IDEAS AND PERSPECTIVES

Stoichiometric relations in an ant-treehopper mutualism

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Abstract

Carbon : nitrogen : phosphorus (C : N : P) stoichiometry can underlie physiological and life history characteristics that shape ecological interactions. Despite its potential importance, there is much to learn about the causes and consequences of stoichiometric variation in terrestrial consumers. Here we show that treehoppers (*Publilia modesta*) tended by ants (*Formica obscuripes*) contained lower N concentrations than treehoppers on plants from which ants were excluded. Ant presence also affected nutrient concentrations in host plants: on plants with ants, leaves contained uniformly low concentrations of N; on plants without ants, N concentrations were low only in the few leaves fed upon by treehoppers at the time of collection. We suggest treehopper feeding reduces leaf nutrient levels and ants positively affect treehopper abundance, producing a top–down effect on plant quality. Determining the causes of these stoichiometric changes should help elucidate factors guiding the dynamics of conditional mutualisms between ants and homopterans.

Keywords

Ants, conditional, membracid, mutualism, nitrogen, phosphorus, *Publilia*, *Solidago*, stoichiometry, treehoppers.

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INTRODUCTION

Outcomes of conditional mutualisms often depend on varying ecological factors (Bronstein 1994, 2001; Herre *et al.* 1999). Such conditionality is thought to be widespread in ant-homopteran mutualisms (Cushman & Addicott 1991), in which homopterans produce excretions ('honeydew') collected by ants, and ants provide protection or other services. Ant attendance can positively affect the size of a homopteran aggregation (number of individuals on a host plant) depending on several factors including ant abundance (Cushman & Whitham 1991; Morales 2000) and natural enemy levels (Bristow 1984). Ant attendance can also influence characteristics of individual homopterans such as growth rate (Bristow 1984; Stadler & Dixon 1998) by inducing changes in feeding behaviour (Banks & Nixon 1958) and the quantity or quality of honeydew that homopterans produce (Yao & Akimoto 2001). Discovering these individual-level effects, and determining their causes and consequences, may elucidate mechanisms underlying the distribution and strength of ant-homopteran associations.

Here, we investigate how ant attendance affects the size of aggregations of the treehopper, *Publilia modesta*, and the carbon : nitrogen : phosphorus (C : N : P) stoichiometry of *P. modesta* individuals. Stoichiometry can underlie traits that affect how organisms interact with their environment (Sterner & Elser 2002): one example is the causal relationship between P concentration and growth rate. Although this relationship is taxonomically widespread (Elser *et al.* 2003), little is known about patterns, causes, or consequences of stoichiometric variation in most terrestrial consumers.

Ant attendance could affect treehopper stoichiometry by at least two mechanisms. First, ant presence could lead to an increase in P in treehoppers if ant protection creates conditions for rapid growth, and growth rate in treehoppers is tied to body P concentration. When tended by ants, some homopterans increase feeding rates (Banks & Nixon 1958) and may grow faster (El-Ziady 1960; Bristow 1984; Flatt & Weisser 2000); however, nothing is known about how such changes are related to homopteran stoichiometry. Second, ant attendance could decrease N and P concentrations in treehoppers if treehoppers produce more nutrient-rich

honeydew when ants are present. Ants can induce changes in the production rate or sugar composition of honeydew (Fischer & Shingleton 2001), but little is known about how ants affect honeydew N and P concentrations. However, N-rich honeydew may help treehoppers attract ants because many ants are particularly responsive to amino acid levels in honeydew-like substances (Kay 2002). In this study, we show that ant presence decreases the concentration of N, but not of C or P, in treehoppers. We also show that ant presence is associated with an overall reduction in leaf N concentrations, which we argue results from treehopper feeding.

METHODS

We conducted field work at Cedar Creek (Anoka County), MN, USA. *Publilia modesta* feeds on a variety of plants, but was found only on *Solidago gigantea* at our study site. Eggs are laid on host plants early in the growing season and emerging nymphs apparently rarely leave their natal plant before becoming adults (Morales 2000). Throughout the western United States, *P. modesta* has only a single generation per year (Kopp & Yonke 1973). Nymphs in our study fed primarily by piercing leaves (rarely through stems).

