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Extrafloral nectar content alters foraging preferences of a predatory ant

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We tested whether the carbohydrate and amino acid content of extrafloral nectar affected prey choice by a predatory ant. Fire ants, *Solenopsis invicta*, were provided with artificial nectar that varied in the presence of carbohydrates and amino acids and were then provided with two prey items that differed in nutritional content, female and male crickets. Colonies of fire ants provided with carbohydrate supplements consumed less of the female crickets and frequently did not consume the high-lipid ovaries of female crickets. Colonies of fire ants provided with amino acid supplements consumed less of the male crickets. While a number of studies have shown that the presence of extrafloral nectar or honeydew can affect ant foraging activity, these results suggest that the nutritional composition of extrafloral nectar is also important and can affect subsequent prey choice by predatory ants. Our results suggest that, by altering the composition of extrafloral nectar, plants could manipulate the prey preferences of ants foraging on them.

Keywords: extrafloral nectar; mutualism; nutrition; *Solenopsis invicta*

1. INTRODUCTION

Mutualisms often involve a delicate balance between the costs and benefits for each species involved (Bronstein 1998; Ferriere *et al.* 2002). In food-for-protection mutualisms, a species of plant or honeydew-producing insect exchanges food resources (e.g. extrafloral nectar or honeydew) with a predator for protection from herbivores, competitors or other predators (Bronstein 1998). In these mutualisms, the species that provide protection are frequently generalist predators that consume a wide range of insect prey (Kaplan & Eubanks 2005). Yet, particular herbivores, competitors or predators may be more of a threat than others to plants or honeydew-producing insects. In these situations there would be a strong selective advantage for traits that allowed plants or honeydew-producing insects to influence the prey choice of the predatory arthropods that provide protection.

One way that plants or insects providing the food resource could influence the behaviour of the species providing protection is through the nutritional composition of extrafloral nectar and honeydew. The concentrations of carbohydrates and amino acids provided in these food resources by plants and honeydew-producing insects can vary temporally, spatially and among species (Heil *et al.* 2000; Bluthgen *et al.* 2004). This variation in food resources could have important implications for the mutualist-partner providing protection, because nutritional deficiencies in extrafloral nectar or honeydew need to be remediated through consumption of insect prey (Dussutour & Simpson 2009). Hence, any variation in the concentrations of carbohydrates and amino acids in the food provided by a plant or honeydew-producing insect could alter the prey selection of the predator as it attempts to satisfy its nutritional requirements.

We tested the hypothesis that the nutritional content of mutualist-provided food resources affected prey choice in a predatory ant, the red imported fire ant (*Solenopsis invicta*). Specifically, we tested if the carbohydrate and amino-acid content of extrafloral nectar provided to colonies of fire ants affected their foraging rates on adult male and female crickets, *Acheta domesticus*. Female insects frequently contain higher concentrations of lipids in their bodies than males, owing to high concentrations of lipids in female ovaries, which can account for over 20 per cent of the mass of female crickets (Gilby 1965; Mole & Zera 1993). Hence, we predicted that colonies of fire ants provided with carbohydrate supplements would forage less on female crickets and colonies provided with amino acid supplements would forage less on male crickets.

2. MATERIAL AND METHODS

Red imported fire ants, *S. invicta*, (hereafter 'fire ants') are generalist predators that feed on a wide range of invertebrate and vertebrate prey items. Fire ants also regularly supplement their diet with nectar, extrafloral nectar and honeydew, which contain high concentrations of carbohydrates and low concentrations of amino acids (Helms & Vinson 2008). Colonies of *S. invicta* were excavated from mowed lawns on the campus of Texas A&M University during 9–11 March (College Station, Brazos County, TX, USA). After colonies settled for 48 h, the water dripping method was used to separate the workers, brood and queens from the soil. The colonies collected from the field were used to make standardized laboratory colonies that each consisted of two queens, approximately 50 brood and 1192 ± 62 (mean $\pm$ 1 s.e.) workers. Laboratory colonies were placed in containers (38 cm width $\times$ 55 cm length $\times$ 13 cm height) lined with fluon and provided with a darkened Petri dish for a nest. Colonies were provided with a vial of water and had the plaster of paris substrate in their nest moistened two times per week. Each colony was provided with a single male and female cricket, *A. domesticus*, that was freshly killed three times per week. The colony room was maintained on a 12 : 12 light : dark photoperiod with 40–70% humidity and a daily temperature cycle that included 8 h during daylight at 32°C and 16 h at 24°C.

