ECOLOGY, BEHAVIOR AND BIONOMICS

Common-Garden Experiments Reveal Geographical Variation in the Interaction Among *Crotalaria pallida* (Leguminosae: Papilionideae), *Utetheisa ornatrix* L. (Lepidoptera: Arctiidae), and Extrafloral Nectary Visiting Ants

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Abstract

The study of geographical variation is a key approach to understand evolution of ecological interactions. We investigated geographical variation in the interaction among *Crotalaria pallida* (Leguminosae: Papilionideae), its specialized herbivore, *Utetheisa ornatrix* L. (Lepidoptera: Arctiidae), and ants attracted to extrafloral nectaries (EFNs). First, we used common-garden experiments with plants collected in different sites at different geographical scales to test for differences among populations in *C. pallida* attractiveness to ants. When we compared three populations from Southeast Brazil (150 km apart), the number of visiting ants per plant, and the percent of termite baits attacked by ants, were significantly different among plant populations. In a comparison of populations from SE Brazil and Florida (USA), there was no significant difference between the populations in the number of ants per plant or the frequency of baits attacked. Second, we tested in a common garden if *U. ornatrix* larvae present any behavior to avoid ant predation, and if there were genetic differences among populations. We observed that most larvae moved away from the vicinity of the EFNs (flowers and fruits) to the plant leaves. Of the larvae that moved to leaves, only 10% were attacked by ants while 89% of larvae that stayed near the fruit/flower were attacked. There was a significant difference among populations in the frequency of larvae that moved to the leaves and the frequency of larvae attacked by ants. We discuss the possible causes of the geographical differences observed and propose future research directions in this system.

Introduction

In several systems, the interaction among plants and herbivores is influenced by extrafloral nectaries (EFNs). EFNs are anatomically variable sugar-producing plant structures not directly related to pollination (Koptur 1992, Heil & McKey 2003). Ants attracted to EFNs usually decrease herbivory and increase plant fitness (Del-Claro et al 1996,

Oliveira et al 1999, Rudgers & Gardener 2004, Del-Claro & Torezan-Silingard 2009, Rosumek et al 2009; for an exception, see Nogueira et al 2012). This type of facultative interaction with EFN plants can also directly increase ant colony fitness (Byk & Del-Claro 2011). EFNs occur in more than 90 families of angiosperms and have evolved independently many times (Koptur 1992). In many groups, the presence of EFNs varies even among closely related species

(for example, species in the genera *Ipomea* and *Crotalaria*) (Keeler & Kaul 1984, Flores 2004). A key approach to understand this variation and to untangle the selective pressures responsible for the evolution of EFNs is to look at geographical variation (Rudgers & Strauss 2004, Rios *et al* 2008, Agrawal 2011). Geographical variation in the outcome of ant–plant systems mediated by EFNs may result from differences in the species composition of the associated ant community, variable herbivore pressure, variable ant density on foliage, or variable abiotic factors (Bentley 1976, Horvitz & Schemske 1984, Barton 1986, Smiley 1986, De la Fuente & Marquis 1999, Cogni *et al* 2003, Rudgers & Strauss 2004, Kersch & Fonseca 2005). However, few studies have tested if these differences are genetically based or just plastic responses to differences in the environment (for example, Rios *et al* 2008). These genetic based geographical differences are also important because they are a key prediction of coevolutionary theories (Thompson 2005).

Another key component to understand the evolution of EFNs is the investigation of the ability of herbivores to overcome ant predation. Many herbivores feeding on ant-visited plants possess morphological and behavioral adaptations to deal with frequent encounters with ants on foliage (Heads & Lawton 1985, Freitas & Oliveira 1996, Salazar & Whitman 2001, Sendoya *et al* 2009). In addition, the behavior of the larvae in response to ant attack may determine the outcome of the interaction. For example, larvae jumping from plants to avoid ants may result in less plant herbivory even if the ants are not directly preying them (Rudgers *et al* 2003).

