Plant phenotype influences the effect of ant mutualists on a polymorphic mangrove

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Summary

1. This study examines the effect of ant mutualists on two morphs of the mangrove *Conocarpus erectus*. 'Silver' *C. erectus* plants have a dense layer of leaf hairs, or trichomes, while 'green' plants have very few trichomes. Ants are attracted to extrafloral nectaries (EFNs) produced on the foliage of both high- and low-trichome morphs.

2. High-trichome plants produced fewer and smaller EFNs. *Conocarpus erectus* plants with fewer and smaller EFNs produced less nectar and attracted fewer ants.

3. Ant exclusion increased herbivory and decreased growth in low-trichome plants, but had no detectable effect on herbivory and growth in high-trichome plants.

4. *Synthesis*. This study demonstrates that intraspecific variation in a direct anti-herbivore defence influences the magnitude of the beneficial effect of ants on plants. Individuals with low trichome densities benefitted from ant mutualists while individuals with high trichome densities did not. This likely creates stronger selection for the maintenance of EFN production in low-trichome plants, a conclusion supported by the fact that low-trichome plants produced more and larger EFNs than high-trichome plants, and suggests that the two morphs are on divergent evolutionary trajectories with regard to anti-herbivore defence.

Key-words: anti-herbivore defence, ant–plant mutualism, *Conocarpus erectus*, extrafloral nectaries, herbivory, plant–herbivore interactions, trichomes

Introduction

Mutualisms are frequently context-dependent, and an important question in the study of mutualisms is determining the conditions leading to strong interactions between partner species (Bronstein 1994, 1998). Some of the variation in costs and benefits to mutualistic partners is likely due to phenotypic variation in the partner species. The effect of phenotypic variation on mutualistic interactions has a long history of study in protective ant–plant mutualisms, in which plants provide food and shelter for ants, and ants protect the plants from herbivores (Heil & McKey 2003; Rico-Gray & Oliveira 2007). For decades, ecologists have sought to understand the influence of direct anti-herbivore defences, chemicals or physical structures employed by plants to deter herbivory, on the beneficial effect of ant mutualists. While there are numerous studies comparing the relationship between direct defence and mutualistic ants across species, the paucity of studies exploring this relationship within species and the scarcity of manipulative experiments documenting the effect of ants on plants with different levels of direct defence has limited our understanding of the interaction between direct defence and ant–plant mutualisms.

In 1966, Janzen observed that *Acacia* species with ant associates lack the chemical defences characterizing species without ant associates, and hypothesized that these ant–*Acacia* species lose their direct defences when indirect defence is provided by the ants (Janzen 1966). Since then, a number of studies have documented similar trade-offs between direct defences and ant mutualists between species, reinforcing the idea that plants tend to rely on either direct defence or mutualistic ants, but not both (Heil *et al.* 1999; Heil, Staehelin & McKey 2000; Nomura, Itioka & Itino 2000; but see Heil *et al.* 2002; Eck *et al.* 2001). This pattern suggests that plants with fewer direct defences receive a greater benefit from mutualistic ants. While this is likely to be the case when we compare different species, without information on intraspecific variation in the effects of ants and direct defences on herbivory, the selective forces that generated these trade-offs remain unresolved.

All of the aforementioned studies documenting trade-offs between ant mutualists and physical and chemical defences feature myrmecophytes, plants that offer substantial rewards (food bodies, domatia) and tend to have obligate relationships with their ant mutualists. In contrast to myrmecophytes, many plants attract ants using extrafloral nectaries (EFNs) alone. These EFN-mediated ant–plant mutualisms tend to be more facultative and context-dependent than ant–myrmecophyte

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interactions (Bronstein 1998; Bronstein, Alarcon & Geber 2006), and the low cost of EFN production (e.g. O'Dowd 1979) coupled with the high variability in the beneficial effect of ants attracted to EFNs (Bentley 1976; Horvitz & Schemske 1984; Barton 1986; Becerra & Venable 1989; Bronstein 1998) may complicate the relationship between direct defence and ant mutualists for these plants. To date, there is no evidence of trade-offs between direct defence and EFN production either within (Rudgers, Strauss & Wendel 2004) or between species (Steward & Keeler 1988; Rudgers, Strauss & Wendel 2004). Thus, the influence of direct defence on the beneficial effect of ants may be less pronounced for EFN-mediated ant–plant interactions than for ant–myrmecophyte interactions.

Given the long history of study of the relationship between plant defence and ant mutualists, there are surprisingly few manipulative experiments addressing the effect of variation in direct defence on the benefit of ant mutualists. Stapley (1998) did not find strong evidence that the benefit of mutualistic ants changes when thorns of the myrmecophyte *Acacia drepanolobium* were removed. In other myrmecophytes, induced increases in direct defence in ant-exclusion treatments complicate efforts to explore the effect of the direct defence on the benefits of mutualistic ants (Dyer *et al.* 2004; Moraes & Vasconcelos 2009). Thus, there is little experimental evidence that plants with lower levels of direct defence receive a greater benefit from ant mutualists.

