

Letters

Ants mediate nitrogen relations of an epiphytic fern

Henry David Thoreau once said that ‘nature made ferns for pure leaves to show what she could do in that line’ (Myerson, 1992). Indeed, ferns have thoroughly explored the diversity of leaf function and use the laminar surface as a site for both carbon fixation and reproduction. The fern’s lack of flowers has led most to overlook the potential for fern–animal interactions. Yet recent discoveries of lepidopteron soral crypsis in several tropical ferns (Barker *et al.*, 2005) combined with an increased understanding of fern–herbivore interactions (Balick *et al.*, 1978; Auerbach & Hendrix, 1980; Weintraub *et al.*, 1995; Jensen & Holman, 2000; Mehltreter *et al.*, 2003), and the presence of myrmecotrophy in some species (Rashbrook *et al.*, 1992), demonstrate that fern–animal interactions may be more common than once thought.

Myrmecotrophy is an intriguing and important plant–animal relationship that has significant consequences for plant nutrition, protection, and ecosystem-level processes (Solano & Dejean, 2004; Fiedler *et al.*, 2007; Palmer & Brody, 2007; Sternberg *et al.*, 2007). In the myrmecophytic relationship, host plants typically provide food resources, for example elaiosomes, extrafloral nectaries (Beattie, 1989), and/or suitable nesting spaces (myrmecodomatia) for the ant visitor. In return, it is assumed that ants protect their host plants by removing herbivores and pathogens, attacking competing vegetation (Janzen, 1969) and supplying nutrients (Kaufmann & Maschwitz, 2006).

Myrmecotrophy has been reported in ferns, and species of *Solanopteris* (Forel, 1904; Gómez, 1974, 1977), *Lecanopteris* (Gay, 1991, 1993b; Gay & Hensen, 1992), and *Polypodium* (Koptur *et al.*, 1998) are known to produce potato-like tubers that function as domatia. Limited evidence suggests that nesting ants act to protect host ferns as in the case of *Solanopteris brunei*, where *Azteca* ants become quite aggressive when their host plant is disturbed (Gómez, 1974; and personal observation). Perhaps one of the best known temperate fern–ant relationships occurs in the widespread bracken fern (*Pteridium aquilinum*). This species produces foliar nectaries, and several studies have examined the ecology of this phenomenon, finding limited to no influence of ants on the host plant and vice versa (Tempel, 1983; Heads & Lawton, 1984; Lawton & Heads, 1984; Heads, 1986; Rashbrook *et al.*, 1992). Such results have added to the general rejection of the importance of fern–ant relationships.

Apart from serving as a protective mechanism, ants may also contribute to host plant nutrition. While there are a large

number of papers dealing with ant gardens and host plant interactions (Kaufmann & Maschwitz, 2006), quantification of nutrient exchange between ants and their plant host in natural conditions has not been widely demonstrated in epiphytic taxa and less so in ferns. Gay (1993a) conducted an elegant series of labeled nitrogen (N) laboratory experiments clearly demonstrating nutrient exchange between ants and host plants in the fern genus *Lecanopteris*. In this study, ant-derived nutrients were taken up through the inner walls of the domatia and, in at least one species, via roots produced inside such domatia. While Gay (1993a) demonstrated uptake, the study did not demonstrate the relative importance of this relationship to the overall nutrient budget of the host plant. In another example of N exchange in a myrmecophytic epiphyte, Treseder *et al.* (1995) demonstrated that the Asclepiad *Dischidia major* (Vahl) Merr. may derive up to 39% of its carbon and 29% of its N budget from ants that it hosts in specialized domatia (Treseder *et al.*, 1995). Here we describe a previously unknown cryptic relationship between the fern *Antrophyum lanceolatum* and the ant *Pheidole flavens* and comment on its ecological significance.

