



## Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists



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Food-for-protection mutualisms can provide resources that subsidize ecological dominance, although their stability often depends on the nutritional match between the traded food and traits to which it is allocated. A well-studied food-for-protection mutualism is the protection by Argentine ants, *Linepithema humile*, of hemipteran mutualists in return for honeydew, which is generally assumed to be a nutritionally imbalanced food, high in carbohydrates and low in protein. We tested an alternative hypothesis, that the nutritional value of honeydew depends on the composition of life history traits related to survival (worker maintenance) and colony growth (brood production). Using a factorial design, we manipulated ant colony access to aphids, baseline levels of nutritional deprivation, and, by adding or subtracting queens, the ability to invest in growth. We found that aphid access primarily benefited colonies on high-protein, low-carbohydrate diets, suggesting colony nutritional state influences the value of aphid associations. Unexpectedly, we found that worker survival and brood production were positively correlated across treatments, and both decreased with the ants' percentage of nitrogen mass, a proxy for body protein. Moreover, the workers' percentage of fat mass was generally lower in brood-producing colonies, particularly in high-protein treatments, suggesting strong competition among investments for limited carbohydrates. These results suggest that carbohydrate access generally constrains Argentine ant life history and clarifies the links between the nutritional composition of food rewards and the stability of ecologically important mutualisms.

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Mutualisms are diverse and widespread, and can provide resources that facilitate the ecological dominance of interacting species (Bronstein, 1998; Bruno, Stachowicz, & Bertness, 2003). Mutualisms are also fertile ground for conflict, although their stability can be enhanced when partners receive resources that match dietary needs (Hoeksema & Schwartz, 2003; Schwartz & Hoeksema, 1998). Food derived from mutualists is especially valuable when it provides nutrients that are otherwise in scarce supply. If these nutrients enable concurrent investment in traits with overlapping nutritional compositions, food from mutualists may relax life history trade-offs (Yoshida, 2006). Linking the composition of food rewards to traits that underlie mutualism stability may thus provide general insights into how nutrition shapes these ubiquitous and ecologically important species interactions (Simpson & Raubenheimer, 2012).

Ants are model systems for exploring how mutualisms affect trait expression and response to allocation trade-offs. First, the colonial structure of ant colonies makes it easy to study how diet composition shapes life history decisions, which are reflected in readily quantifiable traits such as the number, mass and chemical composition of individual ants (Davidson, 2005; Mooney & Tillberg, 2005; Smith & Suarez, 2010). Second, ants are generally among the most abundant taxa in terrestrial habitats and often owe their ecological dominance to food provided by mutualists (Ness, Mooney, & Lach, 2010). For instance, ants often engage in food-for-protection mutualisms, protecting diverse plants and insects in return for carbohydrate-rich, protein-poor resources (Heil & McKey, 2003). Here, we link life history allocation and diet composition in mutualisms where ants protect hemipterans (e.g. aphids, scale, mealybugs) in exchange for carbohydrate-rich honeydew.

Ant colonies have diverse nutritional demands that are met by multiple nutrients. Recent work has demonstrated the critical role of interactions among dietary protein and carbohydrates in colony

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performance (Dussutour & Simpson, 2009, 2012; Kay et al., 2014). Such nutritional interactions can result if demands for life history traits such as growth and survival are met by different nutrients. For example, several studies have shown that workers shunt protein to brood but retain carbohydrates (Howard & Tschinkel, 1981; Markin, 1970; Sorensen & Vinson, 1981; Weeks, Wilson, Vinson, & James, 2004), and that low protein:carbohydrate diets affect brood production more than worker survival (Dussutour & Simpson, 2009; Kay, Zumbusch, Heinen, Marsh, & Holway, 2010). However, recent studies on invasive ant species have shown that brood production can have carbohydrate requirements equal to or greater than adult worker maintenance (Shik & Silverman, 2013; Wilder, Holway, Suarez, & Eubanks, 2011).

Most notably for the current study, Wilder et al. (2011) manipulated dietary nutrition and access to an aphid mutualist in the red imported fire ant, *Solenopsis invicta*, and found that carbohydrates limited both growth and maintenance more than did protein. If generalizable, this result suggests that carbohydrate availability may largely determine the nutritional benefits of resources for ants in food-for-protection mutualisms, and thus nutrition-based trade-offs between colony life history traits may be more direct than predicted by multinutrient models (Yoshida, 2006). However, the extent to which these nutritional rewards are assimilated by workers or brood en route to performance benefits remains unknown, as does the impact of colony life history requirements on these allocation decisions.

