Commentary

Scaling-up evaluation of field functioning of arbuscular mycorrhizal fungi

An estimated 90% of terrestrial plants form symbiotic associations with soil fungi, and the majority of those plant species belong to families that characteristically form associations with arbuscular mycorrhizal (AM) fungi (Smith & Read, 1997). The function of these associations is largely based upon the transfer of carbon (C) from the plant to the fungus, and upon the transfer of mineral nutrients, mainly phosphorus (P), from the fungus to the host plant. Arbuscular mycorrhizal fungi may confer other benefits, including improved soil aggregate stability, plant–water relationships and resistance to plant pathogens, but these benefits have rarely been quantified in an ecosystem context (Newsham *et al.*, 1995).

'... , the costs and benefits ascribed to the association in microcosms are especially challenging to confirm in nature.'

In microcosms, the plant's benefit from mycorrhizal associations varies with species of AM fungi (Van der Heijden *et al.*, 1998a). This variation in benefit is ecologically relevant (Bever *et al.*, 2002) as it explains plant diversity responses to manipulations of fungal diversity (Van der Heijden *et al.*, 1998b). However, the costs and benefits ascribed to the association in microcosms are especially challenging to confirm in nature (Johnson *et al.*, 1997). Field experiments have focused on agricultural monocultures and prevalent species of AM fungi (e.g. *Glomus intraradices*) (Lekberg & Koide, 2005). Studies of mycorrhizal functioning in more complex natural systems are lacking (Read, 2002).

Quantification of the cost–benefit of individuals and consortia of AM fungi is pivotal for understanding C and nutrient cycling in the context of ecosystem adaptation to global change (Fitter *et al.*, 2000). Although mycorrhizal research has advanced in the study of P and C exchanges at discrete scales of size and organization, studies of function at lower scales have not yet explained AM fungal interactions for individual genotype, phenotype and environment (Miller & Kling, 2000). New and creative in-field approaches for measuring AM fungus and host responses are crucial for assessing the temporal and spatial variabilities of mycorrhizal interactions.

Pringle & Bever's study, in this issue of *New Phytologist* (pp. 162–175), focuses on AM fungal species as a determinant of the benefit for plant species in a North Carolina grassland. Their experimental design substantiates the use of lower-scale evaluation of phenotype for AM fungi based on the finding that the mycorrhizal responsiveness of plant species under growth chamber conditions is correlated with that response in the highly divergent field environment. They conclude that AM fungus identity influences the survival and fitness of grassland plants by demonstrating that certain fungal species consistently promote the growth of a diverse set of plants. To define fungal phenotype in the field they confine the evaluation of plant response to the timescale over which the roots are still predominantly colonized by the introduced AM fungus. The consistency of the mycotrophic response across a range of plant species, as well as environmental conditions, validates estimation of a 'functional phenotype' in the field based on the growth response under controlled conditions.

Attempting to estimate a 'functional phenotype' might be questioned in light of recent evidence for AM fungus–host specificity (Klironomos, 2003) and wide variation in plant vs fungal pathways for P acquisition among host–fungal interactions (Smith *et al.*, 2004). If root colonization is not a good predictor for P uptake or the host response, measurement of lower-level mechanisms for nutrient uptake in mycorrhizal plants may not readily scale-up to the field level. Nevertheless, definition of a growth response phenotype for AM fungi across different host, soil and fertility conditions may provide a practical approach for assessment of their functioning under natural conditions.

Mediation by AM fungi of vegetation responses to environmental change will require experiments at a minimum of two scales to develop and test models for mycorrhizal functioning (Huston, 1999). Experimental designs should either (1) integrate multiple mechanisms at the landscape scale and include such measures as mycorrhizal influences on net primary production, evapotranspiration and nutrient cycling or (2) integrate measures of AM fungal diversity into assessment of ecosystem function. Extension of lower-level processes that determine ecosystem dynamics must be accomplished with smaller-scale experiments such as implantation of plant species of varying

