Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants

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Predicting the outcome of competitive interactions is a fundamental goal in ecology. Ecological stoichiometry, which relates nutrient balance to ecological processes, provides a framework for identifying mechanistic links among macronutrient availability, nutritional physiology and competitive performance. Because carbohydrates serve as a principal metabolic fuel, carbohydrate scarcity may impinge upon behaviours affecting competitive dominance (e.g. aggression, activity) to a greater extent than deficiencies of protein or other nutrients used preferentially for growth. Here, we tested this prediction with a diet manipulation study involving laboratory colonies of Argentine ants (*Linepithema humile*), a widespread and aggressive invasive species. The availability of both sucrose and insect prey influenced brood production and worker survival after three months. However, colonies became less aggressive and less active only when deprived of sucrose (but not prey). Scarcity of sucrose (but not prey) was also associated with reduced fat mass in individual workers. These data provide the first experimental support that carbohydrate scarcity compromises aggression and activity in ants, and illustrate, in principle, how access to carbohydrate-rich resources (e.g. plant exudates, hemipteran honeydew) might influence behavioural investments that contribute to competitive performance. Such investments might be especially important for invasive ants, given their aggressiveness and tendency to interact with honeydew-producing Hemiptera.

Keywords: aggression; behavioural dominance; *Linepithema humile*; macronutrients; nutrient imbalance

1. INTRODUCTION

Models of resource competition applied to terrestrial animals have been based almost exclusively on demographic or single resource currencies, such as food or energy (Schoener 1983; Brown *et al.* 2004). However, certain animals, bees and ants for example, consume chemically distinct food resources, such as nectar and protein-rich pollen or prey. Recent work in ecological stoichiometry, the study of nutrient and energy balance in ecological processes (Sterner & Elser 2002), suggests that physiological regulation in the face of such nutritional complexity can mediate patterns of ecological interactions (Anderson *et al.* 2004; Kay *et al.* 2005; Elser 2006). One link between nutritional physiology and ecological processes arises when imbalances of nutrients with distinct biochemical properties constrain responses to specific environmental exigencies. For example, dietary protein: carbohydrate ratios can influence the effects of ingesting plant secondary chemicals (Simpson & Raubenheimer 2001; Behmer *et al.* 2002) and the consequences of pathogen infection (Lee *et al.* 2006) as a result of the distinct nutritional roles of proteins and carbohydrates. Macronutrient imbalance could similarly influence investments that underlie competitive prowess, given differences

in the suitability of macronutrients as fuel for dominance-related behaviours (Davidson 1997; Kay & Adler 2003).

Ants provide an ideal system to test how macronutrient availability affects the costs and benefits of competitive dominance. Considerable evidence suggests that resource competition strongly influences population and community dynamics in ants (Hölldobler & Wilson 1990). Ants exhibit a variety of territorial strategies and often engage in interference competition with conspecifics as well as with other species. Dominance behaviours include nest raiding, ritualized aggression, use of chemical defences and caste-specific defensive roles (Hölldobler & Wilson 1990). Comparative studies on ants (e.g. Davidson *et al.* 2003) suggest associations between behavioural dominance and the relative availability of carbohydrates, which serve as an important metabolic fuel for most insects (Dadd 1985). Yanoviak & Kaspari (2000), for example, found that ants in the canopies of tropical rainforests, where carbohydrate resources are abundant (Tobin 1991), exhibited greater activity and aggression compared with litter ants from paired sites in Panama. Similarly, Davidson (1998) hypothesized that the high levels of activity and aggression exhibited by invasive ants may be linked to their well-established reliance on carbohydrate-rich honeydew produced by Hemiptera (Newell & Barber 1913; Way 1963; Tennant & Porter 1991; Helms & Vinson 2002; O'Dowd *et al.* 2003).

Although these comparative studies suggest associations between diet and behavioural dominance, the

mechanistic underpinnings of such correlations have received surprisingly little experimental attention. Here, we test the relationship between macronutrient availability and behavioural dominance with a diet manipulation experiment on the invasive Argentine ant (*Linepithema humile*). The Argentine ant presents an ideal system for this study because it is a highly active and behaviourally dominant invader that aggressively displaces native ant species in its introduced range (Ward 1987; Suarez *et al.* 1998; Human & Gordon 1999). Moreover, Argentine ants rely extensively on plant-based liquid food, especially honeydew (Way 1963; Markin 1970). Despite these proclivities, it is unknown how carbohydrate availability relates to the aggressiveness and high levels of activity typically exhibited by these and other invasive ants.

