

## Carbohydrate regulation in relation to colony growth in ants

A. Dussutour\* and S. J. Simpson

School of Biological sciences, The University of Sydney, NSW 2006, Australia

\*Author for correspondence (e-mail: adussutour@usyd.edu.au)

Accepted 30 April 2008

### SUMMARY

**Ants and all social insects are faced with a nutritional challenge: the food entering the colony is brought by only a small number of its workers but is shared among all members of the colony. In this study, we investigated how ants maintain carbohydrates supply at both a collective and an individual level in response to changes in the concentration of available sucrose solution, colony demography and larval growth. We manipulated the concentration of sugar solutions available to ant colonies (dilute, medium and concentrated solutions) over extended periods and measured the capacity of colonies to maintain sugar supply through compensatory feeding. First, we demonstrated that ants regulated carbohydrate intake at a collective and individual level. Initially, ants consumed most and recruited fastest in response to more concentrated than to dilute sugar solutions, but over time this pattern reversed, such that the number of ants that fed and the volume ingested by each ant was a negative function of sugar concentration in the diet. Second, we found that ants became better at regulating their carbohydrate intake with the production of larvae in the nest. When the number of larvae was experimentally doubled, the ants regulated their consumption of carbohydrates more accurately than when the number of adult workers was doubled, suggesting that larvae play an important role in providing nutritional feedback to workers. Finally, we showed that ants defended a carbohydrate 'intake target' by allowing them to select among sugar solutions of different concentration.**

Key words: ants, carbohydrate, compensatory feeding, foraging, nutrition.

### INTRODUCTION

In social insects, colony nutrition is a decentralized homeostatic process, with workers and larvae regulating their nutrition individually rather than the general nutritional needs of the colony (Cassill and Tschinkel, 1999). Liquid food is exchanged from individual to individual, not in a chain-of-transfer in which donors press food upon passive recipients, but in a chain-of-demand in which recipients solicit food from donors. The chain of demand begins with immobile, legless larvae expressing signs of hunger. These signals are transmitted to adult workers feeding the larvae, and thence to foragers, which leave the nest to collect appropriate foods.

However, foragers and other workers have their own nutritional needs, which differ from those of larvae. Worker ants need carbohydrates as a source of energy (Markin, 1970; Schneider, 1972; Wilson and Eisner, 1957), whereas larval growth relies more heavily on proteins (Cassill and Tschinkel, 1999; Markin, 1970; Sorensen and Vinson, 1981). Hence, numerous authors have shown that the distribution of food among the different individuals of the colony indeed depends upon the type of food collected (Abbott, 1978; Howard and Tschinkel, 1980; Howard and Tschinkel, 1981a; Howard and Tschinkel, 1981b; Sorensen and Vinson, 1981; Sorensen et al., 1981; Sorensen et al., 1985; Sudd, 1967; Wilson, 1971). When sugars are introduced into the nest they are retained and utilized primarily by the workers. Very little is fed immediately to the larvae (Markin, 1970); rather, sugars are retained by the workers for 24 h before reaching the larvae (Sorensen and Vinson, 1981) such that the overall ratio of distribution is 40% to larvae and 60% to workers. Much more of the protein food that enters the nest reaches the larvae and only a small amount is utilized by the workers (Sorensen and Vinson, 1981).

Ants and all social insects are therefore faced with a nutritional challenge. On one hand, colonies need to adjust their harvesting strategy to the internal demands for nutrients within the nest, where larvae and workers have different needs. On the other hand, the food entering a social insect colony is brought by only a small number of its workers: the foragers. So how do foragers' reactions to food encountered outside the nest relate to the nutritional demands of the nest as a whole and themselves as individuals?

At an individual level, once a forager of the ant *Lasius niger* encounters a food source, the decision to drink or not appears to depend only on the nature of the food (protein or carbohydrates) with no evidence of larval influence (Portha et al., 2004). A substantial fraction of the individuals do not ingest proteins, whereas nearly all ants ingest sugar. Moreover, once an ant has decided to drink, its decision to return to the nest relies on a single rule of thumb, the critical volume rule (Mailleux et al., 2000), whatever the type of food and the presence of larvae in the colony. A scout needs to drink up to its critical volume of food before returning to the nest. However, as expected from studies on the control of meal size in non-social insects (Bernays and Simpson, 1982; Simpson and Raubenheimer, 1995), other work in ants has shown that this critical volume is influenced by the concentration of sugar solution (Bonser et al., 1998; Josens et al., 1998), viscosity (Josens et al., 1998), distance (Bonser et al., 1998) of the food from the nest, and starvation level (Josens and Roces, 2000).

