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### Authors

Treseder, Kathleen K  
Davidson, Diane W  
Ehleringer, James R

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## Absorption of ant-provided carbon dioxide and nitrogen by a tropical epiphyte

Kathleen K. Treseder\*, Diane W. Davidson & James R. Ehleringer

Department of Biology, University of Utah, Salt Lake City, Utah 84112, USA

ALTHOUGH ant-plant mutualisms have been described in many ecosystems, the magnitude of the direct benefits from such relationships are hard to quantify. In Bako National Park, Sarawak, Malaysia, stunted 'kerangas' forests occur on nutrient-poor sandstone hills<sup>1-3</sup>. As trees are widely spaced and have a sparse leaf area, a significant amount of light reaches the tree trunks and enables a diverse community of epiphytes to thrive there<sup>4</sup>. One of these epiphytes, *Dischidia major* (Vahl) Merr. (Asclepiadaceae), has evolved unusual methods for enhancing carbon and nitrogen acquisition. We show here that a mutualistic relationship exists between ants of the genus *Philidris* and their host, *D. major*. Using stable isotope analysis, we calculate that 39% of the carbon in occupied host plant leaves is derived from ant-related respiration, and that 29% of the host nitrogen is derived from debris deposited into the leaf cavities by ants.

In addition to small coin-shaped leaves, *D. major* has evolved sac-like 'ant leaves' (Fig. 1), in which ants of the genus *Philidris*<sup>5</sup> (Dolichoderinae) frequently raise young and deposit debris (faeces, dead ants and scavenged insect parts)<sup>4,6</sup>. Adventitious roots from *D. major* grow through the cavity opening and proliferate wherever debris has accumulated<sup>4</sup>. It has been proposed

that the *Dischidia-Philidris* relationship is mutualistic and that *D. major* uses ant debris as a nitrogen source<sup>4</sup>; also, the stomata on the internal surfaces of leaf cavities<sup>7,9</sup> may absorb ant-respired carbon dioxide and thereby reduce transpirational water loss<sup>8,9</sup>, but neither of these suggestions is supported by experimental evidence.

We tested these hypotheses by measuring stable isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of ants, hosts and substrates, and by capitalizing on differences in the isotope composition of possible nutrient sources. This approach enabled us to quantify the benefit to hosts from their symbiotic ants.

The  $\delta^{13}\text{C}$  value of *Dischidia* depends on both the  $\delta^{13}\text{C}$  of the source  $\text{CO}_2$  ( $\delta^{13}\text{C}_{\text{CO}_2}$ ) and discrimination during carbon fixation ( $\Delta$ ), thus  $\delta^{13}\text{C}_{\text{plant}} = \delta^{13}\text{C}_{\text{CO}_2} - \Delta$ . *D. major* has obligate crassulacean acid metabolism (CAM)<sup>10,11</sup>, and we found that uninhab-

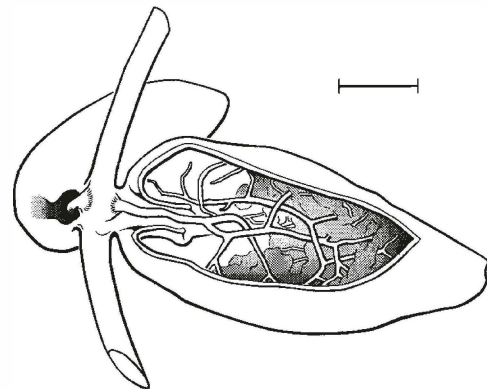


FIG. 1 Two ant-occupied leaves of *Dischidia major*: one is cut away to show adventitious roots and ant debris. The modified ant leaves are rolled up to form an enclosed abaxial surface and a cavity accessible through a small basal opening near the petiole<sup>4,6</sup>. Scale bar, 1 cm.

\* Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305, USA.

ited plants had a mean  $\delta^{13}\text{C}_{\text{plant}}$  value of  $-16.0\%$ , typical of CAM plants<sup>12</sup>. Given an atmospheric  $\delta^{13}\text{C}_{\text{CO}_2}$  value of  $-7.9\%$  (ref. 13),  $\Delta$  is calculated as  $8.1\%$ .

Ant respiration and decomposing debris are additional possible sources of  $\text{CO}_2$  in ant-occupied leaves and could alter  $\delta^{13}\text{C}_{\text{CO}_2}$ . The carbon isotope ratio of animals (and their respiration) is essentially the same as that of their food<sup>14,15</sup>. *Philidris* ants feed on the exudates of Homoptera<sup>4</sup> that ingest the phloem of  $\text{C}_3$  rainforest trees. Subsequently, the ants have low  $\delta^{13}\text{C}$  values averaging  $-25.9\%$  (Fig. 2).  $\delta^{13}\text{C}_{\text{CO}_2}$  used for photosynthesis by *D. major* could vary from  $-7.9\%$ , if no ant-derived  $\text{CO}_2$  is taken up, to  $-25.9\%$ , if ant-derived  $\text{CO}_2$  accounts for all fixed  $\text{CO}_2$ . The corresponding  $\delta^{13}\text{C}_{\text{plant}}$  values would be expected to vary from  $-16\%$  to  $-34\%$ .