Materials and methods

Study site and tissue sampling

Antrophyum lanceolatum (L.) Kaulf. is an understory epiphyte, and plant tissues were collected between 1 and 2 m above the ground from host trees growing at La Selva Biological Station in Costa Rica. To determine the occurrence of ant infestation in this fern species, we sampled 93 individual plants with seven or more leaves for the presence/absence of nests. We evaluated nutrient exchange between *P. flavens* and *A. lanceolatum* by measuring the natural abundance stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of plants occupied by ants, plants free of ant colonies, and ant wastes produced in ant nests. We collected eight leaf samples from ant-unoccupied plants, seven leaf samples from ant-occupied plants and their associated wastes. To extract ant wastes, rhizome mats were separated with dissecting probes to remove ant nest material. All waste and plant material was placed in glassine envelopes and dried to a constant mass at 70 °C. Tissues from each leaf or waste sample were pulverized and analyzed for total C, total N, and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios at the University of Florida Stable Isotope Laboratory on a Finnigan MAT Delta IRMS isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA, USA) operated in automatic trapping mode after combustion of samples in an elemental analyzer (Carlo Erba Instrumentazione, Milan, Italy). The reference CO_2 was calibrated against

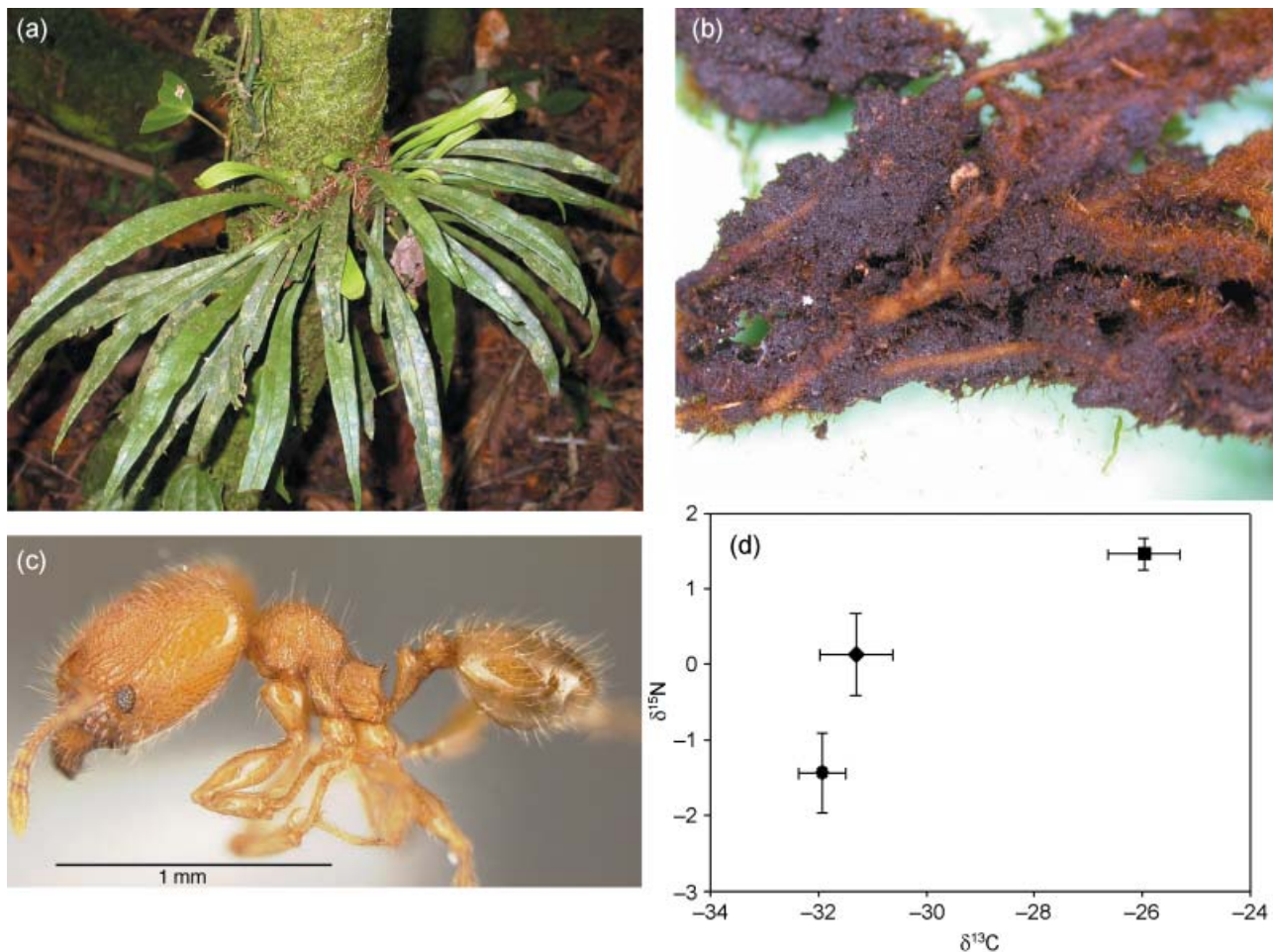


Fig. 1 Ant–fern interactions in a Costa Rican rain forest. (a) The understory epiphytic fern *Antrophyum lanceolatum* (L.) Kaulf. (b) Image of the rhizome mass of *A. lanceolatum* with ant debris created by a nest of *Pheidole flavens*. (c) *Pheidole flavens* collected from the root mass; in large ferns nests can contain over 100 ants. Bar, 1 mm. (d) Results of the nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope survey. Error bars represent standard error. Squares, ant debris; diamonds, plants with ants; circles, plants without ants. *Pheidole flavens* identification and photo courtesy of John Longino Evergreen State College.

standard Pee Dee belemnite (PDB). The measurement precision is better than 0.2‰ for C and 0.4‰ for N.

Mixing models