At a collective level, workers recruit nestmates to a food source at different rates depending upon food type (Cassill and Tschinkel, 1999; Portha et al., 2002), food concentration [*Solenopsis saevissima* (Wilson, 1962; Cassill and Tschinkel, 1999); *Solenopsis geminata* (Hangartner, 1969); *Lasius niger* (Beckers et al., 1993); *Monomorium* and *Tapinoma* (Szlep and Jacobi, 1967); *Tetramorium*

*impurum* (Verhaeghe, 1982); *Myrmica sabuletti* (de Biseau et al., 1991) (for a review, see Detrain et al., 1999)] and hunger level (Mailleux et al., 2006). In general, workers recruit more workers when they are starved, more strongly to sucrose than to protein, and more strongly to concentrated than to dilute solutions. At a collective level, the presence of larvae increases the mobilization of foragers to sucrose or proteinaceous solutions and consequently increases the sugar and protein collected by workers (Brian, 1972; Portha et al., 2002).

A major challenge for any animal is maintaining an appropriate amount and balance of nutrients ingested to meet requirements in the face of a nutritionally heterogeneous environment and changing demands of growth, development and reproduction. Extensive studies on non-social insects have elucidated the nutritional regulatory strategies and mechanisms employed by a range of insects and other animals (e.g. Raubenheimer and Simpson, 1999; Simpson and Raubenheimer, 2000; Simpson et al., 2004). Insects have been shown to possess separate appetites for protein and carbohydrate, which underlie an ability to compensate for changes in nutrient density in foods and to select among nutritionally complementary foods to achieve a nutritional 'intake target'. How social insects such as ants maintain nutrient supply at both a collective and an individual level in response to changes in the nutritional composition of available foods, colony demography and larval growth is not known (but see Kay, 2004). Such an understanding would provide an important extension to models of collective behaviour and to the study of nutritional ecology. As a first stage, in the present paper we investigated how ants maintain intake of sugar at a collective level and individual level. It is well known that sugars are phagostimulatory to larvae and workers, with higher volumes of concentrated than of diluted sugar solutions being ingested in the short term (Cassill and Tschinkel, 1999). However, for colonies (or individuals) to regulate sugar intake in the longer term, larger volumes of diluted than of concentrated solutions must be ingested if these are all that are available in the environment. None of the studies performed on nutrition in ants to date has exceeded 48 h (e.g. Glunn et al., 1981; Kay, 2004; Portha et al., 2002; Portha et al., 2004; Sorensen et al., 1985), and Markin (Markin, 1970) showed that it could up to 5 days before larvae received carbohydrates from workers. Hence, we manipulated the concentration of sugar solutions available to ant colonies over extended periods and measured their capacity to maintain sugar supply to the colony through compensatory feeding. We first investigated the role of colony growth on carbohydrate regulation and followed the carbohydrate intake of the colony as a whole from when the first eggs were laid to the first appearance of pupae. Second, we investigated the role of colony size and number of larvae on carbohydrate regulation to determine whether the number of 'mouths' or the presence of larvae affect carbohydrate regulation. Third, we measured the carbohydrate 'intake target' [*sensu* Raubenheimer and Simpson (Raubenheimer and Simpson, 1993)] of a mature colony by allowing workers to

select among sugar solutions of different concentration. Finally, we studied carbohydrate regulation at an individual level when foragers were either in contact or not with their nestmates.

## MATERIALS AND METHODS

### Species and rearing conditions

The ponerine ant genus *Rhytidoponera* also named green headed ants is distributed throughout Australia and its neighbouring islands. Colonies of *R. metallica* (Smith) are found under rocks, in decaying logs, in leaf-litter and superficial layers of soil (Haskins and Haskins, 1979; Ward, 1986). In *R. metallica*, nest founding occurs mainly by budding (Haskins and Haskins, 1979) and is associated with the usual presence of multiple fertilised egg-laying workers (gamergates) in the colony (Ward, 1986). Haskins and Haskins (Haskins and Haskins, 1983) mentioned that from 5 to 15% of the females become gamergates in *R. metallica*. As with most ponerines, workers are monomorphic (Haskins and Haskins, 1979).