An ideal system to address geographical variation in EFNs and behavioral responses of the herbivore is the interaction between *Crotalaria pallida* and *Utetheisa ornatrix* L. Previous studies in this system have addressed chemical and behavioral aspects of the sequestration of plant defensive pyrrolizidine alkaloids by this specialist herbivore (reviewed by Eisner & Meinwald 1995). More recently, studies have addressed the role of *C. pallida* EFNs in attracting defensive ants (Ferro *et al* 2006, Guimarães *et al* 2006), and geographical variation and local adaptation in the interaction between the plant and the herbivore (Cogni & Futuyma 2009, Cogni *et al* 2011). Here, we introduce a novel approach in the system that combines aspects of geographical variation and differences mediated by EFNs and the attracted ants. We looked at the interaction among *C. pallida*, *U. ornatrix*, and EFN-visiting ants in a geographical context. Specifically, using a common-garden approach, we asked the following questions: (1) Are there genetic differences among populations in *C. pallida* attractiveness to ants and the frequency of ant attack to simulated herbivores? (2) Does the difference depend on the geographical scale? (3) Do *U. ornatrix* larvae

present any behavior to avoid ant predation? (4) Are there genetic differences among *U. ornatrix* populations in the behavior to avoid ant predation?

Materials and Methods

Study system and populations studied

Crotalaria pallida is an annual plant that might have been introduced in the New World from Africa (possibly around 500 years ago) (Cogni & Futuyma 2009) but the current pantropical distribution is obscured by widespread cultivation (Polhill 1982). In the Neotropics, it occurs at high densities from southern Brazil to the southeastern USA (Flores 2004, Cogni & Futuyma 2009). The generalist pod-borer *Etiella zinckenella* (Treitschke; Lepidoptera: Pyralidae), and the specialist *U. ornatrix* are the two main natural enemies of *Crotalaria* plants in the Neotropics (Cogni 2010, Cogni *et al* 2011). *Utetheisa ornatrix* uses *C. pallida* as its main host in many locations (Ferro *et al* 2006, Eisner & Meinwald 1995, Cogni *et al* 2011). Because the larvae prey on the seeds, and up to 20% of *C. pallida* fruits may be damaged in the field, *U. ornatrix* can have a significant impact on the fitness of *Crotalaria* plants (Ferro 2001, Ferro *et al* 2006, Cogni *et al* 2011). *Crotalaria pallida* has EFNs located at the base of the pedicel (Díaz-Castelazo *et al* 2005, Melo *et al* 2010a, 2010b). The EFNs remain active from the early development of flowers to formation of mature fruits and the ants constantly patrol the flowers and fruit pods and expel *U. ornatrix* larvae that are outside the fruit (Ferro *et al* 2006, Guimarães *et al* 2006). The EFNs can also attract predaceous wasps that prey upon *U. ornatrix* larvae (J. R. Trigo, personal observation). Another major defense trait in *Crotalaria* plants is the constitutive presence of pyrrolizidine alkaloids (PAs), which have deterrent and toxic effects on generalist herbivores (Macel 2010, Trigo 2010). The specialist *U. ornatrix* is able to sequester PAs from *Crotalaria* plants; sequestered PAs protect all developmental stages against predators and are modified by males into a courtship pheromone (Eisner & Meinwald 1995, Conner 2009, Cogni *et al* 2012, Hoina *et al* 2012). Previous studies in the system have shown that local adaptation of *U. ornatrix* to *C. pallida* depends on the geographical scale (Cogni & Futuyma 2009), and that *C. pallida* resistance traits vary spatially (Cogni *et al* 2011).

We collected *C. pallida* seeds and *U. ornatrix* adults in three sites in the state of São Paulo, Southeastern Brazil, and in one site in Central Florida, USA, as previously described (Cogni & Futuyma 2009, Cogni *et al* 2011). These sites will be referred as CAM for Campinas-SP (22°45′12″S, 47°03′20″W), BOT for Botucatu-SP (22°46′45″S, 48°24′14″W), JUQ for Juquiá-SP (24°19′55″S, 47°38′15″W), and FL for

Archbold Biological Station in Central Florida (27°11′13″N, 81°20′19″W).

Ant visitation experiments

Plants from each site were grown in a greenhouse as in Cogni & Futuyma (2009) and used in two common-garden experiments. The localities of both common-garden experiments, the Mata de Santa Genebra and the Zoológico de Mogi Mirim, did not have naturally grown *Crotalaria* plants, and were relatively near the site where the CAM population was collected.