If *D. major* incorporates  $^{13}\text{C}$ -deficient carbon dioxide respired by the ants or their debris, colonized leaves should have lower  $\delta^{13}\text{C}$  values than do uncolonized leaves. In addition, as colonies deposit debris inside a leaf only after they have raised brood there for some time<sup>4</sup>, ant leaves with debris should have had a longer time to accumulate ant-respired  $\text{CO}_2$ . Overall, completely vacant leaves should have the highest  $\delta^{13}\text{C}$  values (about  $-16.0\%$ ), followed by ant-occupied leaves that are debris-free, and finally, by debris-filled leaves. The three groups followed the expected pattern (Fig. 2). Therefore individuals of *D. major* take up significant amounts of carbon from *Philidris*.

To estimate the fraction of plant carbon derived from ant-related respiration (per cent  $\text{CO}_2$  from ants), we used the two-member mixing model:

$$\% \text{CO}_2 \text{ from ants} = \frac{\delta^{13}\text{C}_{\text{plant}} + \Delta - \delta^{13}\text{C}_{\text{CO}_2,\text{atm}}}{\delta^{13}\text{C}_{\text{ant}} - \delta^{13}\text{C}_{\text{CO}_2,\text{atm}}} \times 100\%$$

where  $\delta^{13}\text{C}_{\text{CO}_2,\text{atm}}$  and  $\delta^{13}\text{C}_{\text{ant}}$  are the isotope compositions of the atmosphere and ants, respectively. We calculated the fraction of plant carbon derived from ant-related respiration as  $39\%$  ( $\pm 8\%$ ,  $n=5$ ) for ant-occupied, debris-filled leaves;  $27\%$  ( $\pm 4\%$ ,

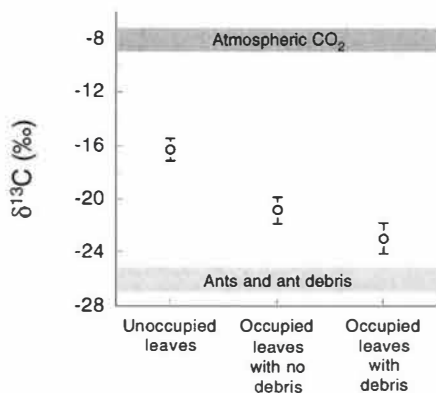


FIG. 2 Mean carbon isotope values  $\pm 1$  s.e. for *Dischidia major* ant leaves and the potential  $\text{CO}_2$  sources for these leaves (atmospheric  $\text{CO}_2$  ( $-7.9\%$ ), ants ( $-25.9\% \pm 0.3$ ,  $n=6$ ), or ant debris ( $-25.9\% \pm 0.9$ ,  $n=3$ ); shaded bars). Values differed significantly for the three groups of ant leaves ( $H_{[5,5,6]}=9.510$ ,  $P<0.009$ ).  $\delta^{13}\text{C}$  values of ant-related debris, a possible source of carbon dioxide, did not differ significantly from those of the ants ( $U_{[3,6]}=12$ ,  $P=0.731$ ).

**METHODS.** Material was collected along the Lintang trail in Bako National Park, Sarawak, Malaysia in August 1993. With an isotope ratio mass spectrometer (Delta S, Finnigan MAT at SIRFER, University of Utah, Salt Lake City), isotope ratios of ants, leaves and ant debris were measured on 2–3 mg samples. Isotope compositions are reported in  $\delta^{13}\text{C}$  values as  $\delta^{13}\text{C} = \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \right) \times 1,000\%$ , where  $^{13}\text{C}/^{12}\text{C}_{\text{sample}}$  and  $^{13}\text{C}/^{12}\text{C}_{\text{standard}}$  are the isotope ratios of the sample and standard, respectively<sup>25</sup>.

$n=6$ ) for ant-occupied leaves without debris; and  $4\%$  ( $\pm 4\%$ ,  $n=6$ ) for vacant leaves.