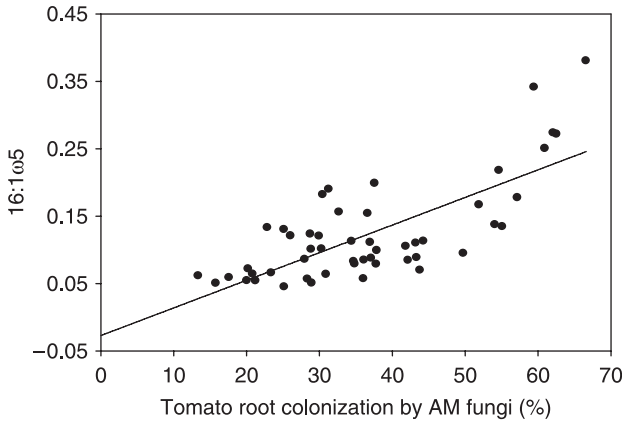

Fig. 1 Relationship of the fatty acid 16:1ω5 content in tomato roots to the observed proportion of colonization of tomato roots in field plots by arbuscular mycorrhizal (AM) fungi. Results are from year 5 of the 5-yr cropping study in southeast Florida. Curve fit: $r^2 = 0.54$. Data from C. Rasmann *et al*., unpublished.

life histories colonized by representative AM fungi from that ecosystem, as validated by Pringle & Bever. Lower-scale experiments need to be designed to test each component of fitness of the symbiotic partners under relevant environmental and spatial scales in the field. The design could involve the use of *in situ* root observation windows, mesh dividers, or bags and isotopic tracers and signature fatty acids (e.g. Fig. 1) to assess the dynamics of nutrient and C exchanges. Measurements of C and P exchanges under controlled conditions are well refined and have been successfully extended to the field (Olsson *et al*., 1999; Schweiger & Jakobsen, 1999; Johnson *et al*., 2001).

Even with novel approaches for scaling up the evaluation of functioning in field studies, the dynamics of C and P in mycorrhizal associations in the field will be difficult to interpret under complex conditions in plant communities, as illustrated by the recent study of Li *et al*. (2008). In their attempt to integrate the phenomenon of plant vs AM pathways for root P acquisition into the consideration of cost–benefit of wheat mycorrhizas, they concluded that mycorrhizal responses of plants grown singly may not apply at the population level. Thus, consideration of functional diversity under natural conditions of plant and fungus competition remains a challenging frontier in mycorrhizal biology.

James H. Graham
University of Florida, Citrus Research and Education Center, Lake Alfred, FL 33850, USA
(tel +1863 9561151; fax +1863 9564631; email jhg@crec.ifas.ufl.edu)

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Resolving uncertainty in the carbon economy of mycorrhizal fungi

Mycorrhizal fungi are amongst the most important soil microorganisms in the terrestrial carbon cycle. In ecto-, arbuscular and ericoid mycorrhizal fungi, the quantity of carbon allocated to the fungus can be substantial (Smith & Read, 2008) and transfer occurs very rapidly, often within hours of fixation by the plant. The evidence for this comes from experiments that differ in design such that a variety of assumptions and limitations are introduced to different extents. These may include the omission of particular pathways, such as respiration (shoot, root or fungal), consideration of carbon movement in only one direction (not surprisingly, typically plant to fungus) and the use of fungal strains that are essentially 'laboratory rats' and which might give a somewhat biased view of mycorrhizal functioning in nature. In this issue (pp. 176–184), Cameron *et al.* combine clever experimental design with plants and fungi removed from the field to overcome many of these limitations to provide data on the carbon 'cost' of a mycorrhizal symbiosis, which can (almost) be considered a complete budget. Their study is all the more valuable because it quantifies carbon fluxes between the adult green orchid *Goodyera repens* and its mycorrhizal endophyte *Ceratobasidium cornigerum*. Despite their widespread distribution, diversity, intriguing ecology and importance for conservation and horticulture, orchids and their mycorrhizal fungi remain poorly studied groups.

'... the polarity of carbon movement between plant and fungus dramatically switches between the achlorophyllous juvenile stages of the plant and the adult stage when it becomes fully autotrophic.'