Regional scale. In the first experiment, we studied the three Brazilian populations. Forty-two plants from each of the three sites were transferred from the greenhouse to the borders of the forest fragment of Mata de Santa Genebra in Campinas-SP (22°49′38.91″S, 47°06′08.21″W), in a common-garden design. We placed 42 blocks of three plants each at 10-m intervals; each block had one plant from each of the three sites. Plants were 30 cm from each other within blocks. Plants were transferred on 15 October 2005, and the experiments were carried out on 25–27 October. Plants in the same block were similar in size, phenological stage (flowers or young fruits), and number of unripe pods. For each plant, the total number of ants per plant was counted during a 30-s interval. All observations were performed in the morning (between 09:00 and 12:00); therefore, only diurnal ant species were sampled. To estimate ant aggressiveness, a termite worker (*Nasutitermes* sp.) was glued on a reproductive stem of each plant and observed if it was attacked by ants during 10 min (as in Cogni *et al* 2003). The time until the attack was also recorded. The variables were compared among localities using the nonparametric randomized block Friedman test for the number of ants visiting plants, a χ^2 test for the frequency of termites attacked by ants, and the nonparametric Kruskal–Wallis test for the time an ant took to attack a termite bait. The relative frequency of ant species was compared among plant origin sites by a χ^2 test, after excluding *Crematogaster* sp. that was only observed twice.

Continental scale. In a second experiment (February and March 2009), we studied plants from one site from Brazil (CAM) and the Florida site. Twenty-five plants from each site were transferred from a common garden to a grass field at the Zoológico Municipal de Mogi Mirim "Luiz Gonzaga Amoêdo Campos" in Mogi Mirim-SP (22°26′45.91″S, 46°57′17.7″W). The same procedures described above were used, except that the ant counts were repeated five times on alternate days, and the results are presented as averages. The mean number of visiting ants for each original site was compared by a paired *t* test. The frequency of termites attacked by ants was compared by a χ^2 test. The relative frequency of ant species was compared between plant origin sites by a χ^2 test, after excluding species with an average frequency of less than one.

Larval behavior experiments

Another set of experiments, also using a common-garden design, was carried out to test inter population differences in *U. ornatrix* larvae behavior to avoid ant predation. These experiments used only larvae from the three Brazilian populations (regional scale). We did not studied differences in larvae behavior between the sites from Brazil and Florida (continental scale) because we did not have permits to carry out experiments with imported larvae in the field. Moth colonies from each of the three Brazilian sites were established from more than 40 adults collected in the field, and kept in the laboratory with more than 25 adults at any single time. We fed larvae from all sites on fresh green *C. pallida* seeds. Fresh seeds, from plants grown in a greenhouse from seeds collected in a field site in Itatiba-SP, were provided every other day. Third instars from each site were randomly placed on the same block of plants transferred to the field in 2005, and observed for 10 min. Each larva was placed on a reproductive stem near the flowers or fruits (the plant part where active EFNs occur). We recorded the number of larvae that moved to a different location on the plant, the number that were attacked by ants, and the larva response to ant attack. Forty-two larvae from each site were used. We carried out control experiments placing the larvae on the leaves instead of flowers/fruits, and more than 95% of larvae stayed on the leaves and were not attacked by ants (data not shown). The frequencies of each behavioral category were compared among sites by a χ^2 test.

Results

Ant visitation experiments

Regional scale. In the experiment comparing plants originally from the three Brazilian sites, the number of ants per plant was significantly different among plant populations (Table 1; Friedman test, $\chi_r^2 = 12.636$; $df = 2$; $P = 0.002$). Attacks to termite baits by ants were significant different among populations (Table 1; $\chi^2 = 8.320$; $df = 2$; $P = 0.016$). The time an ant took to attack a termite bait did not differ among plants from the three sites (Table 1; Kruskal–Wallis test, $H = 1.019$; $P = 0.601$). Four ant species were observed

Table 1 Ant visitation and predation behavior in *Crotalaria pallida* plants originally from three sites from the state of São Paulo, SE Brazil, in a common garden.

	BOT (*n*=42)	CAM (*n*=42)	JUQ (*n*=42)
Median number of ants (1st–3rd quartiles)	0 (0–1)	1 (0–1)	1 (0–2)
Frequency of ants			
Brachymyrmex sp.	2	4	2
Camponotus sp.	6	10	13
Crematogaster sp.	1	0	1
Pheidole sp.	8	8	8
Proportion of termites attacked	0.26	0.40	0.57
Median time to attack termites (s; 1st–3rd quartiles)	57 (35–122)	43 (10–173)	47 (21–80)

The frequency of each ant species is represented by the number of plants from each population visited by each ant species. For statistics, see "Results".