Here we measure how resources from aphid mutualists are allocated within incipient colonies of the Argentine ant, *Linepithema humile*, a widespread and destructive invasive species (Wetterer, Wild, Suarez, Roura-Pascual, & Espadeler, 2009) whose ecological dominance is often associated with interactions with hemipteran mutualists (Holway, Lach, Suarez, Tsutsui, & Case, 2002). Access to aphids not only increases the growth rate of *L. humile* propagules more than does its access to insect prey (Shik & Silverman, 2013), but carbohydrate-rich nectar can also promote worker survival (Kay et al., 2010) and aggression (Grover, Kay, Monson, Marsh, & Holway, 2007), as well as the invasive spread (Rowles & Silverman, 2009) and establishment of massive supercolonies (Tillberg, Holway, LeBrun, & Suarez, 2007). However, small propagules are poor competitors (Hee, Holway, Suarez, & Case, 2000; Sagata & Lester, 2009), and are likely under intense pressure to increase colony growth rate by maximizing brood production and worker survivorship. And yet, little is known about how nutrients are allocated to survival or growth during this most tenuous life history stage of an ant colony (Oster & Wilson, 1978).

We used a factorial design with incipient *L. humile* colonies to manipulate access to aphids, baseline levels of nutritional deprivation, and, by adding or subtracting queens, the ability to invest in growth. This design allowed us to test whether the presence of brood reduces carbohydrates available for adult workers, and whether the value of aphid honeydew depends on access to nutrients elsewhere in the diet. Moreover, if carbohydrates similarly limit brood production and worker maintenance, we predicted that honeydew from hemipteran mutualists would decrease allocation trade-offs at a critical stage early in a colony's life history.

We further linked the nutritional value of honeydew with mutualism stability, monitoring aphid growth rates to test the prediction that colonies deprived of dietary protein would shift from tending aphids to consuming them (Cushman & Addicott, 1991). Since hemipterans can also alter the composition of their honeydew when tended by ants, increasing amino acid content in their honeydew (Yao & Akimoto, 2002) and reducing the nitrogen content in body tissue (Kay, Scott, Schade, & Hobbie, 2004), we also tested whether the nitrogen content of aphid body tissue reflects protein demands of attendant ant colonies.

## METHODS

### Experimental Set-up

We collected Argentine ants from Research Triangle Park, North Carolina, U.S.A. (35°51'N, 78°49'W) on 8 September and 14 October 2011, extracting them from leaf litter into flouon-coated containers with plaster nests, and ad libitum 25% sucrose-water and freshly killed German cockroaches, *Blattella germanica*. Argentine ants are well suited for the experiments described below because invasive populations typically form massive supercolonies with many polydomous nests, each with many queens, which reproduce by budding (Holway & Case, 2000). On 22 and 23 December, we set up 80 incipient colonies, each with 200 workers, and allowed them to colonize plastic cups filled with potting soil and a 2-week-old cotton plant (*Gossypium hirsutum*) inside a 19.4 litre bucket lined with flouon and covered with a lid of fine mesh. Cotton plants have small numbers of extra floral nectaries and nectar consumption by ants was likely minor relative to consumption of aphid honeydew (Mondor, Rosenheim, & Addicott, 2008; Shik & Silverman, 2013; Wilder et al., 2011). As described below, we used a three-way factorial design with each successive treatment (aphid, queen, diet) applied to half of the experimental colonies by stratifying across the previous treatments. We kept all nests in a climate-controlled laboratory on a 12:12 h light:dark cycle, at 26 °C and 65% RH.

### Manipulating Access to Aphids

On 15 December, we inoculated half the cotton plants ( $N = 40$ ) with cotton aphids, *Aphis gossypii*, and then removed all but 100 aphids per plant on 22 December. Cotton aphids are widespread plant generalists and known mutualists of Argentine ants (Mondor et al., 2008; Powell & Silverman, 2010). We added 30 ml of water to the soil of all plant cups every third day during the experiment and frequently inspected control (–aphid) plants to verify they lacked aphids. Every 2 weeks, we planted three seeds of *G. hirsutum* in the soil of each cup to ensure aphids had ad libitum plant material on which to expand their populations.