Up-flow, down-flow

The experimental design employed by Cameron *et al.* utilized two identical sets of microcosms that enabled quantification of carbon flux from plant to fungus by subjecting shoots to $^{14}CO_2$, and from fungus to plant by exposing external mycorrhizal mycelium to a ^{14}C-labelled algal amino acid mixture. The latter pathway is generally ignored in studies of mycorrhizal associations (e.g. Johnson *et al.*, 2002), but this is potentially unsafe, regardless of mycorrhizal type. In ectomycorrhizas, for example, Frank (1894) suggested that the fungi could access organic nitrogen, and subsequent experimental work has demonstrated this to be the case. It has since been suggested that this heterotrophically obtained carbon may reduce demands on host plant photosynthate (Taylor *et al.*, 2004). A similar situation also exists for ericoid associations, which like many ectomycorrhizal plants are often found in soils that contain quantities of organic forms of nutrients far in excess of inorganic forms and where compounds such as simple amino acids are rapidly recycled (Smith & Read, 2008). Furthermore, evidence is now accruing that suggests that arbuscular mycorrhizal plants may also be able to access organic nitrogen sources (Hodge *et al.*, 2001; Rains & Bledsoe, 2007), although the specific role of the fungi in taking up amino acids directly has yet to be demonstrated. Given that many temperate grassland soils also contain large amounts of simple organic nitrogen compounds, it seems possible that fungus to plant transfer of carbon may occur in these systems, and so this pathway should not necessarily be ignored.

In the case of *Goodyera*, Cameron *et al.* found that carbon did move in both directions, but that the minimum flux from plant to fungus was nearly 6-fold greater than the maximum flux from fungus to plant. It is noteworthy that the loss of plant assimilate from fungal respiration was small (0.4% of the total carbon fixed by the plant) compared with estimates from both ecto- (Heinemeyer *et al.*, 2007) and arbuscular (Johnson *et al.*, 2002) mycorrhizal systems, and compared with the proportion respired (2.5%) from the amino acid source. Although one can make some criticisms of their experimental design, such as the addition of the ^{14}C-labelled amino acid mixture to a small spatially distinct part of the mycelium, the clear message from the data is that adults of this species of orchid and their mycorrhizal fungi behave like a true mutualism. This is an important finding for a number of reasons, not least because the polarity of carbon movement between plant and fungus dramatically switches between the achlorophyllous juvenile stages of the plant and the adult stage when it becomes fully autotrophic. Elucidation of the genetic control of this switch by taking similar approaches to those currently used in studies of arbuscular mycorrhizal symbioses (Krajinski & Frenzel, 2007) must be a promising future line of inquiry. This may tell us more not only about the functioning of orchid mycorrhizas, but also about the placement of mycorrhizal associations along the mutualism–parasitism continuum (Johnson *et al.*, 1997).

Contradictory findings

The results of Cameron *et al.*, along with those of their previous study (Cameron *et al.*, 2006), are at odds with the only other investigations of plant to fungal carbon flux in *G. repens* (Hadley & Purves, 1974; Alexander & Hadley, 1985). The outcome of these earlier studies was that recent assimilate from adult plants did not move to the fungus, even though their experimental designs were broadly similar to those used by Cameron *et al.*

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(2006, 2008). Cameron *et al.* (2006) suggested that some of the differences in environmental conditions, notably temperature, in these experiments could explain the different results, but this does not seem to be a very satisfactory explanation for such contradictory evidence. It is vital that the earlier studies of Hadley and colleagues are not ignored but are considered alongside those of Cameron *et al.* to inspire future research on the functioning of orchids and other mycorrhizal types. The influential paper by Nancy Johnson and colleagues (Johnson *et al.*, 1997) suggests an important role of the interaction between environment and genotype (of both host plant and fungus) in placing mycorrhizal associations along the mutualism–parasitism continuum, and, given the relative simplicity of the *G. repens* mycorrhizal symbiosis and its experimental tractability, further investigation of the potential for genotypic controls of these contradictory responses is required. The potential lack of any carbon 'pay-back' to some genotypes of *C. cornigerum* when the host plant is in the adult stage may have considerable consequences for the fitness of the fungus and the stability of the association.