The current study takes advantage of a polymorphism in a physical defence trait of an EFN-bearing mangrove. *Conocarpus erectus* Linnaeus, 1753 (Combretaceae) generally occurs in two morphs – 'silver' plants have a dense layer of trichomes on the leaf surface, while 'green' plants have very few trichomes (Semple 1970; Correll & Correll 1982). The high-trichome morph has lower levels of herbivory than the low-trichome morph, suggesting that trichomes are an effective direct defence (Schoener 1987, 1988; Spiller & Schoener 1996; Agrawal & Spiller 2004). High-trichome plants are expected to receive less of a benefit from ant mutualists because they are already defended by trichomes. In other words, trichomes and mutualistic ants may be redundant with respect to their effects on herbivory. If mutualistic ants have a greater positive impact on fitness in low-trichome plants, this redundancy can drive selection for higher nectar production in low-trichome plants than high-trichome plants. In this paper, observational studies are used to test the hypothesis that low-trichome plants produce more nectar and attract more ants, and an ant-exclusion experiment is used to test the hypothesis that ants have a greater beneficial effect on low-trichome plants.

Materials and methods

STUDY SYSTEM

Conocarpus erectus is a supratidal mangrove that occurs in tropical and subtropical regions around the world. The high- and low-trichome morphs are considered subspecies, rather than separate species, due to the presence of intermediate phenotypes in large parts of the range of *C. erectus* (Semple 1970). While there is some plasticity

in the number of trichomes produced by *C. erectus*, changes from one morph to the other have only been observed under extreme circumstances – high-trichome plants temporarily sprout foliage with reduced trichome densities following the removal of all above-ground biomass by hurricanes or pruning (Spiller & Agrawal 2003; Agrawal & Spiller 2004). Under less extreme circumstances (including the removal of all leaves from a plant) high- and low-trichome phenotypes are maintained (Agrawal & Spiller 2004; T. W. Schoener, unpubl. data). Both high- and low-trichome plants possess small (1–4 mm in length), oblong EFNs on the petiole near the base of each leaf blade (Kemis 1984). It is possible that high-trichome plants possess additional anti-herbivore defences that are lacking in low-trichome plants and that these would also contribute to the observed differences in herbivory between the two morphs; however, low-trichome plants have tougher leaves than high-trichome plants (Agrawal & Spiller 2004), and there does not appear to be a systematic difference between the two morphs in total tannin concentration (L. Yang, unpubl. data).

This research was conducted in the islands of the Exuma chain in the central Bahamas. *Conocarpus erectus* is one of the most common shoreline plants on the islands in this region, with high- and low-trichome morphs present in roughly equal proportions. While plant growth occurs year round in this subtropical environment, it is highest during (and directly after) the summer rainy season. Extrafloral nectaries on *C. erectus* in this region are visited by a variety of insects, including ants, wasps, moths, beetles and flies. The most common ant visitors are *Camponotus tortuganus* Emery, 1895; *Brachymyrmex obscurior* Forel, 1893; *Crematogaster lucayana* Wheeler, 1905; *Crematogaster steinheili* Forel, 1881; *Dorymyrmex pyramicus* Roger, 1863; *P. pallidus* Smith 1855; and a *Paratrechina* sp. Honeydew-producing insects are very rare on *C. erectus* in the study area, so EFNs are the primary attractant for ant visitors (J. Piovia-Scott, personal observation).

OBSERVATIONAL STUDIES

In order to test the hypothesis that high-trichome plants produce less nectar and attract fewer ants I conducted three observational studies. The first relates the amount of nectar produced to the length (a proxy for size) of EFNs in the two morphs. The second relates ant density to the length of EFNs in the two morphs. Finally, the third compares the number and length of EFNs produced by the two morphs.

In order to get more detailed information on nectar production in high- and low-trichome plants, I collected nectar from 16 high-trichome and 15 low-trichome plants in September 2009. Nectar was collected from two to four stems per plant in the following manner. First, any accumulated nectar was removed from EFNs on selected stems. Then, mesh bags were placed over the selected shoots for 24 h in order to prevent removal by ants and other insects. After this 24-hour period, the bags were removed and accumulated nectar was collected from EFNs on the youngest (most apical) five leaves of each bagged shoot using microcapillary tubes. In addition to measuring the volume of nectar produced, I measured the sugar content of nectar using a hand-held refractometer (Thermo Fisher Scientific Inc., Pittsburg, PA, USA). Active EFNs on the youngest five leaves on each stem were counted and measured, and the relationships between EFN length and nectar production were analysed using linear models with plant morph, total length of EFNs, and their interaction as the independent variables, and nectar volume and sugar content as the dependent variables in separate analyses. Non-significant ($P > 0.05$) predictors were removed from the final model. Regressions were forced through zero,

as plants without EFNs produce no nectar, and dependent variables were log-transformed to meet model assumptions.

