

## Variation in the use of orchid extrafloral nectar by ants

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**Summary.** The relation between ant-plant specificity and the use of host plants as a resource was investigated in the facultative, myrmecophytic orchid, *Caularthron bilamellatum* (Rchgf.) Schult. Using stable isotopes, we determined the portion of the ants' diets derived from host plants. We documented that six ant species inhabiting the orchid: (1) derived nutritional benefit from host orchids, and (2) had species-specific levels of extrafloral nectar use. Proportionate contribution of extrafloral nectar to ant diets ranged from 11 to 48%. These results demonstrate extreme interspecific differences in the nutritional benefits received by ants from host orchids. Interspecific differences in nutritional benefits from orchid nectar may be affected by colony size, nutritional needs, behavioral ecology of the ants, and the abundance of alternate food sources.

**Key words:** Ant-plant interactions – Carbon isotope – Extrafloral nectar – *Caularthron* – Mutualism

Many plants have specialized structures that house or feed ant associates in exchange for possible advantages in herbivore control or nutrient deposition (Beattie 1985). Although a few cases of extreme specialization exist (Janzen 1966; Schupp 1986), most associations between ants and host plants are unspecialized and opportunistic (Schemske 1982; Beattie 1985; Fisher and Zimmerman 1988). The extent of ant-plant specificity may be directly related to the dependency of ants on host plants as a resource (Schemske 1982). Therefore, a central question is: what portion of the ants' diets are derived from host plants? Stable isotopes have recently been recognized as an important tool in exploring many questions in ecology (see Rundel et al. 1989 for review). Among these uses are their application as non-radioactive tracers in food web studies and trophic interactions.

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Stable carbon isotope analysis demonstrated extreme interspecific differences in the use of extrafloral nectar by six ant species inhabiting the myrmecophytic orchid, *Caularthron bilamellatum*, (Rchgf.) Schult..

Pseudobulbs of *C. bilamellatum* are lined with a layer of parenchymous cells which desiccate at the onset of the first dry season, forming a hollow chamber. Ants enter the pseudobulbs through preformed basal slits and use these chambers as nest sites. In addition, ants are commonly seen at the extrafloral nectaries located at the base of the leaves, and on the peduncles, buds, pedicels, and fruits (Fisher and Zimmerman 1988). Since most extrafloral nectaries are located on the inflorescences, more extrafloral nectar is available for consumption by ants during the reproductive phase. During the year of this study (1987), orchids began producing inflorescences in January and again in November. We found 11 species of ants inhabiting *C. bilamellatum*; the six most common ant species were studied here. The association between the orchid and the ants appears to be an opportunistic one since the frequencies of all ant species nesting in the orchids do not differ from their frequencies on the host trees in the absence of orchids (Fisher and Zimmerman 1988).

### Methods

#### Study site

Investigations were conducted at the Smithsonian Tropical Research Institute facilities at the Barro Colorado Nature Monument from January through December 1987. The Monument is a protected area of lowland semi-deciduous tropical forest with an annual rainfall averaging 2600 mm. The Nature Monument is located within the Panama Canal watershed in central Panama, consisting of three mainland peninsulas and a 15 square km island that was formed by the flooding of Gatun Lake in 1914. See Croat (1978) and Leigh et al. (1982) for further description of the area. Study orchids were located on *Annona glabra* trees (Annonaceae) along the margins of Gatun Lake.

### Carbon isotope analysis

Orchid nectar consumption was monitored by sampling ant colonies at monthly intervals. The carbon isotopic composition of the whole body of an animal has been shown to reflect the isotope composition of its diet, and therefore, it is possible to perform dietary analysis based on the determination of the  $^{13}\text{C}/^{12}\text{C}$  ratio of animal carbon (DeNiro and Epstein 1978). This ratio, expressed as  $\delta^{13}\text{C}$  values, is defined as:

$$\delta^{13}\text{C} = \left[ \frac{^{13}\text{C}/^{12}\text{C}_{\text{Sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{Standard}}} - 1 \right] \times 10^3$$