To test in detail how nutrient imbalance affects behaviour, we independently manipulated the levels of carbohydrates (sucrose) and proteins (insect prey) available to experimental colonies of Argentine ants. Like other insects (Simpson *et al.* 2004), ants are able to mediate nutritional imbalance through compensatory feeding (Kay 2004). However, independent variation in nutritional sources can produce substantial physiological changes in ant colonies (Bono & Herbers 2003; Kay *et al.* 2006). Our central hypothesis is that carbohydrate scarcity will compromise aggression and activity, but that prey availability will be either of ancillary importance or unrelated to these performance measures. Because colony-level behaviours are influenced by the demography and size of the colony itself, we also examine how sucrose and prey availability affect brood production, worker survival and worker condition. We predict that (i) variables relating to colony size will be affected by the availability of both sucrose and prey and (ii) increasing sucrose availability will lead to higher worker lipid stores, which are known to influence foraging propensity. The factorial approach used in this study allows us to quantify the separate and interactive effects of two key macronutrients on the behaviour and performance of a widespread, abundant and highly aggressive invasive species.

2. MATERIAL AND METHODS

We collected ants for experimental *L. humile* colonies from 63 sites in San Diego Co., CA in January 2006. Collecting sites were at least 200 m apart, and ants from each site served as the source of material for one experimental colony. Each experimental colony consisted of five queens, approximately 1000 workers and roughly similar amounts of brood. We reared colonies in circular plastic containers (28 cm in diameter $\times$ 10 cm in height) lined with Fluon to prevent ants from escaping. In each container, we placed three nest chambers (glass test tubes: 16×150 mm) half full of water and stopped with cotton; we covered the nest chambers with aluminium foil to keep them dark. We reared colonies under standard laboratory conditions (12 : 12 hour light : dark cycle at approximately 20°C). Prior to the start of the experiment, we fed colonies 20% sugar water weight-in-weight (w/w) and freeze-killed crickets (*Acheta domestica*) ad libitum for one to two months.

Diet manipulations consisted of factorial combinations of sucrose and insect prey. These chemically distinct food items represent sources of carbohydrates and proteins, respectively, often consumed by ants (Porter 1989; Evans & Pierce 1995;

Kay *et al.* 2006). Because natural sources of carbohydrates differ in their sugar content (Blüthgen *et al.* 2004; Woodring *et al.* 2004), we chose three concentrations of sucrose: zero (0%); low (2%); and high (20%) (w/w). This same range of sucrose solutions has been successfully used in studies examining diet and colony growth in other ant species (Bono & Herbers 2003; Kay *et al.* 2006). We fed ants sucrose in an agarose gel medium three times a week. We also fed ants three levels of prey: zero (no prey); low (0.5 freeze-killed crickets per week); and high (5 crickets per week). Individual crickets weighed, on average, 0.47 g (wet weight). This prey range approximates that used in previous studies on Argentine ants (Aron *et al.* 2001). We randomly assigned colonies to experimental groups; each group consisted of a different combination of food-type levels. We made water available ad libitum throughout the experiment. Because an earlier study found no relationship between aggression and colony starvation after five weeks (Thomas *et al.* 2005a), we fed colonies treatment diets for 12 weeks. At 10 and 11 weeks, we conducted behavioural assays, and, at 12 weeks, we sacrificed all colonies to measure colony productivity.

(a) *Behavioural assays*

We carried out two behavioural assays on each experimental colony: intraspecific aggression and *per capita* colony-level activity. To quantify aggression levels, we used an established protocol developed in earlier studies (Thomas *et al.* 2005a,b, 2006; see also Roulston *et al.* 2003). For each aggression trial, we haphazardly sampled five workers from an experimental colony and matched them against five workers from a stock colony originating from Lake Hodges, San Diego Co., CA (Suarez *et al.* 2002; Tsutsui *et al.* 2003; Thomas *et al.* 2006). For the duration of the study, we provided the Lake Hodges stock colony with ad libitum 20% sugar water (w/w), freeze-killed crickets (*A. domestica*) and water. The Lake Hodges supercolony is behaviourally and genetically differentiated from the supercolony source for this study (Tsutsui *et al.* 2003; Thomas *et al.* 2006). When paired with workers from Lake Hodges, workers from the supercolony source for the present study are nearly always the aggressors (Tsutsui *et al.* 2003; Thomas *et al.* 2005b). Each trial took place in a 9 cm diameter Petri dish with the 1.4 cm vertical edges lined with Fluon. Once ants were placed in the dish, we scanned ants every minute for 10 min and classified worker behaviours into one of four categories: (i) no interest or ignore, (ii) mandible gaping, avoidance or intense antennation, (iii) aggression (a physical attack by one or both workers, including lunging, biting, holding or pulling of legs or antennae), and (iv) fighting (aggression resulting in death or severe injury; includes the use of chemical defensive compounds). Although there were 10 ants in the Petri dish, almost all aggressive interactions involved pairs of workers. Once a category (iv) fight began, the combatants were removed to prevent a single ant from killing more than one opponent (see Tsutsui *et al.* 2003). To avoid resampling, workers were not returned to their colonies. Observers were blind to the experimental group during all trials.