### Manipulating Queen Number

On 24 December, we added four queens per colony to half of the colonies crossed with the aphid treatment ( $N = 40$ ). The addition of these queens from the parent nests of workers allowed us to manipulate a colony's ability to allocate to growth (brood production) and was day 1 of the experiment. Initial worker mortality, determined by inspecting trash piles for corpses on 25 December, was minimal (mean  $\pm$  SD =  $1.5 \pm 3.3$  dead workers per colony).

### Manipulating Access to Dietary Protein and Carbohydrates

We manipulated each colony's baseline state of nutritional deprivation by providing ad libitum synthetic agar-based diets from Dussutour and Simpson (2008a), with protein:carbohydrate ratios of either a 1:3 (hereafter 'high carbohydrate' diet;  $N = 40$ ) or 3:1 (hereafter 'high protein' diet;  $N = 40$ ). Both diets provided 109 g/litre of protein + carbohydrates. These diet manipulations were crossed with aphid and queen treatments to complete the factorial design. Protein in these diets came from dried whole egg powder, calcium caseinate and whey protein. The digestible carbohydrate was sucrose. Methyl 4-hydroxybenzoate was used to prevent diet spoilage. Vanderzant vitamin mixture provided micronutrients. For details, see [Supplementary material](#). We used treatment protein:carbohydrate ratios of 1:3 or 3:1 because these

ratios span the optimal range accepted by *L. humile* and other ants in prior experiments (e.g. Dussutour & Simpson, 2008b; Kay, Shik, van Alst, & Kaspari, 2012; Kay et al., 2010). Beginning 24 December, we provided each colony a petri dish with a 1 g cube of fresh diet and replaced these diet cubes daily.

#### *Measuring Allocation of Biomass to Survival and Growth*

After 40 days, we harvested all colonies by spreading out nesting soil in large trays and counting and collecting all workers, larvae, pupae and queens. We chose 40 days as the end point because this meant that all adult workers represented survivors from the day 1 propagule, given that all colonies were initially introduced without brood, and development time from egg to adult is 40 days at 26 °C (Newell & Barber, 1913). A few new workers were present on day 40, but these were lightly coloured 'callow' workers under 3 days old (Newell & Barber, 1913) that we combined with pupae and larvae for estimates of brood production. We then freeze-dried these colony components at –40 °C for 48 h and weighed each to the nearest 1 µg on a Cahn microbalance.

We used mixed model analyses in SAS (proc mixed, V.9.2, Cary, NC, U.S.A.) to perform ANCOVAs testing for treatment effects on two measures of final colony size: worker mass (dry mass of surviving workers) and worker survival (number of surviving workers). While all ants were from a single supercolony, initial worker size differed between the two collection dates. During set-up, all experimental colonies received 200 workers, but colonies in +aphid treatments received smaller workers from the 14 October collection date, lowering initial colony mass in +aphid colonies ( $F_{1,78} = 34.86$ ,  $P = 0.0001$ ). We thus used initial worker mass nested in aphid treatment as a covariate in the analyses on final colony size and interpreted LS means. A worker sample from one experimental colony was incompletely harvested and excluded from analyses of worker survival and final worker mass.

Initial worker mass nested in aphid treatment was not a significant covariate in mixed model analysis of colony growth (summed dry mass of larvae, pupae, callow workers;  $P = 0.29$ ), so we excluded the covariate from the model examining the effects of aphid and diet treatments on colony growth, and interpreted treatment means.

#### *Measuring Allocation of Nutrients to Survival and Growth*

To examine treatment effects on worker composition, we used the percentage of fat mass to assess the assimilation of dietary carbohydrates, the percentage of nitrogen mass to assess the assimilation of dietary protein, and the percentage of fat/percentage of nitrogen to relate worker performance with the ratio of protein and carbohydrates in worker body tissue. We first used Soxhlet extraction to measure the percentage of fat mass in pre-weighed samples of 20 freeze-dried workers for each colony. We used the method of Smith and Tschinkel (2009) to extract fat with diethyl ether for 16 h, placing workers into perforated gelatin capsules skewered on fine wire, and then weighed them again after drying at 60 °C for 24 h. Two samples were lost during this process because the gelatin capsules cracked open during the extraction process. For each colony, we also measured the percentage of nitrogen mass of a 0.4 mg sample of freeze-dried workers (two to three individuals) and brood (larvae and pupae). We used a Flash EA1112 CN analyser (ThermoQuest, Milan, Italy) that combusts samples with aspartic acid as a standard. Nine worker samples (at most two samples for a given treatment) and one brood sample were lost during nitrogen analyses. Low brood production in one treatment group (see Results) further reduced brood available for analyses of the percentage of nitrogen mass ( $N = 32$ ).