where the standard is the Peedee Belemite carbonate and  $\delta$  is expressed in ‰ (per mil) units with a precision of  $\pm 0.2\%$ . Discrimination of dietary sources using  $\delta^{13}\text{C}$  values is possible because the carbon isotope ratio in plants differs according to the pathway used during the photosynthetic fixation of  $\text{CO}_2$  (Farquhar et al. 1989, O'Leary 1981, Park and Epstein 1960).  $\text{C}_3$  plants fix  $\text{CO}_2$  by way of the Calvin cycle and have  $\delta^{13}\text{C}$  values ranging from  $-23$  to  $-34\%$  (O'Leary 1988, Ehleringer et al. 1986). In tropical regions, as the one studied here,  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plants are generally lower than  $-27\%$  (Medina and Minchin 1980). Plants employing the CAM pathway, much like  $\text{C}_4$  plants, discriminate less against  $^{13}\text{C}$  than plants employing the  $\text{C}_3$  pathway, and thus have  $\delta^{13}\text{C}$  values ranging from  $-10$  to  $-18\%$  (O'Leary 1988, Ehleringer et al. 1986).

We investigated orchids found on *A. glabra* L. trees (Annonaceae) located along shoreline marshes in Gatun Lake in the Panama Canal. Water surrounding the *A. glabra* trees restricted ant foraging to other insects on these trees or to extrafloral nectar produced by *C. bilamellatum*. Therefore, discrimination of dietary sources for the ants was based on two alternate food sources: 1) nectar from a CAM orchid having a distinct  $\delta^{13}\text{C}$  value ( $\delta^{13}\text{C}$  value of *C. bilamellatum* nectar was  $-14.6\%$ ) or 2) other food sources, such as insects that feed on the predominant  $\text{C}_3$  vegetation, having  $\delta^{13}\text{C}$  values ranging from  $-28$  to  $-35\%$  (Sternberg et al. 1989).

We collected ants at the end of each month from January 1987 through December 1987. Five to ten workers of each of the six ant species studied (for taxonomic names see Table 1) were collected at three colonies at three different orchids. During some months, less than three colonies per species were sampled due to the rarity of that particular species. Ants that were visible on each orchid were aspirated, placed in glassine envelopes, dried for three days

at  $50^\circ\text{C}$  and then mailed to the University of Miami for isotopic analysis. Ant species not inhabiting the orchid were collected as controls. The control-ants, collected from the understory on Barro Colorado Island, encounter mostly  $\text{C}_3$  plants and insects that have eaten  $\text{C}_3$  plants, while ants inhabiting the orchid, a CAM plant, may also feed on nectar from that host orchid. Both orchid-ants and control-ants were analyzed for their  $\delta^{13}\text{C}$  values. The average monthly carbon isotope value for each species was used in subsequent analyses.

### Results

The  $\delta^{13}\text{C}$  values of orchid leaves studied here were on the average  $-16.5\%$ . Leaves grown and analyzed during the wet season had an average isotopic value of  $-16.3\%$ , indicating that the orchid is obligate CAM. All orchid-ants had significantly greater  $\delta^{13}\text{C}$  values averaged over the entire year than did the controls (Tukey-Kramer procedure, 0.05 level), indicating that all ant species derived nutritional benefit from their association with the orchid.

The  $\delta^{13}\text{C}$  values of the 6 orchid-ant species and control-ants were averaged over three 4 month periods (Table 1). Grouping of values into 4 month periods facilitates presentation and follows a natural division in phenology of the orchid and seasonality of rainfall. Extrafloral nectar production was greatest during the reproductive phase occurring during the dry season from January to April. The lowest period of nectar production occurred from May through August during the first half of the wet season and when new pseudobulbs were being formed. During September through December, new pseudobulbs reached maximum size and produced extrafloral nectar at the base of each leaf, resulting in the second greatest period of nectar production. For some species, mean  $\delta^{13}\text{C}$  values for the 4 month periods reflected this seasonality of nectar production. *Crematogaster brevispinosa*, *Hypoclinea bispinosa*, *Paratrechina*