For each 10 min trial, we calculated one aggression index ranging from 1 (no aggression) to 4 (intense aggression). We determined the proportion of ants involved in each behavioural category every minute and then calculated the average for each category. We next multiplied the average of each category by the aggression level for that behaviour (i.e. 1–4), and summed these values to obtain a final aggression

Table 1. Two-way ANOVAs for the behavioural assays conducted in this study.

assay	d.f.	MS	F	p
intraspecific aggression				
sucrose	2	1.199	8.617	0.0006
prey	2	0.053	0.382	0.684
interaction	4	0.312	2.241	0.077
residual	54	0.139		
per capita activity				
sucrose	2	45.839	7.392	0.0015
prey	2	7.226	1.165	0.320
interaction	4	0.519	0.084	0.987
residual	54	6.201		

index. An index of 1 indicates no aggression in a trial, whereas an index of 4 means that all ants were fighting at the highest level within the first minute. This index therefore reflects not only aggression intensity, but also how rapidly it occurs.

To assess activity, we measured each colony's ability to explore a three-dimensional structure placed in its nest container. Each structure consisted of three 30 cm long bamboo skewers arranged as a tripod with small paper 'leaves' attached to the skewer tips. Because structures contained no resources, they served as a neutral way to determine a colony's inherent activity level with respect to the exploration of novel space. Argentine ants actively investigated these structures as expected based on the tendency of this species to forage in above-ground vegetation. A new structure was used for each colony to eliminate biases resulting from pheromone deposition. At 5, 10 and 15 min after the start of each trial, we counted the workers present on the structure. For each colony, we computed a time-averaged activity score and then divided this value by the number of living workers in the colony to generate a single *per capita* activity score. Activity trials were conducted after colonies had been starved for 24 hours.

(b) *Colony productivity and worker condition*
Twelve weeks after the experiment began, we sacrificed all colonies to measure the following: number of surviving workers; brood dry mass; per worker fat dry mass; and per worker lean dry mass. For each colony, brood were dried at 40°C for 48 hours. For lipid analysis, we randomly selected at least 30 workers from each colony, dried them to a constant mass and then stored them at −20°C. We determined total lipid content using a modification of Van Handel's (1985) procedure (see also Hahn 2006). Briefly, we homogenized workers with a mortar and Teflon-coated pestle, then extracted lipids from a known mass (± 0.1 µg) of this material using three washes of a 2 : 1 (v/v) chloroform : methanol solvent. After evaporating the solvent from the pooled extracts using dry N_2 gas, we resuspended the lipid residue in 0.5 ml of extraction solvent. We dried a 50 µl aliquot of the lipid extract and immediately treated it with 100 µl of 98% H_2SO_4 at 95°C. After cooling, we added 2.5 ml of phospho-vanillin reagent to each sample and read the A_{525} after 5–30 min. We used a 70 : 30 mixture of glycerol trioleate : glycerol tripalmitate as a standard to estimate fat mass (percentage of dry mass). All samples and standards were run in triplicate. We estimated fat mass per worker in a colony using percentage of fat mass×per worker dry mass,
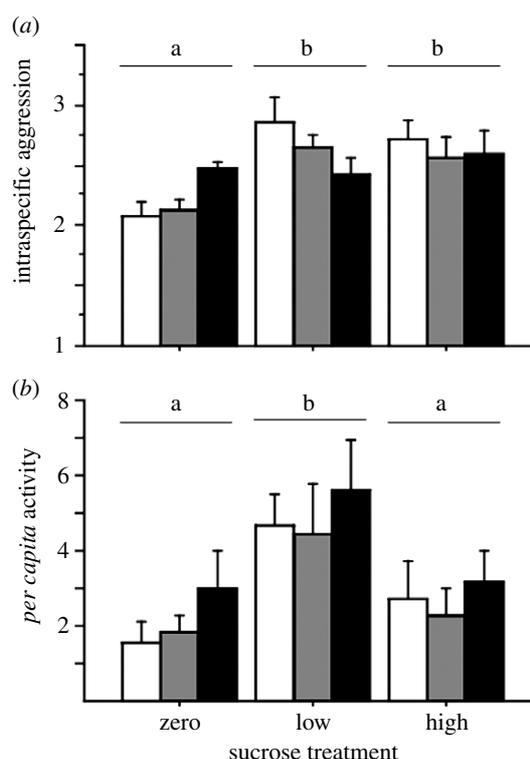