BOT Botucatu-SP, *CAM* Campinas-SP, *JUQ* Juquiá-SP.

visiting the EFNs of *C. pallida*; the relative frequency of species was similar among plants from each of the three sites (Table 1; χ^2=2.16; *df*=4; *P*=0.706). All four species attacked termite baits and *U. ornatrix* larvae.

Continental scale. In the experiment comparing plants originally from Florida and Brazil, the number of ants per plant did not differ between populations (Table 2; *t*=0.099; *df*=24; *P*=0.922). The percent of termites attacked by ants did not differ between populations either (Table 2; χ^2= 0.00; *df*=2; *P*>0.999). Eight ant species were observed visiting the EFNs of *C. pallida*; the relative frequency of species was similar among plants from the two sites (Table 2; χ^2=0.21; *df*=4; *P*=0.995).

Table 2 Ant visitation and predation behavior in *Crotalaria pallida* plants originally from Central Florida (FL) and Campinas-SP, SE Brazil (CAM), in a common garden.

	CAM (*n*=25)	FL (*n*=25)
Mean number of ants±SD	1.42±2.92	1.55±2.26
Frequency of ants		
Brachymyrmex sp.	5.4	6.8
Camponotus sp.1	8.8	9.8
Camponotus sp.2	10	10
Camponotus sp.3	0.4	0.4
Crematogaster sp.	0.8	0
Pheidole sp.1	0	0.6
Pheidole sp.2	0	0.2
Pseudomyrmex sp.	0	0.2
Proportion of termites attacked	0.64	0.64
Median time to attack termites (s; 1st–3rd quartiles)	180 (80–304)	120 (60–172)

The frequency of each ant species is represented by the number of plants from each population visited by each ant species, averaged over 5 days of sampling. For statistics, see "Results".

Larval behavior experiments

When an *U. ornatrix* larva was placed on the reproductive stems of a *C. pallida* plant, the larva stayed near the flower or fruit, or it rapidly moved down the plant until it found a leaf where the larva stayed. There was a significant difference among the sites on the frequency of larvae that moved to the leaves or stayed on the reproductive stem (Fig 1a; χ^2=13.62; *df*=2; *P*=0.001). The behavior of the larvae greatly influenced the chance of being attacked by ants: while just 10% of larvae that moved to leaves were attacked, 89% of those that stayed near fruit/flower were attacked (Fig 1b; χ^2=81.93; *df*=1; *P*<0.001). These patterns resulted in differences in the frequency of larvae attacked by ants among sites (Fig 1c; χ^2=14.0; *df*=2; *P*<0.001). During an ant attack, most of the larvae jumped from the plant as soon as they were detected and first touched by ants. This behavior was effective in most of the attacks. The percentage of larvae that survived an attack also varied among sites (Fig 1d; χ^2=10.35; *df*=2; *P*=0.006).