**Table 1.** Mean  $\delta^{13}\text{C}$  values (mean  $\pm$  95% CI), number of replicates, and percentage of orchid carbon in diets of orchid-ants and control-ants January through December 1987

Ant Species	Jan.-April			May-Aug.			Sept.-Dec.			Yearly Average	
	$\delta^{13}\text{C}$	N	%	$\delta^{13}\text{C}$	N	%	$\delta^{13}\text{C}$	N	%	$\delta^{13}\text{C}$	%
<i>Crematogaster brevispinosa</i>	$-22.4 \pm 0.6$	12	35.6	$-24.3 \pm 0.6$	15	21.8	$-23.3 \pm 0.6$	8	26.9	$-23.4 \pm 0.5^a$	27.3
<i>Hypoclinea bispinosa</i>	$-23.2 \pm 1.1$	8	29.0	$-24.8 \pm 0.7$	5	24.2	$-25.4 \pm 1.0$	5	9.2	$-24.0 \pm 0.7^a$	22.4
<i>Odontomachus laticeps</i>	$-24.3 \pm 0.9$	8	19.9	$-25.5 \pm 1.3$	7	12.1	$-25.1 \pm 0.7$	8	11.8	$-24.9 \pm 0.6^b$	14.9
<i>Paratrechina pubens</i>	$-24.8 \pm 0.3$	9	15.7	$-25.3 \pm 0.5$	10	13.7	$-25.0 \pm 0.3$	11	12.6	$-25.0 \pm 0.3^b$	14.1
<i>Azteca velox</i>	$-26.1 \pm 0.7$	10	5.0	$-25.5 \pm 0.6$	12	12.1	$-24.7 \pm 0.5$	12	15.2	$-25.4 \pm 0.4^b$	10.8
<i>Azteca instabilis</i>	$-24.7 \pm 0.4$	5	16.6	$-26.0 \pm 0.6$	5	8.1	$-25.6 \pm 1.3$	6	7.6	$-25.4 \pm 0.6^b$	10.8
Control spp*	$-26.7 \pm 0.4$	16		$-27.0 \pm 0.6$	25		$-26.5 \pm 0.5$	24		$-26.7 \pm 0.3^c$	

<sup>a, b, c</sup> Yearly average values for each species having different superscript letters are significantly different as determined by the Tukey-Kramer procedure at the 0.05 level

\* Control species were *Ectatoma tuberculatum*, *Eciton burchelli*, *Atta columbica*, *Hypoclinea bispinosa*, *Azteca* sp. 1, *Azteca* sp. 2, *Azteca* sp. 3, *Pseudomyrmex* sp., *Zacryptocerus* sp.