Figure 1. Mean (+s.e.) (*a*) intraspecific aggression and (*b*) *per capita* colony-level activity for Argentine ant colonies reared on diets with different levels of sucrose and insect prey. For each performance measure, the effect of sucrose was significant, but the effect of prey was not. Different lower-case letters indicate significantly different sucrose levels from Tukey tests. White bars, zero prey; grey bars, low prey; black bars, high prey.

and lean mass per worker using per worker dry mass−per worker fat mass.

(c) *Statistical analysis*
We used two-way analysis of variance (ANOVA) to test how sucrose and prey availability affected each dependent variable. We treated food-type levels as fixed factors because the amounts used were set by us and were a non-random subset of those found in nature. For all but one ANOVA, Cochran's tests confirmed homogeneity of variances. Brood dry mass data were the exception and were thus log transformed prior to analysis. Following each ANOVA, we used Tukey tests to compare means across experimental groups (Day & Quinn 1989).

3. RESULTS
(a) *Behavioural assays*
Sucrose deprivation diminished the levels of intraspecific aggression, whereas prey availability had no measurable effect on this performance measure (table 1). Although workers from colonies deprived of sucrose in all cases exhibited aggression, these workers were consistently less aggressive compared with workers from colonies provided with sucrose (figure 1a; two-way ANOVA: $F_{2,54}=8.617$, $p<0.001$). Irrespective of treatment, aggression nearly always consisted of two workers reciprocally fighting; fights of level 3 or 4 tended to remain at the level until the end of the 10 min trial. As with the aggression assays, *per capita* activity also varied in response to the availability of sucrose (two-way ANOVA: $F_{2,54}=7.392$, $p<0.005$)
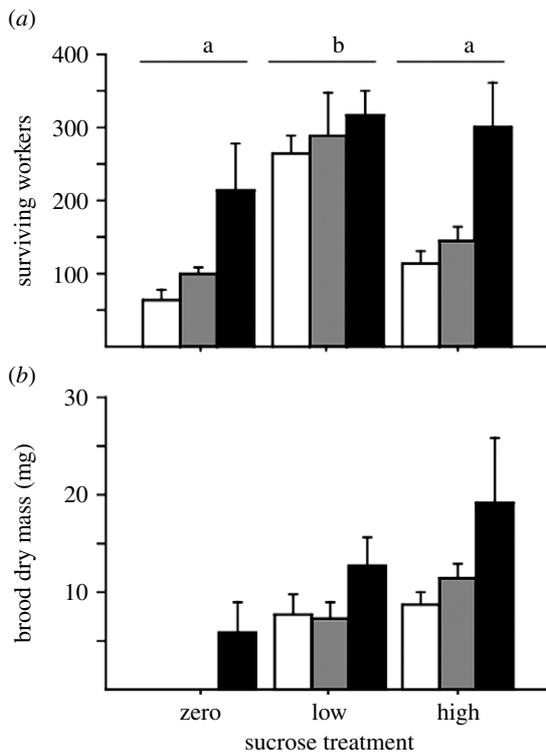
Figure 2. Mean (+s.e.) number of (*a*) surviving workers and (*b*) brood dry mass for Argentine ant colonies reared on diets with different levels of sucrose and insect prey. Both sucrose and prey affected the number of surviving workers. Different lower-case letters indicate significantly different sucrose levels from Tukey tests. (*b*) Two experimental groups in the zero-sucrose treatment produced either few brood (low prey) or no brood (zero prey). With the zero-sucrose treatment excluded, the effect of prey on brood dry mass was marginally significant. White bars, zero prey; grey bars, low prey; black bars, high prey.

but not prey (table 1). Colonies in the low-sucrose treatment exhibited higher *per capita* activity compared with colonies in the high- and zero-sucrose treatments, which did not differ significantly from one another (figure 1*b*).