Twenty-four colonies of 1000 workers of *R. metallica* were collected in January 2007 in Sydney, Australia. These 'mother colonies' were housed in tubes placed in plastic boxes (40×30×15 cm) and were allowed to settle in the lab for 1 month. The nests were regularly moistened and the colonies were kept at 24–26°C under a 12 h:12 h L:D photoperiod. We supplied ants with water and a mixed diet of vitamin-enriched food (Bhatkar and Withcomb, 1970) as well as adult *Drosophila melanogaster*, three times a week.

### Intake regulation and colony growth

First we determined the sucrose and water intake at a colony level as a function of colony growth. We collected 250 workers (including gamergates) from each mother colony and housed them in plastic boxes (20×20×6 cm), the bottoms of which were covered by a layer of plaster moistened by a cotton plug soaked from a water reservoir underneath. Each box was connected to a foraging arena (20×20×10 cm) by a transparent tube. None of these experimental colonies had brood when we started the experiment. All the experiments were carried out at 24–26°C.

The time required for an egg to develop to a pupa was about 6 weeks. During the first week only eggs were present in the colony. The first larvae were present during the second week and pupae were first observed during the sixth week. Accordingly we measured the sucrose and water intake for 6 weeks.

The experiment consisted of three treatments using three different concentrations of sucrose solution (concentrated: 18%, 0.52 mol l<sup>-1</sup>; medium: 9%, 0.26 mol l<sup>-1</sup>; and dilute: 4.5%, 0.13 mol l<sup>-1</sup>). We divided the colonies in four blocks of six colonies. Each block received a different solution each week (Table 1). For example, the first block (colonies A1, A2, A3, A4, A5 and A6) was given the 4.5% solution the first week, the 18% solution the second week, the 9% solution the third week, the 4.5% solution the fourth week, the 18% solution the fifth week and the 9% solution the sixth week.

Table 1. Treatment according to block and week

Treatment	Dilute solution						Medium solution						Concentrated solution						Control										
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6					
Block of six colonies	A	B	C	A	B	C	C	B	A	C	B	A	C	A	B	C	A	B	D	D	D	D	D	D	D	D	D	D	D
Block A	A1, A2, A3, A4, A5, A6																												
Block B	B1, B2, B3, B4, B5, B6																												
Block C	C1, C2, C3, C4, C5, C6																												
Block D	D1, D2, D3, D4, D5, D6																												

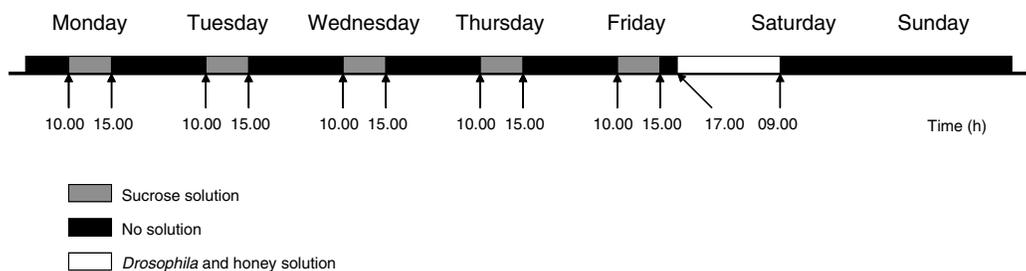


Fig. 1. Experimental timetable. Each colony had access to the sucrose solution for 5 h each day. On the fifth day (Friday) from 17:00 h to the following day (Saturday) 09:00 h, ants had access to protein in the form of 250–300 *Drosophila* and more varied carbohydrates as 15% honey solution. The ants were then deprived until the following Monday at 10:00 h.

The fourth block was used as a control and received a 15% honey solution for 6 weeks.

Each colony had access to the solution for 5 h a day (10:00–15:00 h) for 5 days (Monday to Friday). The fifth day (Friday) from 17:00 h to the following day (Saturday) 09:00 h, ants had access to proteins in the form of 250–300 *Drosophila* and more varied carbohydrates as 15% honey solution. The ants were then deprived until the following Monday at 10:00 h (Fig. 1).

Each day, the solution was placed in the foraging arena in two Petri dishes, each with 1.5 ml solution contained in a small depression within a block of Blu-Tack (Bostik<sup>®</sup>). The ants only had access to one Petri dish; the second was used as a control for measuring and correcting for evaporation. We also provisioned the nest with moistened cotton wool to minimize the water loss. In order to evaluate the colony's intake, the Petri dishes with the solution were weighed every day before they were placed in the foraging arena and again after they were removed. We measured the volume consumed by the colony using density concentration tables. At 25°, 4.5, 9 and 18% sucrose solutions have densities of 1.0158, 1.0340 and 1.0722 g cm<sup>-3</sup>, respectively.