Improving the budget

The major uncertainty in the budget presented by Cameron *et al.* concerns the quantity of carbon allocated to fungal tissues inside the host plant root. By either including or excluding the root and associated intraradical mycelial pool, they estimated a 153-fold difference between the maximum and minimum fluxes of carbon from plant to fungus. Given the extensive fungal development seen in orchid roots (Smith & Read, 2008), a significant proportion of this variation must be a result of allocation to the fungus. This clearly emphasizes the importance of obtaining reliable measurements of both fungal biomass and carbon allocation to it, in host plants roots. This situation is not just specific to orchid mycorrhizas: we know remarkably little about the intraradical biomass of arbuscular and ericoid mycorrhizas. What we do know is largely derived from simplistic calculations based on root and fungal volume or measurement of ergosterol content, which is considered to be a general biomarker of biomass, an approach that must be treated with caution (Olsson *et al.*, 2003). Fine-scale quantitative isotope imaging systems, such as secondary ion mass spectrometry (nanoSIMS; Popa *et al.*, 2007), are now in routine use, but are little used in mycorrhiza research. We must now develop these cutting-edge techniques to quantify nutrients (including carbon) in intraradical fungal structures, whether they are arbuscules, vesicles, or hyphal coils, and reduce the large uncertainties in mycorrhizal carbon budgets.

Chasing the pulse

There can be little doubt that isotope pulse-chase experiments have enabled (and will hopefully continue to enable) significant advances in mycorrhizal functioning to be made, and the experiments reported by Cameron *et al.* (2006, 2008) certainly fit this category. However, one must remember that quantifying carbon budgets on this basis provides information on, firstly, the flux of only recent plant assimilate and, secondly, a very small part of the life of both plant and fungus. The former issue can be overcome by utilizing continuous steady-state isotope labelling. In a recent elegant application of this technique, it was found that there was a differential use of 'old' and 'new' carbon sources by mycorrhizal and nonmycorrhizal *Lolium perenne* plants (Grimoldi *et al.*, 2006), whereby the former allocated a greater proportion of old carbon to their mycorrhizal symbionts. This approach can therefore provide valuable information on the physiological interactions between plant and fungus that would otherwise be ignored by conventional pulse-chase experiments of the sort used by Cameron *et al.* The amount of carbon allocated to mycorrhizal fungi can also only be estimated accurately when carbon fluxes are measured in a range of ecologically relevant environmental conditions and at numerous stages in the lives of the organisms involved and so, ultimately, experiments have to be undertaken in the field. As it associates with only a single mycorrhizal endophyte, *Goodyera* is a prime candidate for developing techniques to undertake these challenging measurements.

David Johnson

Institute of Biological and Environmental Sciences, Cruickshank Building,University of Aberdeen, Aberdeen AB24 3UU, UK (tel +44(0)1224 273857; fax +44(0)1224 272703; email d.johnson@abdn.ac.uk)

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Key words: carbon cycle, continuous labelling, fungal genotype, green orchid, mutualism–parasitism, pulse labelling, respiration.

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*

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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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 $\delta^{15}N$ and $\delta^{13}C$ 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 Instrumentazion, Milan, Italy). The reference CO_2 was calibrated against 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 $\delta^{15}N$ 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 = $(\delta^{15}N_{occupied} - \delta^{15}N_{unoccupied})/(\delta^{15}N_{debris} - \delta^{15}N_{unoccupied})$, where $\delta^{15}N_{occupied}$ is the nitrogen isotopes of plant leaf material with rhizome nests, $\delta^{15}N_{unoccupied}$ is the nitrogen isotopes of plant leaf material lacking rhizome nests, and $\delta^{15}N_{debris}$ is the nitrogen isotopes of waste material extracted from rhizome nests). We did not apply a mixing model to the $\delta^{13}C$ values as there was no difference in $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{15}N$, 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.