In order to determine if plants with more EFNs attract more ants, I surveyed the number of EFNs and the number of ant visitors on 25 plants in one island in September 2006. These plants were selected such that a broad range of EFN numbers was present in the sample; all plants were *c.* 2 m in height. I measured and counted active EFNs on the youngest five leaves on four stems per plant. Ants were surveyed twice, once during the daylight and once at night, by recording the number of each species seen in a 2-min search of the plant. Data were analysed using linear models with plant morph, total length of active EFNs per shoot, and their interaction as the independent variables, and ant abundance as the dependent variable. Non-significant ($P > 0.05$) predictors were removed from the final model. Ant abundances were log-transformed to meet model assumptions.

In order to determine if high-and low-trichome morphs produce different numbers and lengths of EFNs, I surveyed EFNs on two randomly selected low-trichome and two randomly selected high-trichome plants in each of 10 small islands in December 2005. For each tree, I randomly selected 10 shoots. In order to account for variation due to leaf age, I randomly selected one young leaf and one old leaf per shoot for data collection. Young leaves were taken from the terminal end of growing stems, while old leaves were taken from the basal portion of growing stems. I analysed the average number of EFNs and the average length of EFNs per leaf on each plant using separate linear mixed models in SAS PROC MIXED (SAS Institute 1999). Morph and age were fixed factors and island and plant were random factors. Non-significant interactions between fixed effects ($P > 0.05$) were dropped from the final model. Interactions containing random variables were of no *a priori* interest and were not included. I used a variance-component structure for random terms, which assumes that they are independent and additive. Type III sums of squares and Satterthwaite approximations for denominator degrees of freedom were used for tests of fixed effects. Assumptions of homoscedasticity, normality of residuals and normality of random effects were confirmed by examining residual plots and distributions of residuals and random effects. Although the correlation between EFN number and length makes it difficult to determine if morph and leaf age affect each variable independently of the other, I present the results of analyses of both variables for illustrative purposes.

ANT-EXCLUSION EXPERIMENT

In order to test the hypothesis that ants have a greater beneficial effect on low-trichome plants than high-trichome plants, I conducted ant exclusions on both morphs. The ant-exclusion experiment ran from September 2006 to September 2007 and included a total of 36 plants in three different islands. On each island, I selected equal numbers of high-and low-trichome plants ranging from 1 to 3 m in height. High- and low-trichome plants were interspersed with one another, and plants with very few EFNs were avoided, as these plants were expected to attract very few ant visitors. I also avoided plants that had been heavily damaged by storms or human activities in the recent past, as *C. erectus* exhibits plastic responses to these types of disturbance for both trichomes and EFNs (Agrawal & Spiller 2004; J. Piovia-Scott, unpubl. data). On each plant, I selected two experimental branches on which ant exclusions would be feasible. I randomly selected one of them for ant exclusion – the other branch served as a control. Ants were excluded using Tree Tanglefoot (Contech Enterprises Inc., Victoria, BC, Canada), which was applied

on top of a layer of removable tape in order to protect the plant. Most other predators in the system can either fly or jump, and were therefore not likely to be excluded by the tanglefoot barrier (for example, lizards were observed on both ant-exclusion and control branches). The ant-exclusion treatment was initiated for 18 of the experimental plants in September 2006, and data were collected from these plants in December 2006, May 2007 and September 2007. For the remaining 18 plants, the treatment was initiated in May 2007 and data were collected only in September 2007. Tanglefoot barriers were replaced during data-collection periods.

To measure leaf damage, I randomly selected 20 leaves from each experimental branch and pressed and photographed them in the field. Total leaf area and damaged area were measured from digital images on a computer using image-analysis software (Sigma-Scan; Systat Software Inc., San Jose, CA, USA). Damage was classified either as a 'hole' (missing leaf tissue) or a 'scar' (necrotic leaf tissue associated with herbivore attack). I conducted non-destructive counts of EFNs on the newest (most apical) five leaves on each of four randomly selected stems per branch.

I also collected data on plant growth and ant density in September 2007. The period between May and September includes most of the rainy season, when *C. erectus* growth rates are highest (J. Piovia-Scott, unpubl. data). To measure growth, I selected four shoots on each experimental branch and marked the most distal leaves present with ink in May 2007. For each plant, shoots with similar numbers of leaves were selected for growth measurements. In September 2007, I counted the number of new leaves (those more distal than the marked leaves) that had appeared since May (average leaf size was similar for high-and low-trichome plants; $P = 0.83$). I calculated growth as the average number of leaves produced per shoot (shoots that produced flowers were excluded, leading to the omission of two plants from the final analysis for growth). I measured ant density on experimental branches in September 2007 using visual searches. I counted the number of each species present on each branch on six separate occasions (three during daylight hours and three at night), as well as the number of shoots (actively growing stems, including leaves and flowers) on each branch. Densities were calculated as the average number of individuals per shoot. Plant growth and ant density were log-transformed to meet model assumptions.