On 23 July 2003, we chose eight *S. gigantea* ramets (hereafter = plants) that had large numbers of *P. modesta* tended by ants. We haphazardly divided plants into an experimental and a control group ($n = 4$). We removed all ants from experimental plants, and applied sticky trap (Tanglefoot) to the base of stems to prevent ants from returning. We clipped a small amount of mostly senesced vegetation that provided ants with access to experimental plants; similar amounts of vegetation were removed around control plants. We counted treehoppers on each plant after initiating the experiment and every 3–4 days thereafter for 24 days. After 24 days, we separately collected each leaf (and any treehoppers on the leaf) from each plant.

We conducted tissue analyses on samples dried at 60 °C for 48 h. We analysed subsamples of ground leaves and whole treehoppers for carbon and nitrogen concentrations using a Perkin–Elmer 2400 CHN analyser (Perkin–Elmer, Wellesley, MA, USA), and phosphorus concentration using persulfate digestion and ascorbate–molybdate colorimetry. We estimated soluble protein concentration in treehoppers using the direct Lowry procedure (Sigma, St Louis, MO, USA), which involves colorimetry on a sample combined with an alkaline cupric tartrate reagent. In the phosphorus and soluble protein assays, treehoppers were gently crushed with a Teflon-coated rod to expose tissues to reagents. In each assay, we analysed 3–5 haphazardly selected treehoppers from each plant. We also analysed from each plant three leaves with and three leaves without treehoppers on them at the time of our final census; we used all leaves in a category when fewer than three existed.

We used repeated-measures ANOVA to determine whether ant presence affected treehopper aggregation size. To control for initial differences in aggregation sizes, the repeated measure was treehoppers on a plant divided by the maximum number of treehoppers on that plant during the experiment. These proportions were arcsine transformed for analysis. We used nested ANOVAs to compare leaf and treehopper chemistry (with plants nested within ant treatment).

RESULTS

Treehopper aggregation sizes were largest in the initial survey (mean ± SE for all plants = 178.5 ± 31.9) and declined thereafter. Over the course of the experiment, aggregation size decreased more on plants without ants than on control plants (Fig. 1; time × treatment interaction: $F_{6,36} = 3.26$, $P = 0.012$; final means: plants without ants = 36.8 ± 15.8, control plants = 150.3 ± 16.1). In the final survey, the percentage of leaves with treehoppers was significantly lower on plants without ants than on control plants (28 ± 10% vs. 89 ± 2%; $\chi^2 = 5.33$, d.f. = 1, $P = 0.021$), but the number of treehoppers on those leaves did not differ between groups (9.1 ± 4.0 vs. 6.8 ± 0.4; *t*-test with unequal variances: $t = 0.55$, d.f. = 3, $P = 0.61$). We never observed adult treehoppers on our plants during the experiment.

Treehoppers had significantly higher N concentrations on plants without ants than on control plants; treehopper C and P concentrations did not differ between groups (Fig. 2). Differences in N concentration between groups were not explained by body mass differences. Although dry mass was weakly (negatively) correlated with N concentration (log–log relationship; $r^2 = 0.34$, d.f. = 31, $P = 0.056$), treehopper
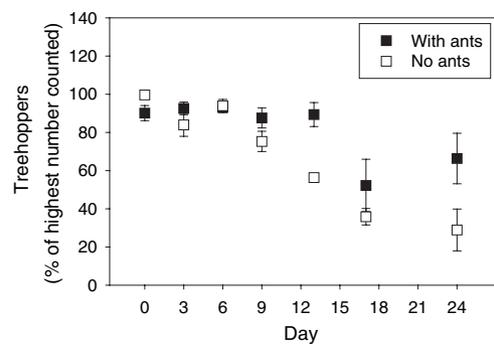