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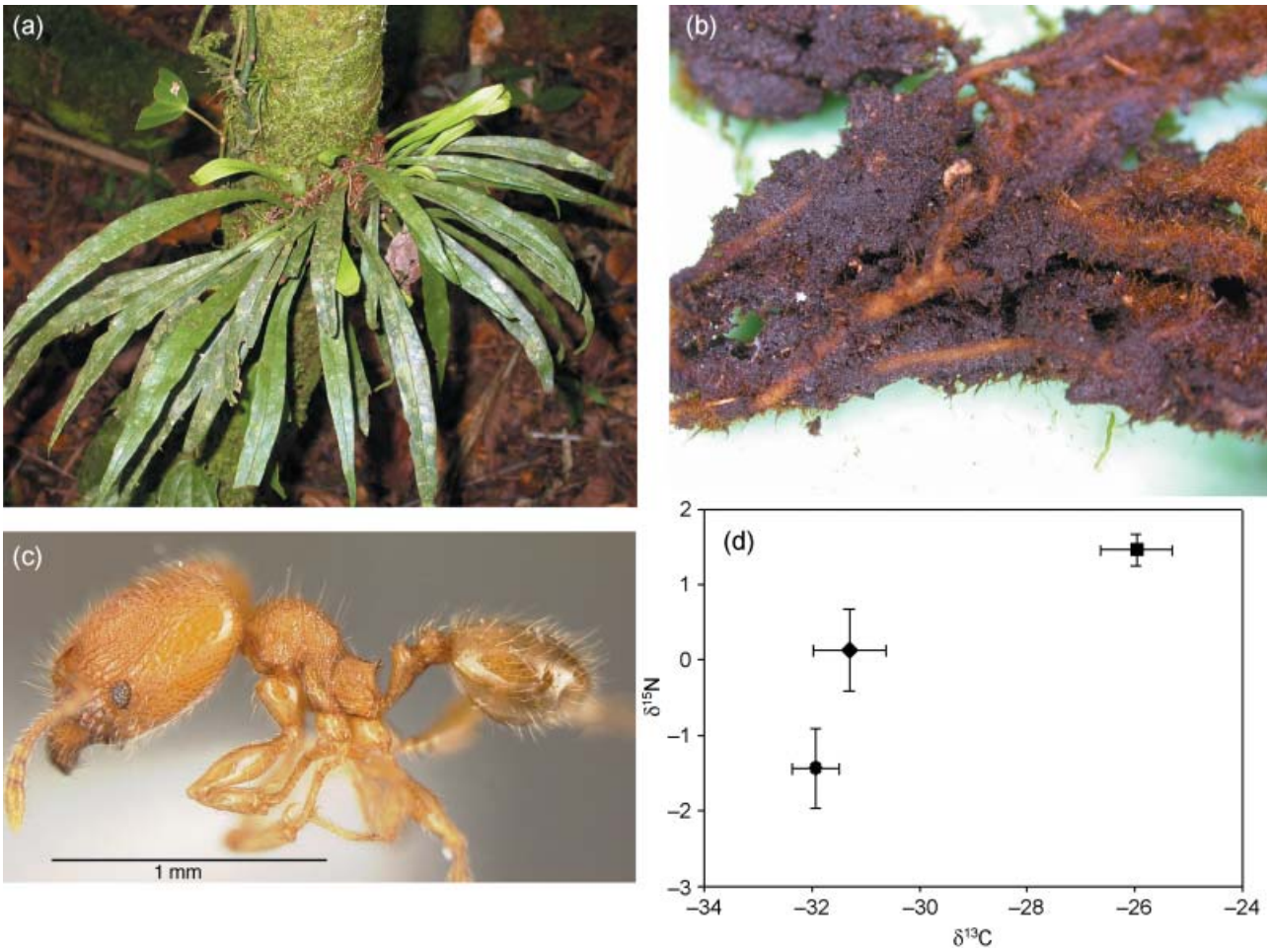

Fig. 1 Ant–fern interactions in a Costa Rican rain forest. (a) The understory epiphytic fern *Antrophyum lanceolatum* (L.) Kaulf. (b) Image of the rhizome mass of *A. lanceolatum* with ant debris created by a nest of *Pheidole flavens*. (c) *Pheidole flavens* collected from the root mass; in large ferns nests can contain over 100 ants. Bar, 1 mm. (d) Results of the nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) 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.