During all the experiments, two colonies per block (A1, A2, B1, B2 and C1, C2) were filmed from above. To assess the number of ants that fed, for each colony we counted the ants in contact with the sucrose solution every 5 min for 5 h. We repeated this procedure each day (5) of each week (6). We chose 5 min as an interval because feeding bouts lasted 270±5 s (mean ± s.e.m.,  $N=1000$ ).

To ensure that there was no block effect (order in which the sucrose solutions were given) we evaluated colony growth and performance. To assess colony growth, we counted the number of ants, pupae and larvae in each colony at the end of the experiment. We then weighed each pupa to evaluate colony performance.

At the end of the experiment we put the workers and their brood back with their mother colony. We allowed the colony to settle for 1 month before doing the second experiment.

#### Intake regulation and brood

We next investigated the role of brood on colony water and sucrose intake regulation. We collected 250 ants from 18 mother colonies and housed them in small nests (see above), yielding a total of 18 experimental colonies. In the first experiment we found that on average a colony of 250 ants produced 150 larvae in 6 weeks. We collected 300 larvae (100 small larvae, 100 medium larvae and 100 large larvae) from the appropriate mother colonies and added them to the experimental colonies in order to double the standard number of larvae. Sucrose and water intake was measured in each experimental colony for 5 days as described for the first experiment. We divided the colonies into three blocks of six colonies. Each block

received a different solution. The food intake observed in this experiment was compared with the food intake observed during the sixth week of the first experiment (250 ants and 150 larvae).

At the end of the experiment we returned the workers and their brood to their mother colony. We allowed the colony to settle for 1 month before undertaking the third experiment.

#### Intake regulation and colony size

Third, we studied the role of colony size on sucrose and water intake regulation. We collected 500 ants from 18 mother colonies and housed them in small nests as above, yielding a total of 18 experimental colonies. Each of these experimental colonies had no brood when we started the experiment. We measured sucrose and water intake in each experimental colony for 5 days as described in the first experiment, again with colonies divided into three blocks of six colonies, each block receiving a different solution. Food intake in this experiment was compared with the food intake during the first week of the first experiment (250 ants and no brood).

#### Intake target

Fourth, we allowed colonies to select between different sugar concentrations, to establish whether they regulated to a particular concentration. We collected 250 ants and 150 brood items from 18 mother colonies and housed them in small nests, producing a total of 18 experimental colonies. These colonies were comparable to the ones observed during the sixth week of the first experiment. Each colony received the 18% and the 4.5% sucrose solutions together for 5 h a day for 1 week (Monday to Friday). We measured the sucrose and water intake in each experimental colony for 5 days as described in the first experiment.

We next repeated this experiment, but this time the colonies were given the three different solutions (4.5, 9 and 18%) together.

#### Individual intake

Lastly, food intake was studied at an individual level. We collected 250 ants and 150 brood items from six mother colonies and housed them in small nests to produce a total of six experimental colonies. These colonies were comparable to those observed during the sixth week of the first experiment. We divided the colonies in three blocks of two colonies. Each block received a different solution for 5 h a day for 1 week (Monday to Friday).

The first day, before giving the sucrose solution to the colony, 50 ants per colony were removed. The workers were collected from the foraging arena and were thus considered as foragers. These ants were weighed and placed individually in Petri dishes (diameter 3 cm) with a droplet (100 µl) of sucrose solution for 1 h, after which each ant was reweighed and the weight gain was computed. Twenty-five