Figure 1 Abundance of *Publilia modesta* treehoppers over a 24-day period as a function of the presence or absence of ants. To facilitate visual assessment of treatment differences, numbers of treehoppers for each plant were transformed to the percentage of the highest number of treehoppers counted on that plant: data points are the mean (± 1 SE) of those percentages for all plants in a treatment.
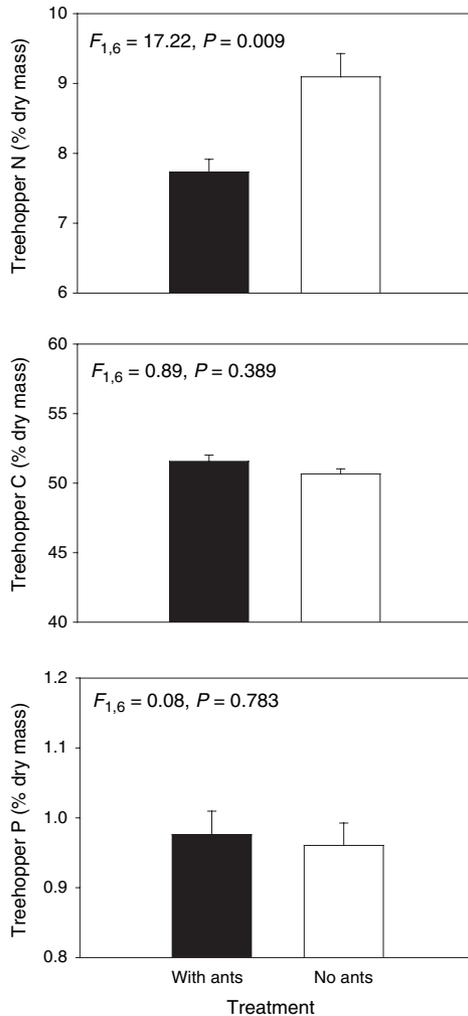
Figure 2 Mean (± 1 SE) N, C and P concentrations in the tissue of treehopper nymphs after a 24-day period in the presence or absence of ants. Data are presented as % of dry mass.

Figure 3 Mean (± 1 SE) N concentration (% of dry mass), P concentration (% of dry mass), and N : P molar ratio in leaves of host plants as a function of two factors: the presence or absence of ants and the presence or absence of treehoppers on leaves.

mass did not differ between treatments ($F_{1,6} = 0.48$, $P = 0.519$), and mean residuals of the relationship between dry mass and N concentration were also higher for treehoppers on plants without ants ($F_{1,6} = 9.21$, $P = 0.029$). Unlike for total N, soluble protein concentration in treehoppers without ant attendants (20.6 ± 1.3%) did not differ significantly from concentrations in tended treehoppers (19.3 ± 1.3%; $F_{1,6} = 1.87$, $P = 0.221$). Assuming protein consists of 17% N, soluble protein contained 39 and 43%, respectively, of the N in untended and ant-tended treehoppers.

Leaf N concentration varied with ant and treehopper presence (Fig. 3). On plants without ants, leaves with treehoppers in the final survey had significantly low N concentrations ($F_{1,6} = 9.85$, $P = 0.02$), marginally less P ($F_{1,6} = 5.73$, $P = 0.054$), and low N : P ratios ($F_{1,6} = 6.63$, $P = 0.042$) than did leaves without treehoppers. Differ-
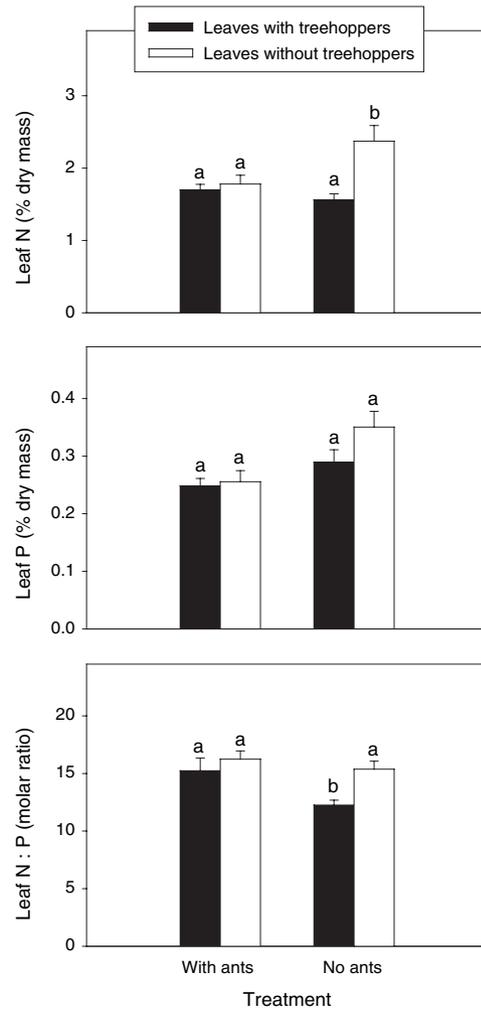
ences in leaf chemistry were substantial: mean N concentration in leaves with treehoppers was 34% lower than in leaves without treehoppers. On control plants, treehopper presence on leaves was not associated with differences in N concentration ($F_{1,6} = 0.30$, $P = 0.602$), P concentration ($F_{1,6} = 0.09$, $P = 0.776$), or the N : P ratio ($F_{1,6} = 0.48$, $P = 0.515$) in leaves. Leaf C concentration did not differ with treehopper presence in ant-excluded ($F_{1,6} = 1.02$, $P = 0.351$) or control plants ($F_{1,6} = 0.56$, $P = 0.484$).

