são estruturadas, mantidas e evoluem. A abundância das espécies, a frequência de a distribuição espacial e temporal e as relações filogenéticas já são considerados importantes mecanismos estruturadores das interações mutualistas formiga-planta ocorrência (Vázquez et al 2009 and referencies). A partir desse estudo, soma-se as relações competitivas como mais um importante mecanismo, ou seja, competição por mutualismos. Além disso, nós transpomos a barreira que considera interação a partir do contato físico entre as espécies e demonstramos que as consequências desse contato podem variar entre espécies parceiras e, mais importante, determinar a efetividade da interação. Portanto, nosso estudo demonstra a importância da experimentação, do comportamento animal e da história natural como ferramentas no estudo de ecologia de interações. Esperamos que futuros estudos possam investigar mais a fundo as relações interespecíficas e enfocar a importância da identidade e história ecológico-evolutiva das espécies.

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