Stable isotope values of both C and N can be used to evaluate food web dynamics of insect–animal interactions. The ratio of natural abundance of $\delta^{15}\text{N}$ isotopes can be used to evaluate nitrogen contribution from consumer to host because consumers and their wastes are often enriched relative to their host plant (Post, 2002). To quantify the fraction of N transfer from ant nests to plants, we applied a two-end-member mixing model (% N from ants = $(\delta^{15}\text{N}_{\text{occupied}} - \delta^{15}\text{N}_{\text{unoccupied}}) / (\delta^{15}\text{N}_{\text{debris}} - \delta^{15}\text{N}_{\text{unoccupied}})$), where $\delta^{15}\text{N}_{\text{occupied}}$ is the nitrogen isotopes of plant leaf material with rhizome nests, $\delta^{15}\text{N}_{\text{unoccupied}}$ is the nitrogen isotopes of plant leaf material lacking rhizome nests, and $\delta^{15}\text{N}_{\text{debris}}$ is the nitrogen isotopes of waste material extracted from rhizome nests). We did not apply a mixing

model to the $\delta^{13}\text{C}$ values as there was no difference in $\delta^{13}\text{C}$ values in occupied and unoccupied plants.

Results and discussion

In the case of most myrmecophytic species, host plants produce highly specialized domatia to house ants. Yet, not all myrmecophytes produce such specialized domatia and we found that 62% of the sampled individuals of the epiphytic fern *A. lanceolatum* (Fig. 1a) harbored the ant species *P. flavens* in their rhizome mats (Fig. 1b,c). In larger fern individuals, these rhizome nests can contain over 100 individual ants and become filled with ant wastes. Such waste material was always highly decomposed (Fig. 1) and we could not determine the identity of the material to verify potential ‘prey items.’ The isotopic signatures of $\delta^{13}\text{C}$ for occupied and unoccupied plants were similar ($F = 4.17$, $P = 0.80$, $df = 8$; Fig. 1) suggesting that the contribution of respired CO_2 from ants to the fern

was minimal. However, in the case of $\delta^{15}\text{N}$, the isotopic values of occupied plants were intermediate between those of ant debris and unoccupied plants ($F = 9.74$, $P = 0.005$, $df = 12$; Fig. 1d). These results suggest that occupied plants receive N from ant sources. Results from the two-end-member mixing model suggest that, on average, ant debris contributed 54.1% (45.6–62.6%) of the N budget of the plant. Variation around the mean is represented by confidence interval calculations following the method presented in Phillips & Gregg (2001).