Data from the ant-exclusion experiment were analysed using linear mixed models. Analyses of leaf damage and EFNs classified island, plant and experimental branch as random factors. Branches were nested within plants, which were nested within islands. Morph, treatment and sampling date were used as fixed factors. This model is analogous to a repeated-measures split-plot ANOVA – trichome density varied between plants, so tests of hypotheses concerning morph used plant-level error terms; treatment varied between branches on a plant, so tests of treatment hypotheses and morph × treatment interactions used a branch-level error term; date varied within branches, as some of them were sampled multiple times, so hypotheses concerning date were examined using the residual error. Models for growth and ant density did not include experimental branch as a random factor or sampling date as a fixed factor, as there was only one sampling date per branch for these response variables. I ran preliminary analyses that included EFNs as a covariate, but it was not significant for any of the response variables examined ($P > 0.1$ in all cases), so it was not included in the final models. Statistical software, model selection procedures, and hypothesis tests are described above.

In order to determine if the low-trichome and high-trichome plants used in this experiment were visited by different ant species,

Table 1. Ants observed on low-trichome and high-trichome plants in the ant-exclusion experiment in September 2007

Species	Low-trichome Number (%)	High-trichome Number (%)
Camponotus tortuganus	394 (56)	343 (40)
Dorymyrmex pyramicus	263 (37)	280 (33)
Brachymyrmex obscurior	43 (6)	123 (14)
Brachymyrmex minutus	0 (0)	17 (2)
Crematogaster steinheili	1 (0)	57 (7)
Crematogaster lucayana	4 (1)	35 (4)
Pseudomyrmex pallidus	2 (0)	3 (0)
Trachymyrmex jamaicensis	0 (0)	1 (0)
Paratrechina sp. A	0 (0)	1 (0)
Unknown	1 (0)	1 (0)
Total	708 (100)	861 (100)

I conducted a MANOVA on the densities of the three most common ant species (Table 1) on control branches. Morph and island were the only factors included in the model. Treatment and branch were not included, as I used only control branches.

Results

OBSERVATIONAL STUDIES

Conocarpus erectus plants with more, larger EFNs produced more nectar and attracted more ants. The survey of nectar production on high-and low-trichome plants showed that both volume of nectar and amount of sugar produced increased with the total length of EFNs ($F_{1,30} = 228.6$, $P < 0.0001$ and $F_{1,30} = 257.6$, $P < 0.0001$, respectively; Fig. 1a). There was no difference between high-and low-trichome plants in the relationship between EFN length and volume of nectar produced (morph × EFN length interaction: $F_{1,27} = 1.13$, $P = 0.30$); the same was true for the amount of sugar produced ($F_{1,27} = 0.42$, $P = 0.52$). Thus, the total length of EFNs is a reasonable proxy for the amount of nectar secreted for both high-and low-trichome plants.

The survey of ants and EFNs found higher ant abundances on plants that had a greater total length of EFNs per shoot ($F_{1,23} = 10.4$, $P = 0.004$; $R^2 = 0.31$; Fig. 1b). This relationship did not differ for low-trichome and high-trichome plants (morph effect: $F_{1,22} = 0.27$, $P = 0.61$; morph × EFN length interaction: $F_{1,21} = 0.48$, $P = 0.50$). Thus, both high-and low-trichome plants secreting more nectar attract more ants. The large, nocturnal ant *C. tortuganus* was the most common ant in this survey (53% of 199 observations), followed by *D. pyramicus* (28%), *B. obscurior* (19%) and *P. pallidus* (0.5%).

Low-trichome plants produced significantly more and larger EFNs than high-trichome plants. The survey of EFNs in 10 islands found that high-trichome plants had 45% smaller EFNs ($F_{1,69.1} = 20.55$, $P < 0.0001$), and 17% fewer EFNs ($F_{1,70} = 8.60$, $P = 0.0045$) than low-trichome plants (Fig. 2). Younger leaves also had more ($F_{1,69.9} = 10.91$, $P = 0.0015$) and larger ($F_{1,68.2} = 42.38$, $P < 0.0001$) EFNs than older leaves.
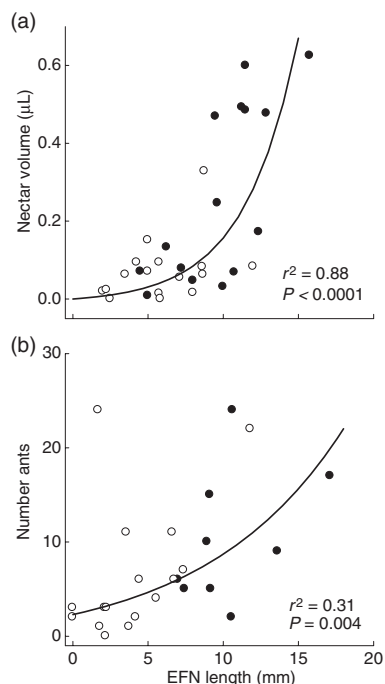

Fig. 1. The relationship between total extrafloral nectary length on the newest five leaves of a shoot and (a) volume of nectar produced by low-trichome (filled circles, $n = 15$) and high-trichome (open circles, $n = 16$) *Conocarpus erectus*, and (b) number of ants observed on low-trichome (filled circles, $n = 9$) and high-trichome (open circles, $n = 16$) *C. erectus*. Neither relationship differed for high- and low-trichome plants (EFN length × morph interaction: $F_{1,27} = 1.13$, $P = 0.30$ and $F_{1,21} = 0.48$, $P = 0.50$ for nectar volume and ant abundance, respectively). The fitted lines from linear regressions of log-transformed nectar volume and ant abundance on EFN length for pooled high- and low-trichome plants are shown.