We used mixed model analyses to perform ANCOVAs testing for treatment effects on workers' percentage of fat mass, percentage of nitrogen mass and percentage of fat/percentage of nitrogen, including initial worker mass nested in aphid treatment as a covariate, and interpreting the LS means. To analyse treatment effects on brood percentage of nitrogen mass, we used mixed models, dropping the nonsignificant covariate initial worker mass ( $P = 0.98$ ), and interpreting means.

#### *Feedbacks to Aphid Mutualists*

On days 7, 21 and 35 of the experiment, we counted all aphids on plants to monitor population growth and test the prediction that protein-starved colonies would consume aphid body tissue rather than aphid honeydew. We used repeated measures ANOVA to examine the effects of queen and diet treatments on aphid number, interpreting the time by treatment interactions. On day 40, we collected and freeze-dried aphids from each plant and used the methods described above to analyse aphid composition. Initial worker mass was not a significant covariate for aphid percentage of nitrogen mass ( $P = 0.30$ ), so we dropped the nonsignificant covariate from the mixed model analysis testing for treatment effects and interpreted means.

## RESULTS

#### *Worker and Brood Composition*

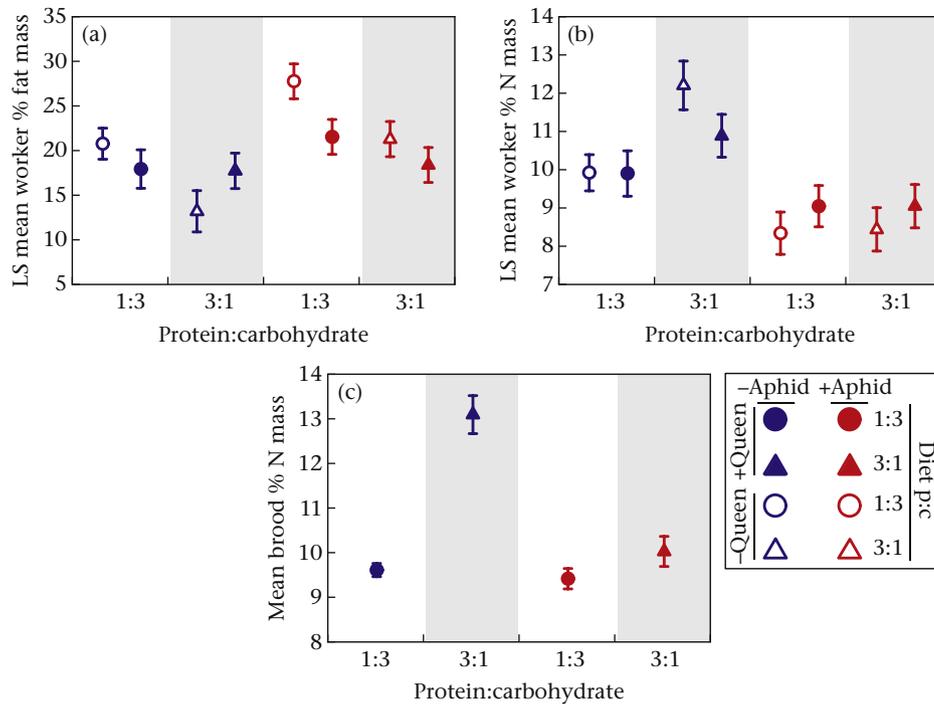
As predicted, worker percentage of fat mass increased with access to both aphids (ANCOVA:  $F_{1,68} = 3.94$ ,  $P = 0.05$ ; Fig. 1a) and high carbohydrate diet (ANCOVA:  $F_{1,68} = 14.78$ ,  $P = 0.0003$ ; Fig. 1a). Access to aphids and diet composition had similar interactive effects on worker and brood percentage of nitrogen mass, a proxy for protein content (Fig. 1, Table 1). Worker percentage of nitrogen mass was about 19% higher in colonies fed the high protein diet without access to aphids than in other treatment combinations (ANCOVA:  $F_{1,61} = 6.26$ ,  $P = 0.015$ ; Fig. 1b). Similarly, brood percentage of nitrogen mass was about 35% higher in the high-protein, –aphid treatment than in other combinations (ANCOVA:  $F_{1,28} = 20.37$ ,  $P = 0.0001$ ; Fig. 1b).