Discussion

At the regional scale (populations from the state of São Paulo), our results clearly show differences in the number of ants visiting plants from the different sites, and that these differences resulted in differences in the frequency of ant attack to simulated herbivores. These differences may be the result of variation among populations in the number of EFNs per plant, the volume of nectar produced, and/or the nutritional value of the nectar. Since the experiment was performed in a common garden with plants grown from seeds, we can infer that these differences are genetic based and not plastic response to differences in the environment. Other *C. pallida* defense traits, such as
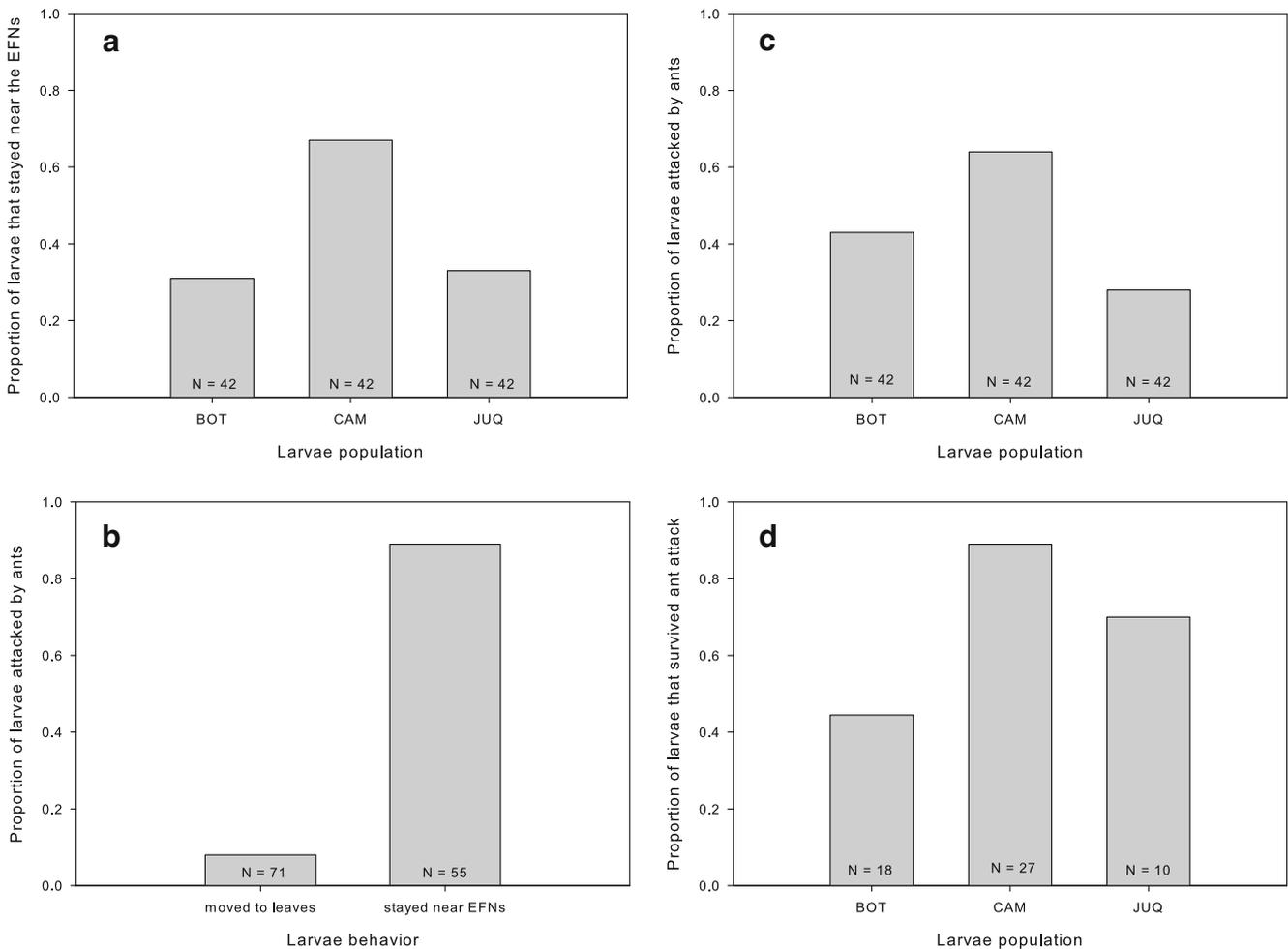
Fig 1 Interpopulation differences in *Utetheisa ornatrix* behavioral response to ant predation. **a** Proportion of larvae that stayed near the EFNs, **b** proportion of larvae that were attacked by ants dependent on plant position, **c** proportion of larvae attacked by ants, and **d** proportion of larvae that survived an ant attack. *Values inside each bar* represent sample size. For statistics, see "Results".

the concentration of PAs, also showed genetic variation among the same sites (Cogni *et al* 2011). In the future, a more complete test of the influence of genetics and plasticity of plants on the attractiveness of the available ant community can be performed by experiments replicated in all the origin sites by reciprocal transplant. We did not find any difference at the continental scale comparison (populations from São Paulo and Florida), which might indicate that the variation depends on the geographical scale (see also, Cogni & Futuyma 2009, Cogni *et al* 2011). However, our results at the continental scale should be interpreted with caution because they depend on the sites sampled. We sampled just two sites to repeat the design of a previous study (Cogni & Futuyma 2009), but our results could have been different if we had sampled a different site from São Paulo (since there were differences among these populations). In both experiments, the ant assemblages visiting the EFNs were similar for plants from the different origins, and they represent typical assemblages of EFN-visiting ants in the Neotropical region (Cogni *et al* 2000, Cogni & Freitas 2002).