ANT-EXCLUSION EXPERIMENT

Overall, low-trichome plants had almost twice as much leaf damage as high-trichome plants (morph effect; $F_{1,29.5} = 27.2$, $P < 0.0001$; Fig. 3a). The effects of ant exclusion on leaf damage differed for high-and low-trichome plants (morph × treatment interaction: $F_{1,28.1} = 5.86$, $P = 0.02$; Fig. 3a). Ant exclusion led to a 21% increase in leaf damage for low-trichome plants (low-trichome control versus low-trichome exclusion: $F_{1,27.1} = 7.86$, $P = 0.01$), but had no effect on leaf damage for high-trichome plants (high-trichome control versus high-trichome exclusion: $F_{1,29.1} = 0.41$, $P = 0.53$).

Ant-exclusion led to a 24% reduction in growth rate for low-trichome plants ($F_{1,33.3} = 8.31$, $P = 0.007$), but had no effect on growth for high-trichome plants ($F_{1,33.4} = 0.45$, $P = 0.51$). This is consistent with the results for leaf damage, although the interaction between morph and treatment was not significant for growth ($F_{1,33.4} = 2.45$, $P = 0.13$; Fig. 3b). Overall, ant exclusion decreased growth by 17% ($F_{1,34.4} = 6.03$, $P = 0.02$; Fig. 3b).

Ant exclusions installed in May 2007 reduced total ant density on experimental branches in September 2007 by 79% (0.09 and 0.02 ants per shoot on control and ant-exclusion branches, respectively: $F_{1,35} = 91.42$, $P < 0.0001$), indicating that the
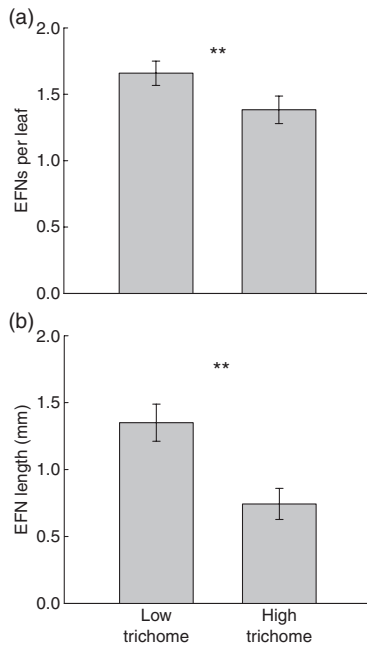
Fig. 2. Extrafloral nectary (EFN) (a) number and (b) length from a survey of 20 low-trichome and 20 high-trichome *Conocarpus erectus* on 10 islands. Means and standard errors are shown. Statistical significance of tests comparing high- and low-trichome plants is shown: *$P < 0.05$, **$P < 0.01$.
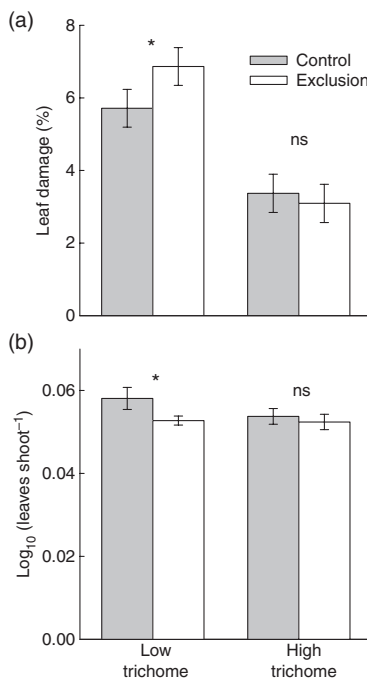

Fig. 3. Difference in (a) leaf damage and (b) growth for ant-exclusion and control branches on low-trichome ($n = 18$) and high-trichome ($n = 18$) *Conocarpus erectus* plants. Least-squares means and standard errors from linear mixed model analyses described in the text are shown for damage; means and standard errors are shown for growth. Statistical significance of tests comparing ant-exclusion and control branches within each morph is shown: *$P < 0.05$, **$P < 0.01$; ns, not significant.

ant-exclusion treatments were largely effective, even after 4 months without maintenance under field conditions. The same three species (*C. tortuganus*, *D. pyramicus* and *B. obscurior*) were the most common ants on both high-and low-trichome plants (Table 1). There was no difference between morphs in the abundance of these three species on control branches (MANOVA: Wilk's Lambda = 0.98, $F_{3,30} = 0.19$, $P = 0.90$). The high-trichome plants selected for this experiment had 15% fewer EFNs than the low-trichome plants ($F_{1,34.3} = 4.30$, $P = 0.046$).