(b) Colony productivity and worker condition
In contrast to the behavioural assays, parameters relating to colony size were affected by both sucrose and prey availability. The number of surviving workers exhibited a pattern similar to that observed for activity (figure 1*a*), in that both sucrose deprivation and sucrose excess had a depressive effect (figure 2*a*). However, prey availability also affected living worker number (figure 2*a*); Tukey tests revealed that colonies in the high-prey treatment had more surviving workers compared with those in the zero- and low-prey treatments.

As with living worker number, brood dry mass at the end of the experiment was affected by the availability of sucrose and prey. Sucrose deprivation largely curtailed brood production, although high-prey levels mitigated this effect (figure 2*b*). As a result of the drastic effects of sucrose deprivation on the standing crop of brood, the complete dataset was unsuitable for two-way ANOVA. Even with the zero-sucrose treatment excluded, there was still a weak trend for brood mass to increase with prey availability (table 2; figure 2*b*; two-way ANOVA:
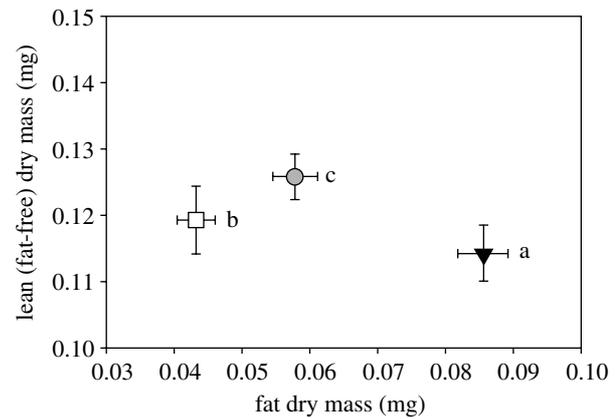
Figure 3. Mean (±s.e.) per worker lean (fat-free) dry mass (mg) versus mean (±s.e.) per worker fat dry mass (mg). Different lower-case letters indicate significantly different sucrose levels for fat dry mass from Tukey tests. Prey level did not significantly alter fat mass. Square, no sucrose; circle, low sucrose; down-triangle, high sucrose.

Table 2. Two-way ANOVAs for colony production and worker condition.

variable	d.f.	MS	F	p
number of surviving workers				
sucrose	2	143 539.191	13.175	<0.0001
prey	2	96 032.762	8.815	0.0005
interaction	4	10 522.095	0.966	0.434
residual	54	10 894.862		
brood dry mass (zero-sucrose treatment excluded)				
sucrose	1	0.990	3.259	0.079
prey	2	0.954	3.142	0.055
interaction	4	0.060	0.199	0.821
residual	36	0.304		
per worker lean mass				
sucrose	2	0.001	2.562	0.088
prey	2	0.001	3.731	0.031
interaction	4	0.001	2.550	0.050
residual	50	2.83×10^{-4}		
per worker fat mass				
sucrose	2	0.009	41.771	<0.0001
prey	2	6.23×10^{-6}	0.030	0.971
interaction	4	4.43×10^{-4}	2.164	0.086
residual	50	2.04×10^{-4}		

$F_{2,36} = 3.142$, $p = 0.055$). However, unlike the case for surviving worker number, high-sucrose levels did not have an inhibitory effect (figure 2*b*). In fact, in the three high-prey treatment groups, brood mass increased steadily with sucrose level (figure 2*b*; one-way ANOVA: $F_{2,20} = 5.422$, $p < 0.05$).

Macronutrient availability had contrasting effects on worker condition. Per worker fat dry mass was unaffected by prey level, but increased stepwise with increasing sucrose level (table 2; figure 3). Compared with ants in the zero-sucrose treatment, ants in the high-sucrose treatment had more than twice the fat mass, on average. In contrast, per worker lean dry mass increased with prey level but not with sucrose level, although there was an interaction between these two variables (table 2). Analysing the effects of prey for each sucrose level separately revealed that prey level significantly affected lean mass only under complete

sucrose deprivation (one-way ANOVA: $F_{2,15} = 6.635$, $p < 0.01$). In the zero-sucrose treatment, workers in the high-prey treatment had higher lean mass compared with workers in the zero- and low-prey treatments (Tukey tests).