DISCUSSION

Ant presence positively affected the size of *P. modesta* aggregations (Fig. 1). Ants have been shown to benefit *Publilia* aggregations in other systems (Cushman & Whitham

1989; Del-Claro & Oliveira 2000), although the existence of this effect can depend on the ecological setting (Billick & Tonkel 2003). We do not know how ants benefited treehoppers: they may have provided protection (Cushman & Whitham 1989; Billick & Tonkel 2003) or enhanced the physiological condition of treehoppers by improving sanitation (Way 1963) or by stimulating higher feeding rates (Banks & Nixon 1958).

Ant presence also affected treehopper stoichiometry: at the time of the final census, N concentration was on average 15% lower in ant-tended treehoppers than in untended treehoppers (Fig. 2). This result is the first to show that the presence of a mutualist can affect the N concentration in an insect. Although little is known about the determinants of insect N concentration (Fagan *et al.* 2002), it likely has important consequences for insect herbivores because they contain much higher N concentrations than their host plants (White 1993). This imbalance may be particularly large for treehoppers and other insects that feed on phloem, which contains very low levels of N (Van Hook *et al.* 1980); phloem-feeders may thus realize substantial fitness benefits by lowering their use of N in tissue construction. Our results suggest that ant-homopteran systems will provide opportunities for better understanding the determinants of tissue N concentration. However, we must emphasize that the extent of our study is limited, as it involved only a short-term manipulation on a small number of host plants.

We suggest three mechanisms that could generate the relationship between ant attendance and treehopper stoichiometry. First, treehoppers may increase N concentration in honeydew to attract or to appease ants, and thus retain less N for tissue construction. Second, treehoppers may alter investment in N-rich structures depending upon the presence of ants. For example, treehoppers without ant protection may produce more cuticle, or cuticle with a higher ratio of protein (*c.* 17% N) to chitin (*c.* 7% N), as an alternative defence against predators. The protein : chitin ratio may be positively related to the degree of sclerotization (cuticular hardening), a key factor underlying the protective function of the cuticle (Chapman 1997). Third, the presence of ants may affect the relationship between treehopper N concentration and survivorship. For example, if N concentration, sclerotization, and cuticular protection are functionally linked, treehoppers with less N may be particularly susceptible to predation in the absence of ants.

Ants and treehoppers had an interactive effective on leaf nutrient concentrations (Fig. 3) that may have resulted from treehopper feeding. Amino acid concentrations in leaves often decrease after attack by phloem feeders (Olmstead *et al.* 1997), which could explain the uniformly low N concentrations in leaves on which treehoppers were feeding at the time of collection. Damage on these leaves from ant-excluded and control plants may have been similar because of the similar number of treehoppers per leaf in each treatment. On plants with ants, leaf N levels were also low in leaves without treehoppers. However, such leaves were rare and were close to leaves being fed upon – they thus may also have been recently attacked. In contrast, many leaves on ant-excluded plants may have escaped recent attack because treehoppers have limited mobility and were rare on these plants. Regardless of the mechanism, ant presence was associated with lower leaf N concentrations in plants because (1) leaves with treehoppers had low N concentrations, (2) plants with ants had proportionally more leaves with treehoppers on them, and (3) leaves without treehoppers had much lower N concentrations on plants with ants than on plants without ants. Plant quality is thought to mediate ant-homopteran interactions through its bottom–up effect on honeydew quality (Cushman 1991). Our results suggest that ant presence may have a top–down effect on plant quality that could increase the cost of producing attractive honeydew, thereby contributing to the dynamic nature of these interactions.

Stoichiometry can generate *a priori* predictions about the dynamics of mutualisms by making clear the mechanisms underpinning trade-offs confronted by each participant (Sterner & Elser 2002). Investigating the consequences of stoichiometric shifts in treehoppers and their host plants should help clarify the material basis of trade-offs faced by *P. modesta*, and lead to a more general understanding of how resource quality guides outcomes of conditional interactions.

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