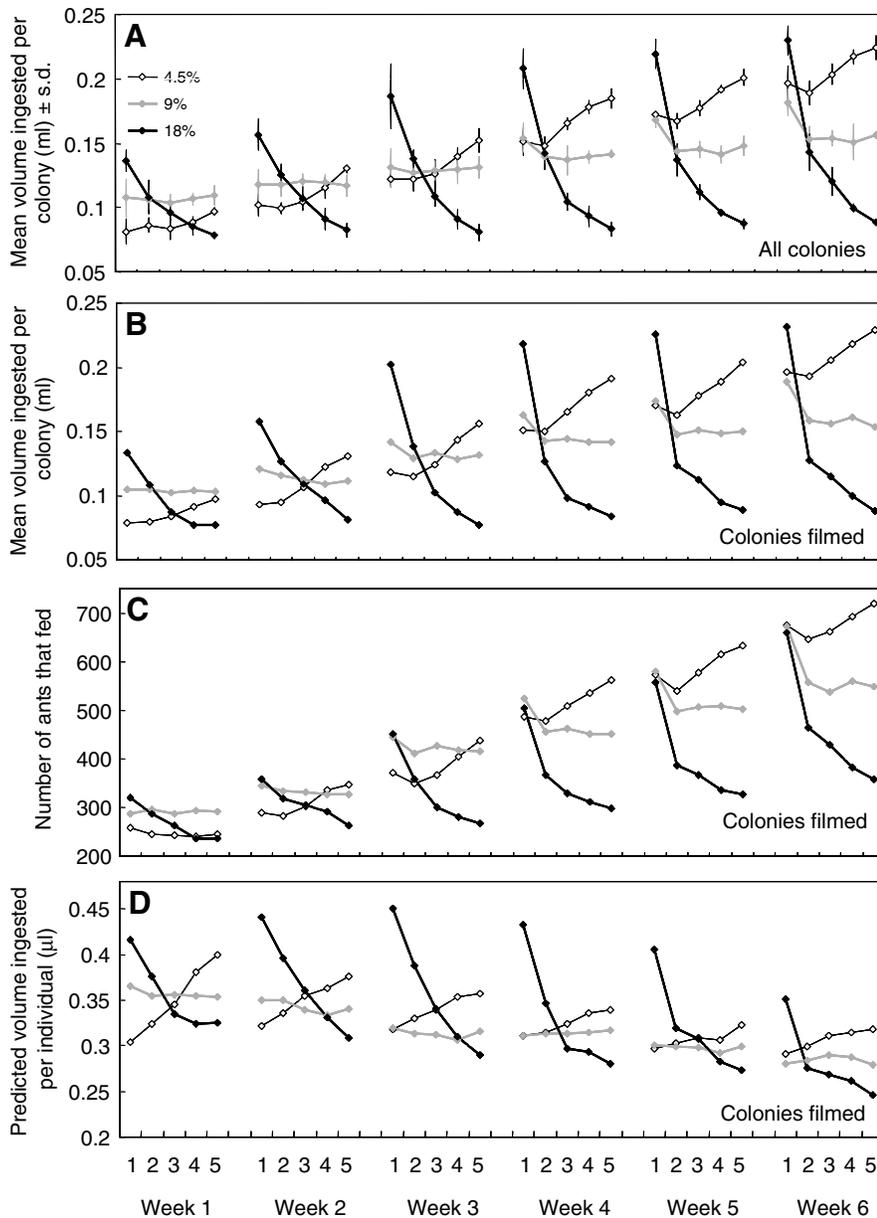


Fig. 2. Mean amount of food ingested per day and per week for each treatment for all colonies (A) and for the colonies filmed (B). (C) Mean number of ants that fed at the food source per day and per week for each treatment. (D) Predicted amount ingested per individual per day and per week for each treatment (two colonies observed for 6 weeks).

ants out of the 50 were marked with paint and placed back with the colony. The 25 ants left were placed together in a Petri dish (diameter 9 cm) with a dental cotton roll soaked in water. The following days we collected the marked ants from the colony and the ones placed in the Petri dish and computed their food intake as for the first day.

Thus we had two groups of ants per colony; the first group was separated from the colony for 1 h a day, whereas the second group had no contact with the colony for the entire week.

## RESULTS

### Intake regulation and colony growth

Data on volume of sugar solutions ingested by the entire colony are presented in Fig. 2A and Table 2. The volume of solution ingested by the colony increased across weeks 1–6, in line with colony growth. The volume of dilute solution ingested increased more across the weeks than did intake of the medium and the concentrated solutions. The volume ingested was also significantly different between days within each 5-day feeding period. Most strikingly,

the volume of concentrated solution ingested decreased from day 1 to day 5 each week, whereas the volume of diluted solution ingested increased and the volume of medium solution remained stable. This pattern indicates compensatory feeding for sugar and became more pronounced with colony growth. The very high intake of the most concentrated solution seen each Monday reflected food deprivation on Sundays.

The number of ants feeding increased across weeks with colony growth (Fig. 2C, Table 2). When the solution was concentrated, the number of ants that fed decreased throughout each week. This decrease became more pronounced with colony growth. By contrast, when the solution was dilute the number of feeding ants increased during the week; a pattern which also became more evident with colony growth. The number of ants remained constant throughout the week for the medium solution but increased from week to week.