#### *Worker Survival, Brood Production and Colony Growth*

Worker survival and brood production responded similarly (but not identically) to treatments, and both were similarly associated with individual ant chemical composition. Worker survival (ANCOVA:  $F_{1,69} = 22.39$ ,  $P = 0.0001$ ; Table 2, Fig. 2a) and final mass (ANCOVA:  $F_{1,69} = 13.42$ ,  $P = 0.0005$ ; Table 2) were generally lower in colonies without aphids (Table 2, Fig. 2a), and brood production was particularly low in colonies without aphids on the high protein diet (ANCOVA:  $F_{1,36} = 16.13$ ,  $P = 0.0003$ ; Fig. 2b, Table 2).

#### *Carbohydrate Supply and Allocation Trade-offs*

The association between all measures of worker composition and the potential for brood production (queen treatment) interacted with diet and aphid treatments. Worker percentage of fat mass was particularly high (ANCOVA:  $F_{1,68} = 5.42$ ,  $P = 0.023$ ;  $F_{1,68} = 3.99$ ,  $P = 0.050$ ; Fig. 1a), worker percentage of nitrogen mass was particularly low (ANCOVA:  $F_{1,61} = 4.16$ ,  $P = 0.046$ ; Fig. 1b), and worker survival was particularly high (ANCOVA:  $F_{1,69} = 19.27$ ,  $P = 0.0001$ ;  $F_{1,69} = 8.15$ ,  $P = 0.006$ ; Fig. 2a) when aphid access and high carbohydrate diet were provided in queenless colonies (Tables 1, 2). Worker survival was also positively associated with both individual percentage of fat/percentage of nitrogen ( $R^2 = 0.81$ ,



**Figure 1.** Effects of aphid access (+/-), diet (protein:carbohydrate ratio, p:c) and queen presence (+/-) on (a) worker percentage of fat mass, (b) worker percentage of nitrogen mass and (c) brood percentage of nitrogen mass in Argentine ants. Values are means  $\pm$  SE.

$N = 8$ ,  $P = 0.002$ ; Fig. 2a) and mass of brood production ( $R^2 = 0.39$ ,  $N = 39$ ,  $P = 0.0001$ ; Fig. 2c). Brood production was negatively associated with percentage of nitrogen mass and positively associated with aphid access and high carbohydrate diet (Fig. 2b). Combined, these results suggest that resource allocation trade-offs between workers and brood are particularly pronounced when colonies have limited access to carbohydrates.

#### Aphid Composition and Population Growth

The percentage of nitrogen mass of aphid mutualists was not significantly influenced by the nutritional composition of ant colony diet or colonies' nutritional demands for brood production (Fig. 3a, Table 1). Aphid populations increased over time across all colony treatments (repeated measures ANOVA:  $F_{2,72} = 5.07$ ,  $P = 0.01$ ), levelling out at a mean  $\pm$  SE of  $1135.15 \pm 459.92$  aphids per plant (Fig. 3b). This population growth was independent of queen treatment (repeated measures ANOVA:  $F_{2,72} = 0.52$ ,  $P = 0.59$ ) or diet composition (repeated measures ANOVA:  $F_{2,72} = 1.50$ ,  $P = 0.23$ ).

## DISCUSSION

We use an ant–aphid system to test whether the value of food rewards from a mutualistic association depends on nutritional context and interactions among potentially competing life history demands. We found that, as predicted, aphid mutualists helped ant colonies offset nutritional costs of an imbalanced, high protein diet. However, we found unexpectedly that workers and brood, which traditionally have been assumed to require different nutrients from parent colonies, were both limited by access to aphids and dietary carbohydrates. These results suggest that carbohydrate availability constrains the development of Argentine ants and underscores the importance of hemipteran mutualists in the success of this widely invasive species. Moreover, by clarifying the role of nutrition in a ubiquitous mutualistic interaction, these results suggest that

colony nutritional status influences the foraging decisions of ants, with potential cascading effects across multiple trophic levels to influence whole communities.