Discussion

A number of studies have documented low levels of chemical and physical anti-herbivore defences in plant species that receive protection from ant mutualists, leading many authors to hypothesize that selection favours either traits associated with ant mutualists or alternate defence traits, but not both (Janzen 1966; Heil *et al.* 1999; Heil, Staehelin & McKey 2000; Eck *et al.* 2001). While these between-species comparisons suggest that this may be the case, within-species studies are needed in order to resolve the selective forces leading to divergence between plants that strongly associate with ant mutualists and have few alternate defences and those that have high levels of alternate defence but weak associations with ant mutualists. This study provides the first evidence that intraspecific variation in a direct anti-herbivore defence trait is associated with the selection of a trait associated with ant mutualists, in this case EFN production. *Conocarpus erectus* plants with few trichomes experienced more herbivory and lower growth rates when ants were excluded, suggesting that selection favours high EFN production for the low-trichome morph. On the other hand, plants with many trichomes were not affected by ant exclusion, suggesting that selection does not maintain EFN production in the high-trichome morph. The results of these ant-exclusion experiments are consistent with observational studies showing that the low-trichome morph produces more and larger EFNs than the high-trichome morph, and that plants producing more, larger EFNs produce more EFN and attract more ants. Thus, selection favours strong associations with ant mutualists in plants with few alternate defences and weak associations with ant mutualists in plants with high levels of an alternate defence, creating divergent trajectories for the evolution of the ant-plant mutualism.

WHY DO THE EFFECTS OF ANT EXCLUSION DIFFER FOR HIGH- AND LOW-TRICHOME *C. ERECTUS*?

Ant–plant mutualisms mediated by EFN are frequently facultative and context-dependent (Bronstein 1994, 1998). Interactions between ants and *C. erectus* appear to be no exception – ants consume the nectar from EFNs on both high- and low-trichome morphs, but in this study ants only provided protection from herbivores for low-trichome plants. While the evidence for different effects of ants on growth in high- and low-trichome morphs is less convincing (the interaction is not significant), individual tests are consistent with the herbivory

results – ants enhance growth in low-trichome plants but not in high-trichome plants.

The fact that ants benefited low-trichome plants but not high-trichome plants could be due to a number of factors. It is possible that low-trichome plants could be attended by ant species that are more effective bodyguards than those on high-trichome plants, but the fact that the same ant species were common on both morphs suggests that this is not the case. It is also possible that low-trichome plants are favoured by herbivores that are more susceptible to ants, while high-trichome plants are favoured by herbivores that are ant-resistant. However, the most abundant herbivores are present on both high- and low-trichome plants (Agrawal & Spiller 2004; J. Piovia-Scott, unpubl.data). The most likely explanations for the difference in the effect of ant-exclusion between high- and low-trichome *C. erectus* are: (i) low-trichome plants have higher densities of ants, and (ii) trichomes and ants represent redundant defences. I will examine these hypotheses in turn.

Low-trichome plants in the ant-exclusion experiment produced more EFNs than high-trichome plants, and the observational studies show that plants with more EFNs produce more nectar and attract more ants. While no difference in ant density between morphs was detected in the ant-exclusion experiment, additional sampling may have shown that the low-trichome plants, having more EFNs than the high-trichome plants, attracted more ants. The fact that the observational study showed a relationship between EFNs and ant abundance while the ant-exclusion experiment did not could be due to differences in the way I selected plants for the two studies. For the observational study I selected plants with a wide variety of EFN densities in order to maximize the amount of variation in this explanatory variable, whereas for the ant-exclusion experiment I tried to select plants with high EFN densities in order to maximize my ability to detect ant effects. Other studies have shown that higher levels of nectar production have been associated with increased ant densities (Heil *et al.* 2001; Itino *et al.* 2001) and thereby better protection from herbivores (Rudgers & Strauss 2004; Heil *et al.* 2009). In addition to having fewer EFNs, it is also possible that high-trichome plants receive fewer ant visitors because trichomes interfere with ant activity. However, neither the ant-exclusion experiment nor the observational study provide any support for this hypothesis – high-trichome plants appear to recruit just as many ants per unit EFN as low-trichome plants.

The difference in the effect of ant-exclusion on high- and low-trichome *C. erectus* may also be the result of redundancy between ant bodyguards and leaf trichomes. In other words, high-trichome plants may not benefit from ant attendance because they are already effectively defended by trichomes. This hypothesis is supported by the fact that low-trichome plants are subject to higher levels of herbivory than high-trichome plants (this study, Schoener 1987, 1988; Agrawal & Spiller 2004). Higher levels of damage may allow low-trichome plants to receive more of a benefit from their ant bodyguards.

Additional support for this hypothesis comes from the fact that EFNs were never a significant covariate for growth or leaf damage in preliminary analyses of the data from the ant-exclusion experiment. This suggests that the difference in the effect of ant-exclusion on high- and low-trichome plants may not have been due to differences in nectar production. Interestingly, ant mutualists do not completely compensate for lower trichome densities, as the presence of ants does not reduce damage in low-trichome plants to the level of observed for high-trichome plants.