We used artificial extrafloral nectar to supplement the nutrition of fire ant colonies. The artificial nectar mimicked the chemical composition of extrafloral nectar of *Passiflora* spp. (Lanza 1988, 1991) and consisted of 1 l of water mixed with carbohydrates (108 g sucrose, 90 g glucose and 53 g fructose) and amino acids (0.0232 g aspartic acid, 0.512 g glutamine, 0.0404 g glutamic acid, 0.0194 g histidine, 0.0436 g isoleucine, 0.04 g leucine, 0.118 g phenylalanine, 0.368 g proline, 0.0704 g tryptophan and 0.1122 g tyrosine). Each colony was provided with a new 5 ml vial of one of four experimental treatments weekly: water only ($n = 12$), the amino-acid component ($n = 15$), the carbohydrate component ($n = 13$) or the complete artificial extrafloral nectar (carbohydrates and amino acids) ($n = 14$).

Three weeks after the start of the experiment, we examined the foraging of fire ants on male and female crickets. The time period after which we conducted the foraging experiment (21 days) was too short for the experimental treatments to have affected colony size because the duration of larval growth and pupation is approximately 30 days at 32°C (Tschinkel 2006). On one of the regularly scheduled feeding days, we freeze-killed male and female crickets, weighed them, impaled a single male and female together on an insect pin and placed it in a container 10 cm from the fire ant colony (figure 1). We counted the total number of ants recruiting to the crickets after 150 min as a measure of foraging activity. After 24 h, we removed the unconsumed remnants of the male and

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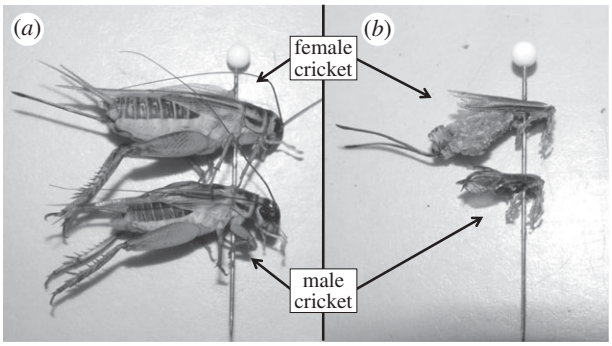

Figure 1. (*a*) Photographs displaying whole male and female crickets on an insect pin and (*b*) the unconsumed remains from male and female crickets that had been fed upon by fire ants, *S. invicta*, whose diet was supplemented by carbohydrates. The unconsumed ovaries can clearly be seen in the unconsumed remains of the female cricket, which was typical for female cricket remains from treatments where fire ants were provided with carbohydrate supplements.

female crickets, dried them at 60°C for 24 h and separately weighed male and female remnants. A separate set of unconsumed crickets were weighed wet and dry to calculate a calibration equation that allowed us to calculate the estimated dry mass of crickets used in the feeding trials and the dry mass of male and female crickets consumed. Since male and female crickets were available simultaneously, we first used multivariate analysis of covariance to test if the consumption of male and female crickets (dependent variables) was affected by carbohydrate and amino acid supplementation (independent variables). Foraging activity was used as a covariate because there was substantial variation in foraging activity among colonies within each treatment. We then used univariate two-factor analysis of covariance (ANCOVA) with foraging activity as a covariate to test how carbohydrate and amino acid supplements affected foraging on male and female crickets. Separate ANCOVAs were used to examine foraging on male and female crickets. We did not directly compare consumption of male and female crickets because female crickets are significantly larger than male crickets.

3. RESULTS

Total consumption of crickets matched the degree of supplementation with highest consumption by fire ant colonies with no supplements (115 ± 12 mg; mean $\pm$ s.e.), lowest consumption by colonies with both carbohydrate and amino acid supplements (77 ± 11 mg) and intermediate consumption when only one type of supplement (carbohydrate $= 112 \pm 11$ mg; amino acid $= 110 \pm 11$ mg) was provided ($F_{4,40} = 66.7$, $p < 0.001$).

Multivariate analysis revealed overall effects of carbohydrates (Wilks' $\lambda = 0.79$, $F_{2,39} = 5.20$, $p = 0.01$) and amino acids (Wilks' $\lambda = 0.85$, $F_{2,39} = 3.45$, $p = 0.04$) with no interaction (Wilks' $\lambda = 0.90$, $F_{2,39} = 2.11$, $p = 0.13$) on the consumption of female and male crickets (figure 2). Univariate tests were used to further examine the nature of these effects for female and male crickets separately.