For ant-related  $\text{CO}_2$  to contribute so significantly to the carbon balance of the plant, the  $\text{CO}_2$  concentration inside ant-occupied leaves should be raised above atmospheric values. By having interior stomata fed by an increased  $\text{CO}_2$  concentration, the plant probably significantly increases its water-use efficiency (photosynthetic carbon gain to transpirational water loss). The capture of ant-respired  $\text{CO}_2$  may increase carbon gain (enhancing growth) and also reduce transpiration by curbing stomatal activity. Moreover, the interior stomata transpire into a partially enclosed cavity, where relative humidity should be higher than the surrounding atmosphere, resulting in further reduction in transpiration. Although *D. major* grows in a tropical climate, water loss is a concern because the epiphytes have no access to soil water.

*D. major* also exploits ant-deposited debris as a nitrogen source, and we used nitrogen isotope analysis to quantify the extent of this (the other likely nitrogen source, rainwater, has  $\delta^{15}\text{N}$  values different from ant-provided debris). *Dischidia nummularia*, which does not possess ant leaves but grows on the same host trees as *D. major*, has access to the same nitrogen sources, with the exception of the ant-provided debris. We found that the mean  $\delta^{15}\text{N}_{\text{plant}}$  value of *D. nummularia* was  $-3.5\%$ ; in contrast, debris deposited in the ant leaves of *D. major* was significantly more enriched in  $^{15}\text{N}$  ( $U_{[5,2]}=0$ ,  $P<0.05$ ; Fig. 3). This is expected, given that ant debris is composed mostly of scavenged insect parts<sup>4</sup>, and animal  $\delta^{15}\text{N}$  values tend to be  $3\%$  greater than those of their food sources<sup>16</sup>. *D. major* leaves were significantly enriched in  $^{15}\text{N}$  compared to those of *D. nummularia* ( $U_{[17,2]}=2$ ,  $P<0.05$ ; Fig. 3), suggesting that *D. major* absorbs nitrogen from ant-deposited debris. On average, *D. major* received about  $29\%$  of its nitrogen from ant debris (Fig. 3). Ant-related nitrogen could provide a large benefit to *D. major*, as nitrogen deficiency may be the major factor limiting epiphytic growth in the light-rich kerangas forests<sup>17</sup>.

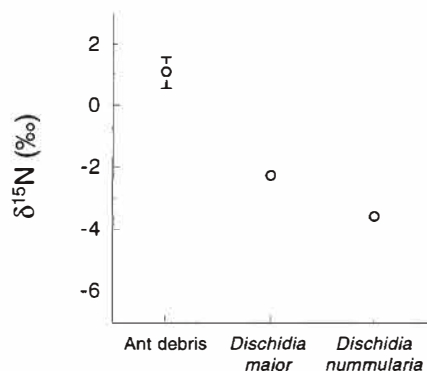


FIG. 3 Mean nitrogen isotope values ( $\pm 1$  s.e.) for ant debris ( $n=5$ ) and random leaves of *Dischidia major* ( $n=17$ ) and *D. nummularia* ( $n=2$ ). Experimental procedure is described in Fig. 2 legend. Standard error bars are not visible when the standard error is smaller than the plot symbol. For *D. major*, we calculated the per cent nitrogen content from ant debris as

$$\% \text{N from ant debris} = \frac{\delta^{15}\text{N}_{D,\text{major}} - \delta^{15}\text{N}_{D,\text{nummularia}}}{\delta^{15}\text{N}_{\text{ant debris}} - \delta^{15}\text{N}_{D,\text{nummularia}}} \times 100\%$$

where  $\delta^{15}\text{N}_{D,\text{major}}$  and  $\delta^{15}\text{N}_{D,\text{nummularia}}$  are the nitrogen isotope ratios of leaves of the two respective species. On average, *D. major* received  $29\%$  of its nitrogen from ant debris. (This estimate is conservative, as both *D. major* and *D. nummularia* often grow roots into ant carton (a m  ch   used for nest building) located on the host tree trunks, and the epiphyte may exploit this source as well<sup>17</sup>.)

Our observations strongly support Janzen's<sup>4</sup> and Huxley's<sup>8,9</sup> hypotheses of mutualism between *D. major* and *Philidris*. In exchange for shelter, ants provide significant amounts of two limiting resources: carbon dioxide and nitrogen. Both features could either expand the realized niche of *D. major*, enabling it to colonize hotter, drier habitats, or could provide *D. major* with a competitive edge over other epiphytes in this nutrient-poor ecosystem.

Finally, epiphytes in other tropical regions (including those of Central and South America, Papua New Guinea, the Philippines and Australia) have various structures occupied by ants<sup>4,8,9,17,23</sup>. In a facultative myrmecophytic relationship involving an ant-occupied orchid from the neotropics, Fisher *et al.*<sup>24</sup> have used stable carbon isotopes to quantify the extent to which ants may forage on their own host plant. We may eventually be able to combine the two approaches and examine reciprocal benefits between plants and ants. □

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