4. DISCUSSION

Previous studies have found multiple effects on insect performance that are associated with specific macronutrient deficiencies and excesses (Raubenheimer *et al.* 2005; Mayntz & Toft 2006; Warbrick-Smith *et al.* 2006). Here, we provide the first experimental evidence that activity and aggression in ants are associated with a *specific* macronutrient imbalance (carbohydrate scarcity). Although the results from this laboratory study should be interpreted cautiously, they demonstrate at least, in principle, that carbohydrates fuel aggression and activity, and that the availability of such resources may influence colony performance, colony fitness and invasion success. Aggression and activity relate directly to measures of performance that affect competitive interactions among ant colonies. Enhanced competitive ability resulting from the monopolization of carbohydrate-rich resources would presumably allow colonies to exert a stronger competitive effect on neighbouring colonies and, eventually, to achieve greater ecological dominance. These processes may be especially important for invasive ants, such as *L. humile*, that are highly aggressive, displace other ants and favour carbohydrate-rich resources (Davidson 1998; Holway 1999; Helms & Vinson 2002).

The positive effects of carbohydrates on *per capita* activity were diminished in the high-sucrose treatment (figure 1*b*). The relatively lean workers in the low-sucrose treatment may have been more likely to engage in foraging compared with the fatter workers in the high-sucrose treatment (figure 3). In social insects, foragers often have lower lipid stores compared with workers in the nest (Porter & Jorgensen 1981; Blanchard *et al.* 2000; Toth & Robinson 2005). Our results suggest that a focus on natural variation in worker-level lipid stores may provide insight into how colony-level nutritional differences contribute to competitive asymmetries.

In addition to effects on worker behaviour, macronutrient availability also affected the number of surviving workers and the size of the standing brood crop. In contrast to the behavioural assays, however, measures of colony production were affected by sucrose and prey availability. As in other studies (Porter 1989; Evans & Pierce 1995; but see Nonacs 1991), increasing levels of prey enhanced worker survival and brood production. The effects of sucrose on worker survival and brood mass were more complex. In particular, colonies in the high-sucrose treatment had fewer living workers, but not reduced brood mass, compared with colonies in the low-sucrose treatment (figure 2). Decreased worker survival in the high-sucrose treatment is consistent with the hypothesis that calorie restriction increases longevity. Raubenheimer *et al.* (2005), for example, found that mortality in *Spodoptera littoralis* (Lepidoptera) increased with carbohydrate intake. In the present experiment, sugar consumption in the high-sucrose treatment (20% sucrose for three months) probably greatly exceeded that typical of colonies under more natural conditions.

Colony size plays a key role in determining competitive performance in Argentine ants (Holway & Case 2001) and other ant species (Hölldobler & Wilson 1990). Although the three-month duration of our study was inadequate to assess long-term trends in colony growth, the joint effects of sucrose and prey on parameters relating to colony size (figure 2) suggest an obvious pathway linking nutrient availability to competitive performance to colony fitness. The relationship between carbohydrate scarcity and reduced activity and aggression (figure 1) indicates that prolonged dietary imbalance may also compromise worker performance in ways that act independently of colony size. Because colony starvation does not influence aggression in the short term (Thomas *et al.* 2005*a*), the results of the present study illustrate that the effects of sucrose deprivation on aggression stem from physiological changes that develop over the course of months. Given that environmental sources of carbohydrates (e.g. aggregations of honeydew-producing insects) often exhibit strong spatio-temporal variability, in order to maximize colony-level fitness, colonies of many ant species may be forced to balance seasonally changing nutritional demands with the costs required to obtain carbohydrate-rich resources when they are most needed.

It will be of great interest to test the hypotheses developed here with additional invasive ant species. Because invasive ants rely extensively on honeydew and other carbohydrate-based resources (Holway *et al.* 2002), our results suggest that access to such food may enhance behavioural dominance, ecological dominance and invasion success. Particularly valuable would be studies that compare invasive ants and phylogenetically paired non-invasive ants with respect to investment in traits requiring carbohydrate fuel (e.g. aggression, activity). Such comparisons are needed to gauge whether the exploitation of plant-based resources provides a general explanation for why invasive ants achieve such spectacular levels of ecological success (Davidson 1998; Helms & Vinson 2002; O'Dowd *et al.* 2003).

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