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

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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 hills1-3. 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 there4. 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 (Dolichoderinae) frequently raise young and deposit debris (faeces, dead ants and scavenged insect parts)4-6. Adventitious roots from D. major grow through the cavity opening and proliferate wherever debris has accumulated4. It has been proposed that the Dischidia-Philidris relationship is mutualistic and that D. major uses ant debris as a nitrogen source4; also, the stomata on the internal surfaces of leaf cavities5-9 may absorb ant-respired carbon dioxide and thereby reduce transpirational water loss8-9, but neither of these suggestions is supported by experimental evidence.

We tested these hypotheses by measuring stable isotope ratios ($\delta^{13}$C, $\delta^{15}$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 $\delta^{13}$C value of Dischidia depends on both the $\delta^{13}$C of the source CO$_2$ ($\delta^{13}$C$_{CO_2}$) and discrimination during carbon fixation ($\Delta$), thus $\delta^{13}$C$_{plant} = \delta^{13}$C$_{CO_2} - \Delta$. D. major has obligate crassulacean acid metabolism (CAM)10,11, and we found that uninhab-

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**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 petiole4-6. Scale bar, 1 cm.
ited plants had a mean $\delta^{13}C_{\text{plant}}$ value of $-16.0\%_o$, typical of CAM plants\(^1\). Given an atmospheric $\delta^{13}C_{\text{CO}_2}$ value of $-7.9\%_o$ (ref. 13), $\Delta$ is calculated as 8.1\%.

Ant respiration and decomposing debris are additional possible sources of carbon in ant-occupied leaves and could alter $\delta^{13}C_{\text{CO}_2}$. The carbon isotope ratio of animals (and their respiration) is essentially the same as that of their food\(^4,5,13\). Phildris ants feed on the exudates of Homoptera\(^3\) that ingest the phloem of C\(_3\) rainforest trees. Subsequently, the ants have low $\delta^{13}C$ values averaging $-25.9\%_o$ (Fig. 2). $\delta^{13}C_{\text{CO}_2}$ used for photosynthesis by D. major could vary from $-7.9\%_o$. If no ant-derived CO\(_2\) is taken up, to $-25.9\%_o$, if ant-derived CO\(_2\) accounts for all fixed CO\(_2\). The corresponding $\delta^{13}C_{\text{plant}}$ values would be expected to vary from $-16\%_o$ to $-34\%_o$.

If D. major incorporates $^{13}C$-deficient carbon dioxide respired by the ants or their debris, colonized leaves should have lower $\delta^{13}C$ values than do uncolonized leaves. In addition, as colonies deposit debris inside a leaf only after they have raised brood there for some time\(^5\), ant leaves with debris should have had a longer time to accumulate ant-respired CO\(_2\). Overall, completely vacant leaves should have the highest $\delta^{13}C$ values (about $-16.0\%_o$), 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 Phildris.

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

\[
\% \text{ CO}_2 \text{ from ants} = \frac{\delta^{13}C_{\text{plant}} + \Delta - \delta^{13}C_{\text{CO}_2,\text{atm}}}{\delta^{13}C_{\text{ant}} - \delta^{13}C_{\text{CO}_2,\text{atm}}} \times 100\%
\]

where $\delta^{13}C_{\text{CO}_2,\text{atm}}$ and $\delta^{13}C_{\text{ant}}$ 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\%, $n = 6$) for ant-occupied leaves without debris; and 4\% ($\pm$4\%, $n = 6$) for vacant leaves.

For ant-related CO\(_2\) to contribute so significantly to the carbon balance of the plant, the CO\(_2\) concentration inside ant-occupied leaves should be raised above atmospheric values. By having interior stomata fed by an increased CO\(_2\) concentration, the plant probably significantly increases its water-use efficiency (photosynthetic carbon gain to transpirational water loss). The capture of ant-respired 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 $\delta^{15}N$ values different from ant-provided debris). Dischidia nummularia, which does not possess any 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 $\delta^{15}N_{\text{plant}}$ value of D. nummularia was $-3.5\%_o$; in contrast, debris deposited in the ant leaves of D. major was significantly more enriched in $^{15}N$ ($U_{17,2} = 0, P < 0.05$; Fig. 3). This is expected, given that ant debris is composed mostly of scavenged insect parts\(^6\), and animal $\delta^{15}N$ values tend to be 3\% greater than those of their food sources\(^16\). D. major leaves were significantly enriched in $^{15}N$ 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\(^17\).

\[\text{FIG. 2 Mean carbon isotope values} \pm \text{s.e. for Dischidia major ant leaves and the potential CO}_2\text{ sources for these leaves (atmospheric CO}_2\text{ (} -7.9\%_o\text{), ants (} -25.9\%_o \pm 0.3, n = 6\text{), or ant debris (} -25.9\%_o \pm 0.9, n = 3\text{); shaded bars). Values differed significantly for the three groups of ant leaves (ANCOVA, } F_{3,5,6} = 9.510, P < 0.009\text{). $\delta^{13}C$ values of ant-related debris, a possible source of carbon dioxide, did not differ significantly from those of the ants (ANCOVA, } F_{1,5,6} = 12, P = 0.733).\]

\[\text{METHODS. Material was collected along the Lintang trail in Bako National Park, Sarawak, Malaysia in August 1993. With an isotope ratio mass spectrometer (Delta S, Finnigan MAT at SIRFUR, University of Utah, Salt Lake City), isotope ratios of ants, leaves and ant debris were measured on 2–3 mg samples. Isotope compositions are reported in $\delta^{13}C$ values as $\delta^{13}C = [(\%^{13}C/\%^{12}C)_{\text{sample}}/(\%^{13}C/\%^{12}C)_{\text{standard}}] - 1 \times 1000\%_o$, where $\%^{13}C_{\text{standard}}$ and $\%^{13}C_{\text{standard}}$ are the isotope ratios of the sample and standard, respectively.}^{5}\]

\[\text{FIG. 3 Mean nitrogen isotope values} \pm \text{s.e. for ant debris (} n = 5\text{) and random leaves of Dischidia major (} n = 17\text{) and D. nummularia (} n = 2\text{). 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}

\[
\% \text{ N from ant debris} = \frac{\delta^{15}N_{\text{D. major}} - \delta^{15}N_{\text{D. nummularia}}}{\delta^{15}N_{\text{D. major}} - \delta^{15}N_{\text{D. nummularia}}} \times 100\%
\]

where $\delta^{15}N_{\text{D. major}}$ and $\delta^{15}N_{\text{D. nummularia}}$ 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 mache used for nest building) located on the host tree trunks, and the epiphyte may exploit this source as well.)
Our observations strongly support Janzen’s⁴ and Huxley’s⁸⁻⁹ 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 ants⁴⁻⁸⁻¹⁷. In a facultative myrmecophytic relationship involving an ant-occupied orchid from the neotropics, Fisher et al.²⁴ 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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