James E. Watkins Jr[1]*, Catherine L. Cardelús[1] and Michelle C. Mack[2]

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

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

Meetings

Arsenic-eaters: by accident or by design

Arsenic: unraveling its metabolism and speciation in plants, Aberdeen, UK, June 2008

In late June 2008 over 70 people gathered at the Douglas Hotel in Aberdeen, Scotland to discuss the metabolism and chemical speciation of arsenic (As). For the participants originating closer to the equator, the surprising midnight blue skies were certainly a treat, particularly when leaving the pub around midnight after some 'off-site' scientific discussion. But putting aside the pleasures of Aberdeen in early summer, what is so interesting about arsenic that would persuade over 70 people to spend 2 days discussing it? Well there are many interesting aspects of arsenic biogeochemistry, some better known than others. For example, numerous reports exist in the literature of arsenic being consumed for medical reasons, the most extreme of which are the 'arsenic-eaters' from the mid-1800s who consumed arsenic compounds to improve their general well-being (for a review see Przygoda *et al.*, 2001). However, there is no doubt that arsenic is best known for its toxicity, which was recognized even by the ancient Greeks. Deliberate arsenic poisoning is now generally a thing of the past. However, as a result of natural and anthropogenic processes arsenic still threatens human societies through its entrainment in the food chain. It was to discuss this issue that participants attended the meeting. Topics ranged across multiple plant species and scales, from the molecular to the field, and covered a diverse set of processes, from arsenic soil chemistry, plant biological and analytical chemistry, to arsenic contamination of food and the practical approaches that could be

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taken to reduce such contamination. It was also clear from the meeting that research into the biogeochemistry of arsenic is perhaps one of a handful of research areas that is naturally moving towards a model in which researchers utilize numerous experimental systems, from traditional genetic models such as *Arabidopsis thaliana*, and 'natural' populations such as the grass *Holcus lanatus* and the fern *Pteris vittata*, to crops such as rice (*Oryza sativa*), for both research and final deployment of new traits such as reduced grain arsenic accumulation. Using these diverse approaches rapid progress is being made in this field, and highlights of this work presented at the meeting are summarized below.

'Not only is the amount of arsenic in the grain crucial to its toxicity, but also the chemical form of the arsenic in the grain plays a critical role.'

Arsenic reduction

It is now well established that plants take up arsenate (As(V)) as a phosphate analog and rapidly reduce it to arsenite (As(III)) in roots. In most plants the As(III) produced by this reductive process is chelated by phytochelatins (PCs) and the majority is stored in the root vacuoles. Also it has recently been demonstrated that As(III) can be effluxed out of the roots in hydroponic systems (Xu *et al.*, 2007). Reduction, PC chelation and storage also occur on uptake of methylarsenate. However, dimethylarsenate appears not to be reduced or complexed, and this may explain its preferential translocation from roots to shoots (Jörg Feldmann, University of Aberdeen, UK). Interestingly, in arsenic-hyperaccumulating plants such as the fern *P. vittata*, after reduction As(III) is preferentially translocated from the roots to the shoots as the As(III) oxyanion arsenite in which form it is stored in the shoot. Plant enzymes capable of reducing arsenate to arsenite have been cloned and enzymatically characterized from *A. thaliana*, *H. lanatus*, *P. vittata* and rice (Bleeker *et al.*, 2006; Ellis *et al.*, 2006; Duan *et al.*, 2007). However, the function of these enzymes *in vivo* remains to be fully understood. In *A. thaliana* knockdown and loss-of-function mutants are both hypersensitive to As(V) (Bleeker *et al.*, 2006; Dhankher *et al.*, 2006) as would be expected if arsenate reduction is necessary to resistance. Furthermore, *A. thaliana* lines ectopically overexpressing the gene that encodes arsenate reductase are more resistant to arsenate. Interestingly, Henk Schat (Vrije Universiteit Amsterdam, the Netherlands) reported at the meeting that these arsenic phenotypes are dependent on the phosphorus (P) status of the plants, with high P reversing these phenotypes. This gave rise to the suggestion that under low P, where loss-of-function of arsenate reductase causes arsenate hypersensitivity, it is As(V) that is the toxic species. However, under elevated P nutrition, where loss-of-function of arsenate reductase causes enhanced As(V) resistance, As(III) is the likely toxic species. Unpublished data on arsenic speciation in both yeast and *A. thaliana* lacking arsenate reductase show that the majority of arsenic is still reduced to As(III) in these organisms, suggesting that there may be several ways to reduce As(V) to As(III) *in vivo*. These issues await further clarification.