What are the possible reasons for the geographical differences reported here? These differences may have evolved by drift or selection. Generally, spatial differences in selection in this type of interaction are likely to occur. The outcome of the interaction (positive, negative, or neutral) depends on the identity and abundance of at least three species assemblages: the visiting ants, the EFNs bearing plants, and the herbivore community (Rudgers & Strauss 2004). In addition, there may be spatial differences in possible fitness costs associated with the production of EFN (Keeler 1985, Rios *et al* 2008). For our system in particular, we have reported that field herbivore incidence on *C. pallida* varies among the sites studied; the average proportion of pods attacked varies from 1.5% to 13% for *U. ornatrix* and from 2% to 9% for *E. zinckenella* (Cogni *et al* 2011). We plan to investigate spatial variation in ant assemblages and other EFN-bearing plants in the community in the future.

Our larval experiments showed that the behavior of moving away from the EFNs is a very effective way to avoid ant predation. This effect of the ants may benefit the plant by reducing damage to fruits and seeds (see Rudgers *et al* 2003). Previous studies in the system have shown that ants cause high predation rates on larvae outside the fruits (Ferro *et al* 2006, Guimarães *et al* 2006), and that the behavior of eating inside the fruit is likely an adaptation to avoid ant predation (Ferro *et al* 2006). It may be interesting to investigate how larval behavior and predation risk by ants vary with ant species (Sendoya *et al* 2009), and with larval size since prey size is a key component to understand predation (Freitas & Oliveira 1996, Cogni *et al* 2002, Cogni & Oliveira 2004a, b). Interestingly, we also found evidence of genetic differences in the behavior of the larvae among populations, with larvae from CAM being less likely to move away from the EFNs. However, these differences may not be stable over time. The study of population structure of *U. ornatrix* with neutral markers resulted in the differentiation among populations from the state of São Paulo in 2005 (the same year we did the EFN experiments), but no differentiation was found in samples of the same populations in 2008 (Cogni *et al* 2011). The difference in population structure between the years suggests a pattern of local population extinction and recolonization, and therefore the differences in behavior we observed may be ephemeral. The differences observed among populations may have evolved by drift or selection. The selection pressure is likely to differ among the populations because *C. pallida* attractiveness to ants varies among populations (as reported here), and the ant assemblages may also vary in the different sites. The selection may also be related to differences among the plant populations in the concentration of PAs (as reported in Cogni *et al* 2011). Since leaves have much lower PA concentration than seeds (Ferro *et al* 2006), moving to the leaves may decrease the amount of PAs sequestered by the larvae. However, ant predation *per se* may not be affected by plant PA concentration. Ferro *et al* (2006) did not find significant differences while comparing predation rates on larvae previously fed on unripe seeds and leaves.

Our results open several questions for future studies. An ideal research program can study a larger number of populations, and sample over different seasons and in multiple years. It can include a common-garden component measuring genetic differences in ant attractiveness to EFNs, as well as PA concentration and other defense traits. It can also include field studies investigating differences in ant attractiveness and other defense traits, differences in herbivore pressure, and differences in ant assemblages. In addition, ant exclusion experiments can test if the trajectory of selection varies among populations. To understand the evolution of the larval behavior to avoid ant predation, future studies can investigate predation rates in the field, larval behavior in the field. On the other hand, common-garden experiments could address how the amount of sequestered PAs may affect behavior and how the population structure of *U. ornatrix* may affect the evolution of interpopulational differences. Therefore, the geographical differences reported here place the *Crotalaria–Utetheisa* interaction as an excellent model system to investigate evolution mediated by EFNs at the population level.