Without specialized domatia to capture expired CO_2 , it was not surprising that ant nests contributed little C to this fern. However, the N contribution was considerable. While N contributions to terrestrial plants have been reported to be higher (Sagers *et al.*, 2000), this fern receives twice the amount of N compared with *D. major* (Treseder *et al.*, 1995), the only other domatia-bearing epiphyte that has been evaluated using isotopes. Such high levels of ant-derived N can provide significant benefit to ferns living on understory tree trunks where N is limited. Our results demonstrate that fern–animal associations can radically improve the nutrient relations of the host ferns. Unlike the fern species in the genera *Lecanopteris* and *Solanopteris*, *A. lanceolatum* invests neither in potentially costly and specialized domatia nor in foliar nectaries or fruiting bodies. Thus, the benefit of hosting ants seems to come at little cost to *A. lanceolatum*. While such N contribution is clearly considerable, the relationship is facultative as ants were absent from 38% of the sampled individuals. In addition, ants did not become aggressive when the host was disturbed.

Apart from the discovery that such an important relationship exists in a lineage of plants often thought to be devoid of animal interaction, these results have a bearing on the potential cryptic nature of plant–animal interactions. The notion that plants lacking domatia may benefit from ant associations is not new (Wagner, 1997); however, our data support the hypothesis that animals may provide substantial nutritional benefit to plants with little if any investment on the part of the plant. Such cryptic associations are likely to go unrecognized yet may be an important component of plant nutrient relations.

James E. Watkins Jr¹*, Catherine L. Cardelús¹ and Michelle C. Mack²

¹Colgate University, Department of Biology, 13 Oak Drive, Hamilton, NY 13346, USA; ²University of Florida, Department of Botany, Gainesville, FL 32611, USA
(*Author for correspondence:
tel +1 315 228 7660; fax +1 315 228 7997; email
j.watkins@mail.colgate.edu)