Food from mutualists is predicted to be especially valuable when it provides nutrients that are either in scarce supply, or essential for supporting investment in fitness-enhancing traits (Hoeksema & Schwartz, 2003; Yoshida, 2006). And, while hemipteran mutualists (and carbohydrates specifically) are known to enhance colony growth rates in some ant species (Helms & Vinson, 2008; Shik & Silverman, 2013; Wilder et al., 2011), this study is the first to link these nutritional benefits of honeydew with the nutrient allocation requirements of colony life history traits. Moreover, previous laboratory studies manipulating dietary macronutrient composition have confirmed that ant colonies, even those of predatory species (e.g. *Rhytidoponera metallica*), not only defend carbohydrate intake targets, but more precisely do so with larvae in the nest (Dussutour & Simpson, 2009). However, the present study provides additional ecological realism because hemipterans are a ubiquitous source of carbohydrates for ant colonies in nature.

We found evidence for a carbohydrate-based growth–maintenance trade-off, as the presence of queens was associated with reduced worker percentage of fat mass, which in turn was associated with lower worker survival. These overlapping carbohydrate requirements of workers and brood highlight the value aphid mutualists for incipient Argentine ant colonies. Aphid access was associated with the greatest nutritional and longevity benefits for workers in queenless colonies, where brood could not compete for harvested honeydew. Moreover, the benefits of aphid access for production of brood in colonies confined to a high protein diet (and the associated decline in brood percentage of nitrogen mass) support the emerging theme in social insect biology that some life history traits may be maximized on more carbohydrate-biased diets than previously hypothesized (Carroll & Janzen, 1973; Davidson, 2005).

Demand for specific nutrients can profoundly influence many species interactions, from herbivorous moose that forage for

**Table 1**

Tests for how access to aphids (Aphid), the protein:carbohydrate ratio of diets (Diet) and the presence of queens (Queen) affect the composition of Argentine ant workers and brood, as well as their aphid mutualists

Tissue	Dependent variable	Treatment	df	F	P
Worker	% Fat mass	Aphid	1	3.94	0.051
		Diet	1	14.78	0.0003
		Queen	1	2.51	0.118
		Aphid* <i>Diet</i>	1	0.17	0.684
		Aphid* <i>Queen</i>	1	5.42	0.023
		Diet* <i>Queen</i>	1	3.99	0.050
		Aphid* <i>Diet</i> * <i>Queen</i>	1	0.57	0.454
		Initial worker mass(aphid)	2	3.50	0.036
		Error	68		
		% N mass	Aphid	1	8.90
	Diet		1	7.06	0.010
	Queen		1	0.00	0.986
	Aphid* <i>Diet</i>		1	6.26	0.015
	Aphid* <i>Queen</i>		1	4.16	0.046
	Diet* <i>Queen</i>		1	0.84	0.363
	Aphid* <i>Diet</i> * <i>Queen</i>		1	0.62	0.435
	Initial worker mass(aphid)		2	2.72	0.074
	Error		61		
	% Fat/% N		Aphid	1	6.89
		Diet	1	17.12	0.0001
Queen		1	0.57	0.451	
Aphid* <i>Diet</i>		1	0.22	0.640	
Aphid* <i>Queen</i>		1	7.96	0.007	
Diet* <i>Queen</i>		1	5.37	0.024	
Aphid* <i>Diet</i> * <i>Queen</i>		1	0.56	0.459	
Initial worker mass(aphid)		2	4.79	0.012	
Error		60			
Brood		% N mass	Aphid	1	26.27
	Diet		1	41.46	0.0001
	Aphid* <i>Diet</i>		1	20.37	0.0001
	Error		28		
Aphid	% N mass	Queen	1	0.77	0.388
		Diet	1	1.48	0.232
		Queen* <i>Diet</i>	1	2.96	0.094
		Error	35		

Univariate mixed models used for all analyses. See methods for details regarding *df* and inclusion of the covariate in analyses.