References

Agrawal AA (2011) Current trends in the evolutionary ecology of plant defence. Funct Ecol 25:4220–432

Barton AM (1986) Spatial variation in the effect of ants on an extrafloral nectary plant. Ecology 67:495–504

Bentley BL (1976) Plants bearing extrafloral nectaries and the associated ant community: interhabitat differences in the reduction of herbivore damage. Ecology 57:815–820

Byk J, Del-Claro K (2011) Ant-plant interaction in the Neotropical savanna: direct beneficial effects on extrafloral nectar on ant colony fitness. Popul Ecol 53:327–332

Cogni R (2010) Resistance to plant invasion? A native specialist herbivore shows preference for and higher fitness on an introduced host. Biotropica 42:188–193

Cogni R, Freitas AVL (2002) The ant assemblage visiting extrafloral nectaries of *Hibiscus pernambucensis* (Malvaceae) in a mangrove forest in Southeast Brazil (Hymenoptera: Formicidae). Sociobiology 40:373–383

Cogni R, Futuyma DJ (2009) Local adaptation in a plant herbivore interaction depends on the spatial scale. Biol J Linn Soc 97:494–502

Cogni R, Oliveira PS (2004a) Patterns in foraging and nesting ecology in the neotropical ant, *Gnamptogenys moelleri* (Formicidae: Ponerinae). Insect Soc 51:123–130

Cogni R, Oliveira PS (2004b) Recruitment behavior during foraging in the neotropical ant *Gnamptogenys moelleri* (Formicidae: Ponerinae): does the type of food matter? J Insect Behav 17:443–458

Cogni R, Raimundo RLG, Freitas AVL (2000) Daily activity of ants associated with the extrafloral nectaries of *Turnera ulmifolia* L. (Turneraceae) in a suburban area in Southeats Brazil. Entomol Mon Mag 136:141–147

Cogni R, Freitas AVL, Amaral Filho BF (2002) Influence of prey size on predation success by *Zelus longipes* (Het: Reduviidae). J Appl Entomol 126:74–78

Cogni R, Freitas AVL, Oliveira PS (2003) Interhabitat differences in ant activity on plant foliage: ants at extrafloral nectaries of *Hibiscus pernambucensis* in a sandy and mangrove forest. Entomol Exp Appl 107:125–131

Cogni R, Trigo JR, Futuyma DJ (2011) Varying herbivore population structure correlates with lack of local adaptation in a geographic variable plant–herbivore interaction. PLoS One 6(12):e29220. doi:10.1371/journal.pone.0029220

Cogni R, Trigo JR, Futuyma DJ (2012) A free lunch? No cost for acquiring defensive plant pyrrolizidine alkaloids in a specialist arctiid moth (Utetheisa ornatrix). Mol Ecol. doi:10.1111/mec.12086

Conner WE (2009) Utetheisa ornatrix, the ornate arctiid. In: ConnerWE (ed) Tigermoths and woolly bears—behavior, ecology and evolution of the Arctiidae. Oxford University Press, New York, pp 1–10

De la Fuente MAS, Marquis RJ (1999) The role of ant-tented extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. Oecologia 118:192–202

Del-Claro K, Torezan-Silingard HM (2009) Insect-plant interactions: new pathways to a better comprehension of ecological communities in Neotropical savannas. Neotrop Entomol 38:159–164

Del-Claro K, Berto V, Réu W (1996) Effect of herbivore deterrence by ants on the fruit set of an extrafloral nectary plant, Qualea multiflora (Vochysiaceae). J Trop Ecol 12:887–892

Díaz-Castelazo C, Rico-Gray C, Ortega F, Ángeles G (2005) Morphological and secretory characterization of extrafloral nectaries in plants of coastal Veracruz, Mexico. Ann Bot 96:1175–1189

Eisner T, Meinwald J (1995) The chemistry of sexual selection. Proc Natl Acad Sci USA 92:50–55

Ferro VG (2001) Padrões de utilização de Crotalaria spp. (Leguminosae, Papilionoideae, Crotalarieae) por larvas de Utetheisa ornatrix (Lepidoptera, Arctiidae). M.Sc. dissertation, Universidade Estadual de Campinas, Campinas, Brazil

Ferro VG, Guimarães PR Jr, Trigo JR (2006) Why do larvae of Utetheisa ornatrix penetrate and feed in pods of Crotalaria species? Larval performance vs. chemical and physical constraints. Entomol Exp Appl 121:23–29

Flores AS (2004) Taxonomia, números cromossômicos e química de espécies de Crotalaria L. (Leguminosae-Papilionideae) no Brasil. Ph.D. dissertation, Universidade Estadual de Campinas, Campinas, Brazil

Freitas AVL, Oliveira PS (1996) Ants as selective agents on herbivore biology: Effects on the behavior of a non-myrmecophilous butterfly. J Anim Ecol 65:205–210