When volume ingested per ant was estimated (volume ingested per day/number of ants recorded feeding), values changed across day and week and differed between solutions (Fig. 2C, Table 2).

Table 2. Results of a three-way ANOVA to test for the effect of the treatment (diluted vs medium vs concentrated solution), the day (Monday to Friday) and the week ( $N=6$ ) at which the measures were done on the volume of sucrose solution ingested per colony

Source of variation	Mean squares	d.f.	<i>F</i>	<i>P</i>
Between colonies				
Treatment (sucrose concentration)	0.028	2	26.13	0.001
Within colonies				
Day	0.060	4	551.27	0.001
Day $\times$ treatment	0.015	8	135.11	0.001
Week	0.002	5	38.49	0.001
Week $\times$ treatment	0.001	10	15.16	0.001
Day $\times$ week	0.007	20	113.57	0.001
Day $\times$ week $\times$ treatment	0.005	40	80.15	0.001

Individual ants would seem to have regulated their sucrose intake better than at the collective level during the first week, showing a pronounced decrease in volume consumed of the concentrated solution and an increase in intake of the dilute solution (compare Fig. 2B and C for week 1). No matter the solution, the volume ingested per ant decreased week after week. This effect appeared to be due to increasing crowding and disturbance around the food source. The probability to be interrupted while feeding was 0.12, 0.20, 0.23, 0.24, 0.28 and 0.35 for the first to the sixth weeks (100 meals followed on day 1 for each concentration and for each week).

Fig. 3 illustrates the dynamics of feeding for each solution throughout the week. The dynamic were almost identical between weeks (three-way ANOVA with repeated measure, week  $\times$  time effect  $F_{295,9558}=3.51$ ,  $P=0.08$ ) so we pooled data for the 6 weeks. When the concentrated solution was introduced to the colony on the first day, the number of ants present at the food source increased exponentially over the first 2 h, indicating a strong recruitment process, and then decreased. This pattern was seen to a lesser extent each day during the week. For the diluted solution, the population at the food source stayed near constant, resulting in a linear accumulation over the 5 h period.

Data presented to this point have been for volume of solution ingested. In Fig. 4 plots of sugar vs water intake are presented for the final day of each week. Each solution is represented as a line in a water/sugar plane. Were ants to have successfully regulated sugar intake in the face of differences in the concentration of solution provided, the intake arrays would have been horizontal. Although complete regulation was not achieved, it is evident that sugar intake was increasingly well conserved as the weeks progressed and the colonies grew.

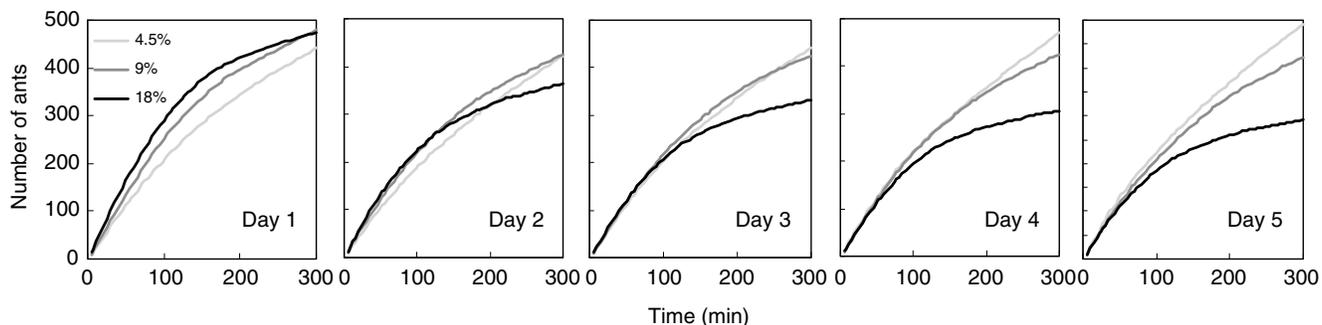


Fig. 3. Dynamics of feeding: cumulative number of ants feeding at the food source for each solution (4.5, 9 and 18% sucrose) throughout the week (the data for the 6 weeks were pooled).