**Table 2**

Univariate mixed models testing for treatment effects on colony life history traits

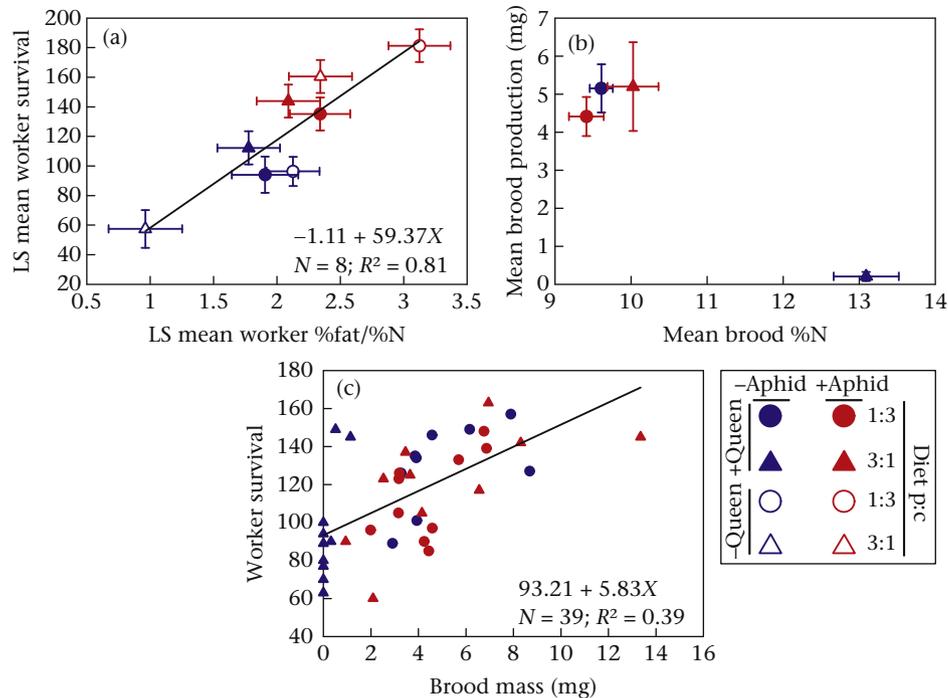
Trait	Treatment	df	F	P
Final worker mass	Aphid	1	13.42	0.0005
	Diet	1	0.80	0.375
	Queen	1	3.68	0.059
	Aphid* <i>Diet</i>	1	1.11	0.295
	Aphid* <i>Queen</i>	1	19.11	0.0001
	Diet* <i>Queen</i>	1	14.21	0.0003
	Aphid* <i>Diet</i> * <i>Queen</i>	1	1.74	0.191
	Initial worker mass(aphid)	2	31.76	0.0001
	Error	69		
	Worker survival	Aphid	1	22.39
Diet		1	1.66	0.202
Queen		1	0.15	0.701
Aphid* <i>Diet</i>		1	0.11	0.738
Aphid* <i>Queen</i>		1	19.27	0.0001
Diet* <i>Queen</i>		1	8.15	0.006
Aphid* <i>Diet</i> * <i>Queen</i>		1	0.83	0.366
Initial worker mass(aphid)		2	34.07	0.0001
Error		69		
Final brood mass	Aphid	1	8.86	0.005
	Diet	1	8.48	0.006
	Aphid* <i>Diet</i>	1	16.13	0.0003
	Error	36		

See methods for details regarding variation in *df* and inclusion of the covariate in analyses.