Guimarães PR Jr, Raimundo RLG, Bottcher C, Silva RR, Trigo JR (2006) Extrafloral nectaries as a deterrent mechanism against seed predators in the chemically protected weed Crotalaria pallida (Leguminosae). Austral Ecol 31:776–782

Heads PA, Lawton JH (1985) Bracken, ants and extrafloral nectaries. 3. How insect herbivores avoid ant predation. Ecol Entomol 10:29–42

Heil M, McKey D (2003) Protective ant–plat interactions as model systems in ecological and evolutionary research. Annu Rev Ecol Evol Syst 34:425–453

Hoina A, Martins CHZ, Trigo JR, Cogni R (2012) Preference for high concentrations of plant pyrrolizidine alkaloids in the specialist arctiid moth Utetheisa ornatrix depends on previous experience. Arthropod-Plant Inte. doi:10.1007/s11829-012-9232-1

Horvitz CC, Schemske DW (1984) Effects of ants and ant-tended herbivore on seed production of a neotropical herb. Ecology 65:1369–1378

Keeler KH (1985) Extrafloral nectaries on plants in communities without ants—Hawaii. Oikos 44:407–414

Keeler KH, Kaul RB (1984) Distribution of defense nectaries in Ipomoea (Convolvulaceae). Am J Botany 71:1364–1372

Kersch MF, Fonseca CR (2005) Abiotic factors and the conditional outcome of an ant–plant mutualism. Ecology 86:2117–2126

Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E (ed) Insect–plant interactions, 4th edn. CRC Press, Boca Raton, pp 81–129

Macel M (2010) Attract and deter: a dual role for pyrrolizidine alkaloids in plant–insect interactions. Phytochem Rev 10:75–82

Melo Y, Machado SR, Alves M (2010a) Anatomy of extrafloral nectaries in Fabaceae from dry-seasonal Forest in Brazil. Bot J Linn Soc 163:87–98

Melo Y, Cordula E, Machado SR, Alves M (2010b) Nectary morphology of Leguminosae senso lato in areas of dry seasonal Forest in Brazil. Acta Bot Brasilica 24:1034–1045

Nogueira A, Guimarães E, Machado SR, Lohmann LG (2012) Do extrafloral nectarines present a defensive role against herbivores in two species of the family Bignoniaceae in a Neotropical savannas? Plant Ecol 213:289–301

Oliveira PS, Rico-Gray V, Días-Castelazo C, Castillo-Guevara C (1999) Interaction between ants, extrafloral nectaries, and insect herbivores in neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in Opuntia stricta (Cactaceae). Funct Ecol 13:623–631

Polhill RM (1982) Crotalaria in Africa and Madagascar. Royal Botanical Garden, Cumbria, UK, 396 p

Rios RS, Marquis RJ, Flunker JC (2008) Population variation in plant traits associated with ant attraction and herbivory in Chamaecrista fasciculata (Fabaceae). Oecologia 156:577–588

Rosumek FB, Silveira FAO, Neves FD, Barbosa NPD, Diniz L, Oki Y, Pezzini F, Fernandes GW, Cornelissen T (2009) Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. Oecologia 160:537–549

Rudgers JA, Gardener MC (2004) Extrafloral nectar as a resource mediating multispecies interactions. Ecology 85:1495–1502

Rudgers JA, Hodgen JG, White JW (2003) Behavioral mechanisms underlie an ant–plant mutualism. Oecologia 135:51–59

Rudgers JA, Strauss SY (2004) A selection mosaic in the facultative mutualism between ants and wild cotton. Proc R Soc Lond B 271:2481–2488

Salazar BA, Whitman DW (2001) Defensive tactics of caterpillars against predators and parasitoids. In: Ananthakrishnan TN (ed) Insect and plant defense dynamics. Science Publisher, Enfield, p 161–207

Sendoya SF, Freitas AVL, Oliveira PS (2009) Egg-laying butterflies distinguish predaceous ants by sight. Am Nat 174:134–140

Smiley JT (1986) Ant constancy at Passiflora extrafloral nectaries: effects on caterpillar survival. Ecology 67:516–521

Thompson JN (2005) The geographic mosaic of coevolution. The University of Chicago Press, Chicago, IL, p 400

Trigo JR (2010) Effects of pyrrolizidine alkaloids through different trophic levels. Phytochem Rev 10:83–98