The number of ants in each colony was not significantly different after 6 weeks between the experimental blocks and was not significantly different from the initial number (two-way ANOVA with repeated measures on time, time effect  $F_{1,20}=2.99$ ,  $P=0.099$  and block effect  $F_{3,20}=0.91$ ,  $P=0.452$ ). The number of larvae and pupae produced after 6 weeks was not different between the four blocks (MANOVA, block effect:  $F_{3,20}=0.37$ ,  $P=0.776$  and  $F_{3,20}=1.17$ ,  $P=0.348$  for the number of larvae and pupae, respectively). The mean pupal mass was not different between the blocks [ANOVA with colony nested within block, block effect  $F_{3,354}=0.08$ ,  $P=0.973$ , colony (block) effect  $F_{20,354}=1.32$ ,  $P=0.159$ ]. There was therefore no effect of order in which the different sucrose solutions were given on colony growth and performance.

#### Intake regulation and brood manipulation

Results of the brood manipulation study are provided in Fig. 5A,B and Table 3. The volume of the dilute solution ingested increased through the week, whereas consumption of the concentrated solution declined; with this pattern being more pronounced when the number of brood items was doubled. Across the entire week ants ingested the greatest volume of diluted solution and the least volume of concentrated solution, especially when the number of brood items was doubled.

#### Intake regulation and colony size

In contrast to the effect of manipulating number of brood, increasing the number of ants to 500 individuals did not modify the pattern observed initially with 250 ants, i.e. the volume of the dilute solution ingested stayed relatively constant through the week, whereas consumption of the concentrated solution declined (Fig. 5C,D, Table 4). The amount of each solution ingested simply increased with colony size.

#### Intake target

When offered two different choices of sugar solutions (4.5% vs 18% or 4.5% vs 9% vs 18%) ants converged on the same intake of sugar and water. The amounts of sucrose and water ingested were not significantly different between the two choice experiments (two-way MANOVA with repeated measure on time, experiment effect  $F_{1,34}=1.51$ ,  $P=0.227$  and  $F_{1,34}=0.429$ ,  $P=0.517$  for sugar and water intake respectively; Fig. 6) and decreased throughout the week (time effect  $F_{4,136}=724.70$ ,  $P<0.001$  and  $F_{4,136}=932.11$ ,  $P<0.001$  for sugar and water intake respectively; Fig. 6). Moreover, the amount of sugar ingested in the two-choice experiments was significantly different from a random choice model (two-way MANOVA with repeated measure on time, choice effect:  $F_{1,34}=89.72$ ,  $P<0.001$  and

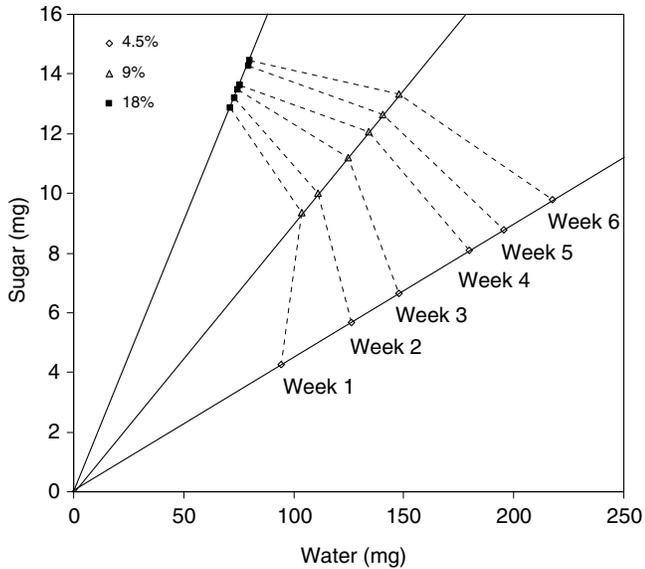


Fig. 4. Sugar vs water intake for the final day (Friday) of each week. Each solution (4.5, 9 and 18% sucrose) is represented as a line in a water/sugar plane. Within each time interval, the intake points are connected with a broken line (the intake array) to demonstrate the nutrient balancing strategy.

$F_{1,34}=126.23$ ,  $P<0.001$ , for the first and the second choice treatments, respectively; Fig. 6).

#### Individual intake

The volume of the dilute solution ingested increased throughout the week, whereas consumption of the concentrated solution declined for the single ants that were kept away from the colony throughout the experiment and those kept with the colony between feeding trials (Fig. 7, Table 5). Across the entire week, ants ingested the greatest volume of diluted solution and the least amount of concentrated solution, especially when they were isolated from the colony (interaction colony influence  $\times$  treatment effect,  $P<0.001$ ; Fig. 7, Table 5).