Arsenic transport

In aerobic environments arsenic is thought to be taken up by a phosphate transporter(s). However, in anaerobic environments, such as during rice cultivation, arsenite is the main species present in the soil and therefore taken up by plants. However, the transport proteins for both arsenite uptake from the soil and transport from roots to shoots were unknown. As reported at the meeting (Fangjie Zhao, Rothamsted Research, UK), and recently published (Ma *et al.*, 2008), this mystery is now solved, at least for rice. Ma and co-authors have elegantly established that arsenite uptake into roots and transport into the xylem occur via the previously characterized silicon transporters OsLsi1 and OsLsi2, which are, respectively, an aquaglyceroporin (a member of the subfamily of nodulin26-like intrinsic proteins (NIPs)) and a silicon/arsenite efflux carrier. Mutation of OsLsi1 causes a significant decrease in arsenite uptake in rice. Furthermore, mutation of the silicon efflux transporter OsLsi2 reduces arsenite transport to the xylem and also accumulation of arsenic in the shoot and grain. Arsenite transport in rice therefore appears to share the same transport pathway as silicon, which explains why rice, a silicon accumulator, also accumulates elevated arsenic. Data reported at the meeting from several different research groups (Thomas Jahn (University of Copenhagen, Denmark), Toru Fujiwara (University of Tokyo, Japan) and Frans Maathuis (University of York, UK)) now also establish that members of the NIP subfamily can act as bidirectional arsenite transporters in other species, including *A. thaliana* and *Lotus japonicus*. Specifically, loss-of-function mutants *nip1;1* and *nip7;1* in *A. thaliana* both show increased resistance to arsenite, and NIP1;1 directly transports arsenite in oocytes, suggesting its direct involvement in arsenite uptake by roots. Furthermore, *NIP5;1* and *NIP6;1* were also shown to increase arsenite sensitivity when heterologously expressed in yeast, although the *in vivo* function of these proteins is not yet clear. Interestingly, NIPs do not appear to be the only means by which plants can transport arsenite. As reported at the meeting (David E. Salt, Purdue University, IN, USA) it would appear that the arsenic-hyperaccumulating fern *P. vittata* transports arsenite via a protein in the bile/arsenite/riboflavin (BART) superfamily, with close homology to the yeast arsenite effluxer

arsenic compounds resistance 3 (ACR3). When heterologously expressed in yeast the *P. vittata PvACR3* gene fully complements the yeast *Δacr3* mutation, and knockdown of *PvACR3* by RNAi in *P. vittata* causes severe arsenite hyper-sensitivity. Interestingly, orthologs of *PvACR3* are absent from all sequenced angiosperm genomes, suggesting that this gene has been lost during the evolution of angiosperms.