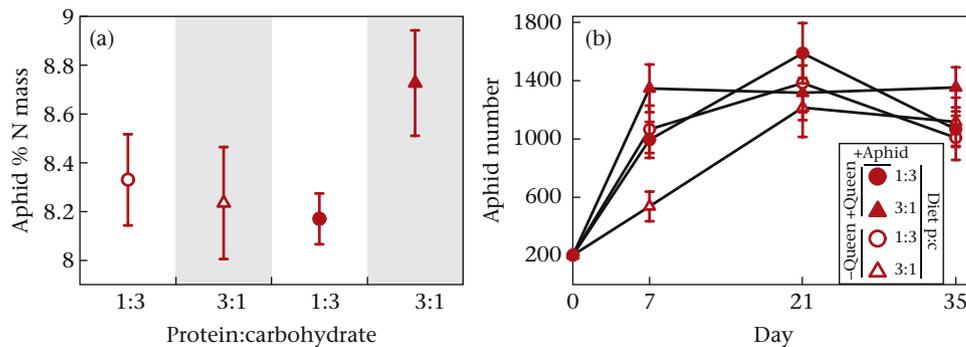
aquatic plants to meet sodium requirements (Belovsky & Jordon, 1981) to predatory spiders that differentially extract macronutrients from prey (Mayntz, Raubenheimer, Salomon, Toft, & Simpson, 2005). Results of the present study link the composition of food rewards to traits that underlie mutualism stability and demonstrate how nutrition shapes these ubiquitous and ecologically important species interactions. For instance, the overwhelming importance of carbohydrates for developing Argentine ant colonies suggests that the ants are under strong pressure to tend, rather than eat, hemipteran mutualists. Indeed, aphid populations grew rapidly across all colony treatments. We note that aphid populations can respond in unpredictable ways to ant tending when measured in the field, and that other factors, such as variation in host plant identity and the abundance of aphid predators and parasitoids, which were controlled for in this laboratory experiment, may interact with the specific macronutrient requirements of Argentine ants to shape aphid population growth rates. Furthermore, a trend towards increased percentage of nitrogen mass of aphid body tissue when paired with +queen colonies fed a high protein diet ( $P = 0.09$ ) suggests that the mutualists may have excreted relatively more carbohydrates in honeydew (e.g. Kay et al., 2004; Yao & Akimoto, 2002). And yet, it remains to be seen whether carbohydrate exudates similarly promote mutualism stability in other ant species that may have different dietary protein:carbohydrate requirements. Evidence from at least one carnivorous ant species, *Solenopsis invicta*, suggests similar benefits derived from carbohydrate exudates (Helms & Vinson, 2008; Wilder et al., 2011).

While the present study contrasts the classic hypothesis of protein fuelling growth and carbohydrates fuelling maintenance, this is not meant to discount the importance of protein for colony growth. For instance, it is possible that Argentine ant larvae are particularly efficient at using protein. Moreover, larvae of other ant species are known to consume a variety of proteins (Wheeler & Martínez, 1995), which can deplete proteins stored in adult ants (Martínez & Wheeler, 1994). And yet, there are many reasons to predict that carbohydrates govern larval development. For instance, moulting Argentine ant larvae must replace their exoskeletons three times (Solis, Fox, Rossi, & Bueno, 2010), and chitin synthesis (and the associated reorganization of tissue during pupation) is energetically costly for larvae of other holometabolous insects, reducing haemolymph titres of trehalose, glucose and lipids (Pant & Kumar, 1979; Siebert, Speakman, & Reynolds, 1993) and fat body glycogen stores (Siebert, 1987). To link aphid-derived resources to colony trait expression, it will be important to understand the physiological underpinnings of carbohydrate usage by all ant castes and life stages.

Ant–hemipteran mutualisms are conspicuous in terrestrial habitats across the planet, and ‘ecological meltdown’ can result when the mutualists support runaway population growth of invasive ants (O’Dowd, Green, & Lake, 2003). And, while honeydew rewards are known to be central to these interactions, much remains unknown about how these carbohydrate-rich subsidies are allocated inside the nest and how they are used to fuel life history investment of whole colonies. These dynamics are key to understanding the relative value of these food rewards, the stability of the mutualisms and ultimately the growth of invasive ant populations. Carbohydrates have also been found to govern the behavioural dominance (Grover et al., 2007), survival (Kay et al., 2010) and establishment (Tillberg et al., 2007) of Argentine ants in an enormous invasive Californian supercolony, and the present study of an unrelated supercolony provides strong evidence that these carbohydrate effects apply broadly across invasive populations. Moreover, these results provide a basis to interpret more conspicuous behavioural interactions among mutualists and their resulting ecosystem impacts, while also moving us towards a general understanding of how nutrition structures ecosystems.



**Figure 2.** Effects of aphid access (+/–), diet (protein:carbohydrate ratio, p:c) and queen presence (+/–) on (a) worker survival, (b) brood production and (c) the relationship between per-colony worker survival and brood production in Argentine ants. Values are means  $\pm$  SE.



**Figure 3.** Testing whether (a) aphid percentage of nitrogen mass or (b) aphid population growth varies with the diet (protein:carbohydrate ratio, p:c) or queen treatment (+/–) experienced by their attendant Argentine ant colony. Values are means  $\pm$  SE.

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## Supplementary Material

Supplementary material for this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2014.06.008>.

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