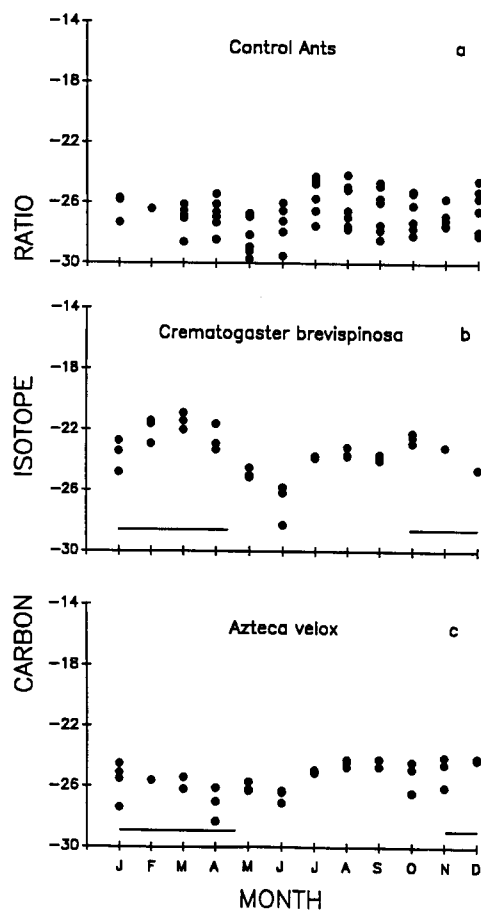


Fig. 1a-c. Monthly  $\delta^{13}\text{C}$  values from January to December 1987 of (a) control-ants, (b) *C. brevispinosa* and (c) *A. velox*.  $\delta^{13}\text{C}$  value of *C. bilamellatum* nectar was  $-14.6\text{‰}$  and the predominant  $\text{C}_3$  vegetation encountered by control-ants had  $\delta^{13}\text{C}$  values ranging from  $-28$  to  $-35\text{‰}$ . The solid line is the reproductive period of the orchid, the time of greatest nectar production. Each dot is the  $\delta^{13}\text{C}$  value of several ants from a single colony for the respective month

*pubens*, and *Azteca instabilis* all showed greater nectar use during the first 4 month period; the seasonal shift in  $\delta^{13}\text{C}$  values of *C. brevispinosa* most resembled the seasonality of nectar production with the lowest level of percent biomass of carbon obtained from nectar,  $21.8\%$ , occurring during the May-August period (Table 1). Statistical analyses were not performed because monthly values were not independent of the previous monthly values and because of a insufficient number of replicates for each month.

Percent biomass of ant carbon obtained from consuming nectar (Table 1) were calculated by using a mass balance equation:  $\% = (\delta_A - \delta_C / \delta_N - \delta_C) \times 100$ , where  $\delta_A$ ,  $\delta_C$ , and  $\delta_N$  are  $\delta^{13}\text{C}$  values of orchid-ants, control-ants and orchid-nectar respectively. Two levels of average yearly nectar use were apparent: *C. brevispinosa* and *H. bispinosa* had biomass percentages of  $27.3\%$  and  $22.4\%$  respectively, which was a significantly greater level of nectar use than the other orchid-ants which had biomass percentages ranging from  $10.8\%$  to  $14.9\%$  (Table 1; Tukey-Kramer procedure,  $0.05$  level).

Monthly  $\delta^{13}\text{C}$  values of control-ant species and two

orchid-ant species are also shown in Fig. 1. *C. brevispinosa* and *Azteca velox* illustrated the two distinct levels of nectar use (Fig. 1). Although all six ant species are not shown, the species not illustrated all fell within the range of levels of extrafloral nectar use by ants shown in Fig. 1. *C. brevispinosa* showed the greatest usage of extrafloral nectar as a food resource.  $\delta^{13}\text{C}$  values of *C. brevispinosa* increased and approached the  $\delta^{13}\text{C}$  values of the orchid during peak periods of nectar production. Calculation of percent biomass for the month of March (Fig. 1) demonstrated that nectar may have comprised up to  $48\%$  of the ants' diet during this peak flowering month. *A. velox* (Fig. 1c) demonstrated the other extreme level of nectar use; unlike *C. brevispinosa*,  $\delta^{13}\text{C}$  values remained constant during the year and nectar comprised only an average of about  $11\%$  of the ants' diet.

## Discussion

In general, the year long ant-nectar interactions exhibited two levels of nectar use. A high use of extrafloral nectar as a food resource was exemplified by *C. brevispinosa*, *H. bispinosa*, and a low use was exemplified by *Odontomachus laticeps*, *Paratrechina pubens*, *A. velox*, and *A. instabilis*. For some species, seasonal shifts in extrafloral nectar consumption followed periods of nectar production. However, there was an increase in nectar use during the months July, August, and September (Fig. 1), which can not be explained by seasonality of extrafloral nectar production.