Arsenic in the food chain

A major route of arsenic poisoning in humans is through the consumption of arsenic-tainted food. There is substantial variation in the natural concentrations of arsenic in soils and groundwater around the world. However, arsenic has also been introduced into the environment by a number of human activities. These include industrial and mining activities, as one might expect, along with other less obvious activities such as the agricultural use of arsenic-based pesticides or the sinking of tube wells in countries such as Bangladesh, which tap into arsenic-contaminated groundwater which is used to irrigate crops such as rice. Andy Meharg (University of Aberdeen, UK) and co-workers demonstrated that rice was far more adept than other cereal crops at taking up arsenic from the soil. This is probably attributable in part to the anaerobic conditions of the soil in the flooded paddy fields where rice is grown, which promote the reduction of arsenate to arsenite, allowing its efficient uptake via aquaglyceroporin/silicon transporters. Arsenic has been shown to accumulate to elevated concentrations in grain (0.3–0.5 μg g^{-1}) from various regions around the world, including Bangladesh (from irrigation water) and the southern central USA (potentially from arsenic-containing pesticides) (Williams *et al.*, 2006, 2007). Meharg indicated that, on the basis of the US Environmental Protection Agencies 2002 recommendations for arsenic toxicity (developed from epidemiological studies performed in Taiwan), people subsisting primarily on rice with these elevated concentrations of arsenic are at a higher risk of cancer. Not only is the amount of arsenic in the grain crucial to its toxicity, but also the chemical form of the arsenic in the grain plays a critical role. Grain with higher concentrations of inorganic arsenic (arsenate and arsenite) is more toxic than grain with a higher proportion of organic arsenic (dimethyl arsinic acid). A major question discussed at the meeting is how to decrease the concentration of arsenic in the rice grain, and specifically how to decrease the concentrations of inorganic arsenic without altering grain yield. These issues were addressed by Adam Price (University of Aberdeen, UK) when he described efforts he is involved in to identify rice varieties with reduced arsenic accumulation in the grain. These experiments included using local rice varieties from various countries, recombinant inbred mapping populations, and an association mapping population grown on arsenic-contaminated fields in a number of countries in Asia. It is hoped that such low-grain-arsenic varieties can be used in breeding programs to introduce low-grain-arsenic traits into elite production varieties. There is also potential for the identification of genes involved in regulating grain arsenic loading which will gives us a greater understanding of both mechanisms of and natural variation in plant–arsenic interactions.

Arsenic phytoremediation

The idea of using plants for the restoration of trace element-contaminated environments can be traced back at least 35 yr to the pioneering work of Tony Bradshaw and co-workers who used metal-tolerant grass races for the restoration of mine tailings. The recent discovery of the arsenic-hyperaccumulating fern *P. vittata* by Ma *et al.* (2001) has opened the door to the possibility of using this fern to remediate, or phytoextract, arsenic from polluted soils. Such phytoextraction strategies have already been extensively tested for both cadmium and nickel using various *Thlaspi* and *Alyssum* species, and work has also been done on developing plants that directly volatilize Hg° as a mercury remediation strategy. Interestingly, the idea of developing plants engineered for the phytovolatilization of trimethyarsine gas as an arsenic remediation strategy was also discussed at the meeting by Barry Rosen (Wayne State University, MI, USA). He also reported the identification of an arsenite methyltransferase (ArsM) from the hot spring acidophilic alga *Cyanidoschyzon merolae* capable of methylating arsenite to trimethyarsine, bringing this potential approach one step closer to application. As was the case with the phytovolatilization of mercury, the toxicology of such an approach for arsenic needs to be carefully evaluated before it can be deployed.

Conclusion

It was clear from this meeting that integration of arsenic research groups focused on basic abiotic/biotic mechanisms, through environmental and food chain arsenic accumulation to restoration technologies, is allowing rapid progress to be made, with translation of basic scientific understanding into practical applications for limiting arsenic uptake into the food chain. The broad significance and general public interest of this work, and the optimum size of the research community, will also facilitate this process. Development of a community cyberinfrastructure was also discussed. This would further enhance community interactions through sharing of data sets, analysis tools, predictive models, educational material and collaborative tools. On a final note, we would also like to take this opportunity to acknowledge the significant contributions Prof. Mark Macnair has made over the last 30 yr in the field of the ecological genetics of arsenic, copper and zinc tolerance and hyperaccumulation in plants. Mark will be retiring from the University of Exeter this year to play croquet, among other things, and the talk he gave at this meeting on 'The ecological genetics of arsenic tolerance in *Holcus lanatus*' was his last as a University Professor. We wish Mark all the best and expect that his postdoc Prof. Andy Meharg (Meeting co-organizer) will continue the good work.

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David E. Salt[1]* and Gareth J. Norton[2]

[1]Department of Horticulture & Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA; [2]Department of Plant and Soil Science, University of Aberdeen, Aberdeen AB24 3UU, UK
(*Author for correspondence:
tel +1 765 496 2112; fax +1 765 464 0391;
email dsalt@purdue.edu)

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Key words: arsenic, metabolism, phytoremediation, reduction, speciation, transport.

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