WHAT DRIVES THE EXPRESSION OF TRICHOMES AND EFNS IN *C. ERECTUS*?

High-trichome plants had fewer and smaller EFNs than low-trichome plants throughout the study area. Since high-trichome plants did not experience increased herbivory or decreased growth when ants were excluded, it is likely that there is weak selection for EFN production in high-trichome plants (Janzen 1966; Rhoades 1979; Simms & Rausher 1987; Mauricio, Rausher & Burdick 1997). Thus, the presence of a trade-off is consistent with the hypothesis that there is redundancy between trichomes and ant bodyguards. Trade-offs between ant mutualists and alternate defences are expected to be less pronounced in EFN-bearing plants than in myrmecophytes due to the low costs of EFN production (e.g. O'Dowd 1979) and the variability in the beneficial effect of ants attracted to EFNs (Bentley 1976; Horvitz & Schemske 1984; Barton 1986; Becerra & Venable 1989; Bronstein 1998). The presence of a trade-off between trichomes and EFNs in *C. erectus* suggests that the beneficial effect of ants on low-trichome plants is relatively strong and consistent, maintaining selection for high levels of constitutive EFN production.

If trichomes and EFNs were completely redundant, and plants with trichomes never received a benefit from ant bodyguards, then high-trichome plants would maximize their fitness by producing no EFNs. While EFNs are less abundant on high-trichome plants than low-trichome plants, they are not totally absent. The most likely explanation for continued EFN production in high-trichome plants is that they receive a benefit from ant mutualists in certain situations. Alternatively, this study may have captured a transient state in which high-trichome plants possess multiple defences but are in the process of losing one of them (Mauricio, Rausher & Burdick 1997), or gene flow between the two morphs may maintain EFNs production in the high-trichome morph.

The independent action of selection on trichomes and EFNs is not the only possible explanation for the observed trade-off. Intrinsic genetic relationships between these two traits, such as negative pleiotropy, could create negative correlations even in the absence of selection based on the ecological effects of the traits (Mole 1994). However, trade-offs between trichomes and EFNs have been observed within the same individuals – high-trichome *C. erectus* plants that have been pruned to the ground sprout leaves with reduced trichome densities and more EFNs (J. Piovia-Scott, unpubl. data) – suggesting that the observed

relationship between these two traits is not the result of intrinsic genetic constraints. Thus, it is likely that selection based on the ecological effects of these particular defence traits has played an important role in generating these trade-offs.

Aside from EFN production, high- and low-trichome morphs differ in other ways that might influence the evolution of anti-herbivore defence traits. For example, the control branches on low-trichome plants in the ant-exclusion experiment tended to grow faster than the control branches on high-trichome plants ($P = 0.07$), in spite of the fact that they sustained higher levels of herbivory. This suggests that the low-trichome morph has a higher tolerance for herbivory than the high-trichome morph. Tolerance is frequently associated with lower levels of resistance (Leimu & Koricheva 2006). For resistance traits in *C. erectus*, trichomes appear to follow this pattern, as they are associated with lower tolerance, but EFNs (this study) and toughness (Agrawal & Spiller 2004) appear to be associated with higher tolerance. Thus, *C. erectus* morphs appear to be most accurately characterized by particular defence syndromes, with high-trichome plants expressing a particularly effective resistance trait (trichomes), and low-trichome plants expressing a suite of different resistance traits (EFNs, toughness) in combination with higher tolerance of herbivory. The relative fitness associated with these different suites of traits may vary from year to year, which may explain the coexistence of high- and low-trichome morphs.

Acknowledgements

References

Agrawal, A.A. & Spiller, D.A. (2004) Polymorphic buttonwood: effects of disturbance on resistance to herbivores in green and silver morphs of a Bahamian shrub. *American Journal of Botany*, **91**, 1990–1997.

Barton, A.M. (1986) Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology*, **67**, 495–504.

Becerra, J.X.I. & Venable, D.L. (1989) Extrafloral nectaries: a defense against ant-homoptera mutualisms? *Oikos*, **55**, 276–280.

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

Bronstein, J.L. (1994) Conditional outcomes in mutualistic interactions. *Trends in Ecology & Evolution*, **9**, 214–217.

Bronstein, J.L. (1998) The contribution of ant plant protection studies to our understanding of mutualism. *Biotropica*, **30**, 150–161.

Bronstein, J.L., Alarcon, R. & Geber, M. (2006) The evolution of plant-insect mutualisms. *New Phytologist*, **172**, 412–428.

Correll, D.S. & Correll, H.B. (1982) *Flora of the Bahama Archipelago*. Liechtenstein, Cramer, Vaduz.

Dyer, L.A., Letourneau, D.K., Dodson, C.D., Tobler, M.A., Stireman, J.O. & Hsu, A. (2004) Ecological causes and consequences of variation in defensive chemistry of a Neotropical shrub. *Ecology*, **85**, 2795–2803.