Carbohydrate supplementation resulted in a significant decrease in the mass of female crickets consumed by fire ants ($F_{1,40} = 7.26$, $p = 0.01$) but no change in the mass of male crickets consumed (figure 2; $F_{1,40} = 1.28$, $p = 0.27$). Unconsumed parts of ovaries were frequently seen in female cricket remains removed from fire ant colonies with carbohydrate supplements, but ovaries were frequently consumed by fire ant colonies that were not supplemented with carbohydrates

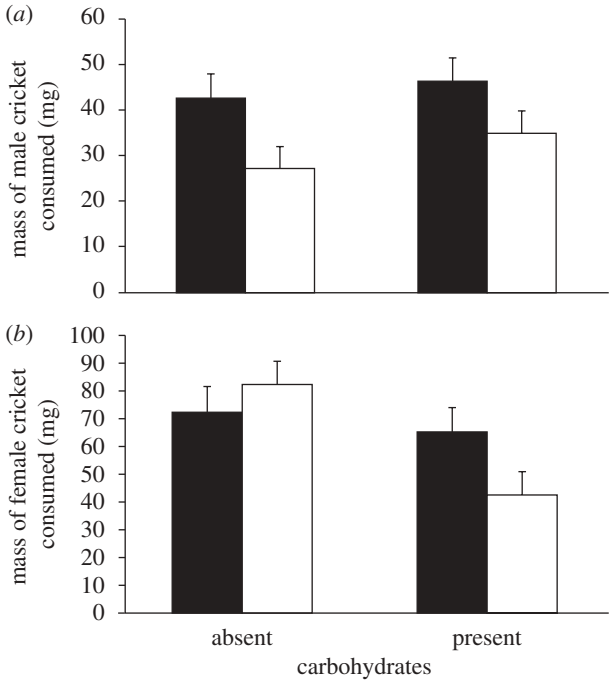

Figure 2. Mass of (*a*) male and (*b*) female cricket consumed by colonies of red imported fire ants, *S. invicta*, provided with or without supplements of carbohydrates and amino acids similar to those found in extrafloral nectar. Filled bar, amino acid absent; unfilled bar, amino acid present.

(figure 1). Amino acid supplementation resulted in a significant decrease in the mass of male crickets consumed ($F_{1,40} = 7.07$, $p = 0.01$) but no difference in the mass of female crickets consumed (figure 2; $F_{1,40} = 0.52$, $p = 0.47$). There were no significant interactions between carbohydrate and amino acid supplementation and the consumption of male ($F_{1,40} = 0.15$, $p = 0.70$) and female ($F_{1,40} = 3.57$, $p = 0.07$) crickets.

4. DISCUSSION

A number of studies have shown that the presence or absence of extrafloral nectar and honeydew affects the foraging activity of ants (Bronstein 1998; Kaplan & Eubanks 2005) but here we show that the nutritional composition (i.e. carbohydrate and amino acid concentrations) of extrafloral nectar is also important and can have a significant effect on prey choice. Colonies of fire ants supplemented with carbohydrates foraged less on female crickets while colonies supplemented with amino acids foraged less on male crickets. That this prey selection occurred between two prey items of the same species indicates that social predators can coordinate fine-scale discrimination among prey. Furthermore, not only did they select between the prey items but they also selectively consumed tissue within a single prey item, as colonies with carbohydrate supplements did not appear to consume lipid-rich female cricket ovaries. Similarly, in spiders and predatory beetles, individuals fed high lipid diets consumed less lipid from prey items (Mayntz *et al.* 2005). While several studies have shown that nutritional state can alter choice of artificial complementary food resources (Raubenheimer *et al.* 2007), this is one of the first studies to show that the

nutritional state of predators can alter prey choice among more natural complementary prey items.

Extrafloral nectar and honeydew are known to vary widely in their concentrations of carbohydrates and amino acids (Heil *et al.* 2000; Bluthgen *et al.* 2004). Much of this variation has been attributed to variation in the costs of producing the resource for plants and honeydew-producing insects (Wackers *et al.* 2005). However, our results suggest that plants or insects could also use variation in carbohydrate and amino acid concentrations to influence the prey selection of the predatory arthropod that provides protection. By changing the composition of extrafloral nectar or honeydew in particular ways, plants and honeydew-producing insects could bias the prey selection of their mutualist partner towards herbivores, competitors or predators that pose the greatest risk to the plant or honeydew-producing insect. Further research is needed on the consequences of variation in extrafloral nectar content for the selection of live prey items by predators and the consequences of this prey choice for the dynamics of ant–plant mutualisms.

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