The carbon isotope values of the ant bodies measured reflect not only the carbon isotope ratio of the food consumed as adults, but the carbon isotope ratio of food fed as larvae. Ant larvae are fed by workers in the colony and for many ant species, studies have documented a preferential distribution of protein to larvae (Vinson 1968, Markin 1970). Adult workers and foragers, in contrast, preferentially consume sugars (Markin 1970). Therefore, the  $\delta^{13}\text{C}$  signature of adult ants reflect the carbon isotope ratio of the food fed as a larvae (potentially proteins from insects with isotopic values similar to  $\text{C}_3$  plants) and the carbon isotope ratio of food eaten as an adult (potentially extrafloral nectar from a CAM orchid).

Interspecific differences in nutritional benefit from orchid nectar may be affected by colony size, nutritional needs, behavioral ecology of the ants, and the abundance of alternate food sources (i.e. insects). For example, observations indicate that the *Azteca* spp. can have larger colonies than *C. brevispinosa* (B. Fisher personal observation). *Azteca* may consume extrafloral nectar as intensely as *C. brevispinosa*, but because of their larger colony size, nectar may comprise only a small portion of the total *Azteca* colony diet. Therefore, *C. brevispinosa* and *Azteca* spp. may use extrafloral nectar at the same rate. If this is the case, and if the benefit against herbivores is related to frequency of ant visitation to nectaries, the benefit derived by the orchid from the *C. brevispinosa* and *Azteca* spp. associates are similar. However, if the

lower significance of nectar in the diet of *Azteca* spp. is due to a lower nectar consumption, the orchid would derive a greater benefit by being occupied by *C. brevispinosa* rather than by *Azteca*.

Seasonal patterns in nectar consumption demonstrated by *Crematogaster brevispinosa*, *Hypoclinea bispinosa*, and *Azteca velox* may represent a change in availability of nectar from flowering season to flowering season. This may be the case for *C. brevispinosa*, whose seasonality of nectar consumption in Figure 1 follows periods of nectar production. For the other five species, seasonality of nectar consumption may represent changes in feeding habits of the ant colony. The difference in nutritional requirements of larvae and adults (Vinson 1968, Markin 1970, Beattie 1985) may determine the percentage of nectar and insects consumed by a colony. Ant colonies with few larvae, and thus a low demand for protein, may consume more nectar than a colony with many larvae. The production of worker castes or reproductives may be seasonal (Macaulay 1967) and may cause the observed shifts in extrafloral nectar consumption (Table 1, Fig. 1).

Alternate food sources such as insects are not in constant abundance in the study area. Insect abundance tends to be lowest during the dry season (Wolda 1978), which corresponds to the period of peak production of extrafloral nectar. Nectar consumption may result in low biomass percentages of orchid carbon in ant diets, but nectar consumption may be critical for colony survival during periods of low insect abundance.

Facultative ant-plants may rely on opportunistic ant species for possible protection or nutrient advantage. By using stable isotopes to distinguish dietary sources for ant associates, we documented interspecific variation in extrafloral nectar consumption. These results suggest that for some ant associates, extrafloral nectar supplies a large fraction of the nutritional requirements of the ant colony during certain months of the year. Despite the interspecific differences in the amount of nectar reward received by ant species, the orchid-ant relationship is not species-specific or dominated by *C. brevispinosa*, the ant species with the highest nectar use; *Azteca* spp. occupied 84% of *C. bilamellatum* in the Monument (Fisher and Zimmerman 1988), and had the lowest nectar use (Table 1).

The limit to specialization in the *C. bilamellatum*-ant association may not be related to the variation or level of dependency of ant species on host plants as a food resource. An alternative explanation for the lack of specificity is that host plant characters are under selection to attract a particular ant species or suite of species which provide protective or nutritional benefits unrelated to the nectar use by ants. Further studies are needed to evaluate the variation in benefits to host plants provided by the different ant species in relation to nectar use. Studies must determine whether or not *C. brevispinosa*, which obtained the greatest percentage of its resources from extrafloral nectar, is also the ant species that has the greatest positive effect on orchid fitness. Previous studies by Rico-Gray and Thien (1986), however, suggest that this may not be the case. They showed

that for the myrmecophytic orchid *Schomburgkia tibicinis* Batem. occupation by *C. brevispinosa* lowered orchid reproductive success.

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