References

- Auerbach MJ, Hendrix SD. 1980. Insect–fern interactions – macro-lepidopteran utilization and species–area association. *Ecological Entomology* 5: 99–104.
- Balick MJ, Furth DG, Cooper-Driver G. 1978. Biochemical and evolutionary aspects of arthropod predation on ferns. *Oecologia* 35: 55–90.
- Barker MS, Shaw SW, Hickey RJ, Rawlins JE, Fetzner JW. 2005. Lepidopteran soral crypsis on Caribbean ferns. *Biotropica* 37: 314–316.
- Beattie A. 1989. Myrmecotrophy – plants fed by ants. *Trends in Ecology and Evolution* 4: 172–176.
- Fiedler K, Kuhlmann F, Schlick-Steiner BC, Steiner FM, Gebauer G. 2007. Stable N-isotope signatures of central European ants – assessing positions in a trophic gradient. *Insectes Sociaux* 54: 393–402.
- Forel A. 1904. In und mit Pflanzen lebende Ameisen aus dem Amazonas-Gebiet und aus Peru, gesammelt von Herrn E. Ule. *Zoologische Jahrbucher Abteilung fur Systematik* 20: 679–707.
- Gay H. 1991. Ant-houses in the fern genus *Lecanopteris* Reinw. (Polypodiaceae) – the rhizome morphology and architecture of *L-sarcopus* Teijsm and Binned and *L-darnaedii* Hennipman. *Botanical Journal of the Linnean Society* 106: 199–208.
- Gay H. 1993a. Animal-fed plants – an investigation into the uptake of ant-derived nutrients by the Far-Eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Biological Journal of the Linnean Society* 50: 221–233.
- Gay H. 1993b. Rhizome structure and evolution in the ant-associated epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). *Botanical Journal of the Linnean Society* 113: 135–160.
- Gay H, Hensen R. 1992. Ant specificity and behaviour in mutualisms with epiphytes: the case of *Lecanopteris* (Polypodiaceae). *Biological Journal of the Linnean Society* 47: 261–284.
- Gómez LD. 1974. Biology of the potato-fern *Solanopteris brunei*. *Brenesia* 4: 37–61.
- Gómez LD. 1977. The Azteca ants of *Solanopteris brunei*. *American Fern Journal* 67: 31.
- Heads PA. 1986. Bracken, ants and extrafloral nectaries. 4. Do wood ants (*Formica-lugubris*) protect the plant against insect herbivores? *Journal of Animal Ecology* 55: 795–809.
- Heads PA, Lawton JH. 1984. Bracken, ants and extrafloral nectaries. 2. The effect of ants on the insect herbivores of bracken. *Journal of Animal Ecology* 53: 1015–1031.
- Janzen DH. 1969. Allelopathy by myrmecophytes – ant *Azteca* as an allelopathic agent of *Cecropia*. *Ecology* 50: 147–153.
- Jensen AS, Holman J. 2000. Macrosiphum on ferns: taxonomy, biology and evolution, including the description of three new species (Hemiptera: Aphididae). *Systematic Entomology* 25: 339–372.
- Kaufmann E, Maschwitz U. 2006. Ant-gardens of tropical Asian rainforests. *Naturwissenschaften* 93: 216–227.
- Koptur S, Rico-Gray V, Palacios-Rios M. 1998. Ant protection of the nectaried fern *Polypodium plebeium* in central Mexico. *American Journal of Botany* 85: 736–739.
- Lawton JH, Heads PA. 1984. Bracken, ants and extrafloral nectaries. 1. The components of the system. *Journal of Animal Ecology* 53: 995–1014.
- Mehlreter K, Rojas P, Palacios-Rios M. 2003. Moth larvae-damaged giant leather-fern *Acrostichum danaeifolium* as host for secondary colonization by ants. *American Fern Journal* 93: 49–55.
- Myerson J. 1992. *Emerson and Thoreau: the contemporary reviews*. Cambridge, UK: Cambridge University Press.
- Palmer TM, Brody AK. 2007. Mutualism as reciprocal exploitation: African plant-ants defend foliar but not reproductive structures. *Ecology* 88: 3004–3011.
- Phillips DL, Gregg JW. 2001. Uncertainty in source partitioning using stable isotopes (vol 172, pg 171, 2001). *Oecologia* 128: 304–304.
- Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718.
- Rashbrook VK, Compton SG, Lawton JH. 1992. Ant-herbivore interactions – reasons for the absence of benefits to a fern with foliar nectaries. *Ecology* 73: 2167–2174.
- Sagers CL, Ginger SM, Evans RD. 2000. Carbon and nitrogen isotopes trace

- nutrient exchange in an ant-plant mutualism. *Oecologia* 123: 582–586.
- Solano PJ, Dejean A. 2004. Ant-fed plants: comparison between three geophytic myrmecophytes. *Biological Journal of the Linnean Society* 83: 433–439.
- Sternberg LD, Pinzon MC, Moreira MZ, Moutinho P, Rojas EI, Herre EA. 2007. Plants use macronutrients accumulated in leaf-cutting ant nests. *Proceedings of the Royal Society B-Biological Sciences* 274: 315–321.
- Tempel AS. 1983. Bracken fern (*Pteridium-aquilinum*) and nectar-feeding ants – a nonmutualistic interaction. *Ecology* 64: 1411–1422.
- Treseder KK, Davidson DW, Ehleringer JR. 1995. Absorption of ant-provided carbon-dioxide and nitrogen by a tropical epiphyte. *Nature* 375: 137–139.
- Wagner D. 1997. The influence of ant nests on Acacia seed production, herbivory and soil nutrients. *Journal of Ecology* 85: 83–93.
- Weintraub JD, Lawton JH, Scoble MJ. 1995. Lithinine moths on ferns – a phylogenetic study of insect-plant interactions. *Biological Journal of the Linnean Society* 55: 239–250.

Key words: ant garden, mixing model, myrmecotrophy, nitrogen, plant–animal interactions, pteridophyte, stable isotopes.