Eck, G., Fiala, B., Linsenmair, K.E., Bin Hashim, R. & Proksch, P. (2001) Trade-off between chemical and biotic antiherbivore defense in the south east Asian plant genus *Macaranga*. *Journal of Chemical Ecology*, **27**, 1979–1996.

Heil, M. & McKey, D. (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology Evolution and Systematics*, **34**, 425–453.

Heil, M., Staehelin, C. & McKey, D. (2000) Low chitinase activity in *Acacia* myrmecophytes: a potential trade-off between biotic and chemical defences? *Naturwissenschaften*, **87**, 555–558.

Heil, M., Fiala, B., Linsenmair, K.E. & Boller, T. (1999) Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften*, **86**, 146–149.

Heil, M., Fiala, B., Maschwitz, U. & Linsenmair, K.E. (2001) On benefits of indirect defence: short- and long-term studies of antiherbivore protection via mutualistic ants. *Oecologia*, **126**, 395–403.

Heil, M., Delsinne, T., Hilpert, A., Schürkens, S., Andary, C., Linsenmair, K.E., Sousa, M.S. & McKey, D. (2002) Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos*, **99**, 457–468.

Heil, M., Gonzalez-Teuber, M., Clement, L.W., Kautz, S., Verhaagh, M. & Bueno, J.C.S. (2009) Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18091–18096.

Horvitz, C.C. & Schemske, D.W. (1984) Effects of ants and an ant-tended herbivore on seed production of a neotropical herb. *Ecology*, **65**, 1369–1378.

Itino, T., Itioka, T., Hatada, A. & Hamid, A.A. (2001) Effects of food rewards offered by ant-plant *Macaranga* on the colony size of ants. *Ecological Research*, **16**, 775–786.

Janzen, D.H. (1966) Coevolution of mutualism between ants and acacias in Central America. *Evolution*, **20**, 249–275.

Kemis, J.R. (1984) Petiolar glands in Combretaceae: new observations and an anatomical description of the extrafloral nectary of buttonwood (*Conocarpus erectus*). *AIBS-BSA, American Journal of Botany*, **71**, 35.

Leimu, R. & Koricheva, J. (2006) A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos*, **112**, 1–9.

Mauricio, R., Rausher, M.D. & Burdick, D.S. (1997) Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology*, **78**, 1301–1311.

Mole, S. (1994) Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. *Oikos*, **71**, 3–12.

Moraes, S.C. & Vasconcelos, H.L. (2009) Long-term persistence of a neotropical ant-plant population in the absence of obligate plant-ants. *Ecology*, **90**, 2375–2383.

Nomura, M., Itioka, T. & Itino, T. (2000) Variations in abiotic defense within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest. *Ecological Research*, **15**, 1–11.

O'Dowd, D.J. (1979) Foliar nectar production and ant activity on a neotropical tree, *Ochroma pyramidale*. *Oecologia*, **43**, 233–248.

Rhoades, D.F. (1979) Evolution of plant chemical defense against herbivores. *Herbivores: Their Interaction with Secondary Plant Metabolites* (eds G.A. Rosenthal & D.H. Janzen), pp. 1–134. Academic Press, New York.

Rico-Gray, V. & Oliveira, P.S. (2007) *The Ecology and Evolution of Ant-Plant Interactions*. University of Chicago Press, Chicago.

Rudgers, J.A. & Strauss, S.Y. (2004) A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 2481–2488.

Rudgers, J.A., Strauss, S.Y. & Wendel, J.E. (2004) Trade-offs among anti-herbivore resistance traits: insights from *Gossypieae* (Malvaceae). *American Journal of Botany*, **91**, 871–880.

SAS Institute (1999) *SAS v8.0*. SAS Institute, Inc., Cary, NC.

Schoener, T.W. (1987) Leaf pubescence in buttonwood - community variation in a putative defense against defoliation. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 7992–7995.

Schoener, T.W. (1988) Leaf damage in island buttonwood, *Conocarpus erectus* - correlations with pubescence, island area, isolation and the distribution of major carnivores. *Oikos*, **53**, 253–266.

Semple, J.C. (1970) The distribution of pubescent leaved individuals of *Conocarpus erectus*: Combretaceae. *Rhodora*, **72**, 544–547.

Simms, E.L. & Rausher, M.D. (1987) Costs and benefits of plant resistance to herbivory. *American Naturalist*, **130**, 570–581.

Spiller, D.A. & Agrawal, A.A. (2003) Intense disturbance enhances plant susceptibility to herbivory: natural and experimental evidence. *Ecology*, **84**, 890–897.

Spiller, D.A. & Schoener, T.W. (1996) Food-web dynamics on some small subtropical islands: effects of top and intermediate predators. *Food Webs: Integration of Pattern and Dynamics* (eds G.A. Polis & K.O. Winemiller), pp. 160–169. Chapman & Hall, New York.

Stapley, L. (1998) The interaction of thorns and symbiotic ants as an effective defence mechanism of swollen-thorn acacias. *Oecologia*, **115**, 401–405.

Steward, J.L. & Keeler, K.H. (1988) Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae). *Oikos*, **53**, 79–86.