#### DISCUSSION

Our study illustrates the extent to which green-headed ants regulate carbohydrate intake and provides compelling evidence that they are defending a nutritional intake target (Simpson and Raubenheimer, 1995) at a collective and individual level. Initially, ants consumed most and recruited fastest to more concentrated (18%) than to dilute (4.5%) sugar solutions, but over time this pattern reversed, such that the number of ants that fed and the volume ingested by each ant was a negative function of sugar concentration in the diet (Fig. 2A). When offered a choice of sugar concentrations, ants selected an intermediate concentration of 13% (Fig. 6).

Such compensatory responses to nutrient concentration have been demonstrated across a variety of insect groups, including grasshoppers (McGinnis and Kasting, 1967; Raubenheimer, 1992; Raubenheimer and Simpson, 1993), cockroaches (Bignell, 1978; Jones and Raubenheimer, 2001) and caterpillars (Lee et al., 2004; Timmins et al., 1988; Slansky, 1993; Slansky and Wheeler, 1989; Wheeler and Slansky, 1991), but not before in a social insect. Ants, and all social insects, are faced with an additional nutritional challenge to solitary species: the food entering a social insect colony is brought by only a small number of its workers and is shared among all members of the colony. Hence, colonies need to adjust their

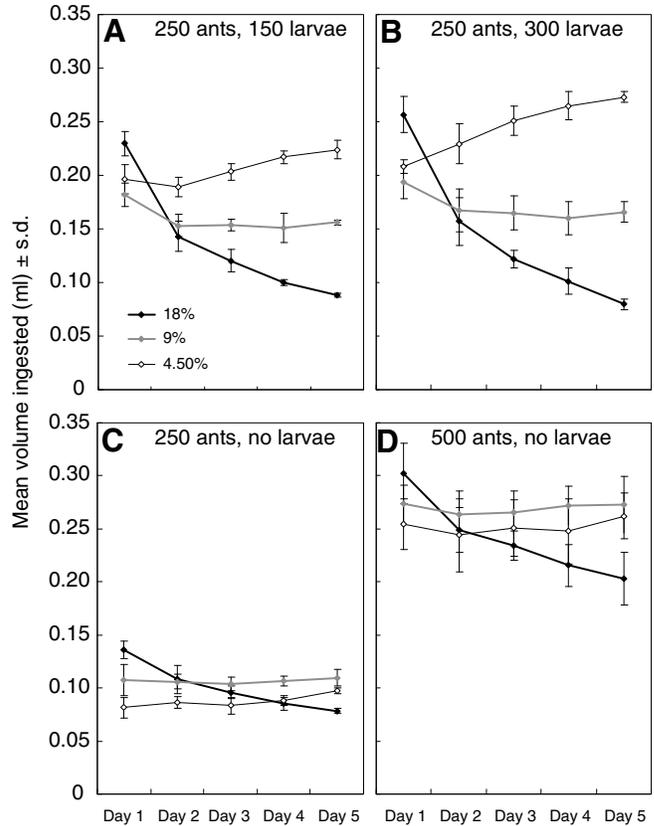


Fig. 5. Mean volume ( $\pm$ s.d.) of each sucrose solution (4.5, 9 and 18%) ingested when the colonies contained: 250 ants and 150 brood items (A), 250 ants and 300 brood items (B), 250 ants and no larvae (C) and 500 ants and no larvae (D) (mean  $\pm$  s.d.).

harvesting strategy to meet the total demand for nutrients within the nest.

A striking finding from the present study was that ants became better at regulating their carbohydrate intake with the production of larvae in the nest, i.e. they came closer to their intake target (Fig. 2A and Fig. 4). When the number of larvae was experimentally doubled, the ants regulated their consumption of carbohydrates more

Table 3. Results of a three-way ANOVA to test for the effect of the treatment (dilute vs medium vs concentrated solution), the day that the measures were done (Monday to Friday) and the manipulation of brood item number (150 vs 300 brood items) on the volume of sucrose solution ingested per colony

Source of variation	Mean squares	d.f.	F	P
Between colonies				
Treatment (sucrose concentration)	0.117	2	272.64	0.001
Brood manipulation	0.017	1	39.08	0.001
Treatment $\times$ Brood manipulation	0.005	2	10.74	0.001
Within colonies				
Day	0.014	4	168.89	0.001
Day $\times$ treatment	0.020	8	249.58	0.001
Day $\times$ brood manipulation	0.000	4	0.70	0.591
Day $\times$ treatment $\times$ brood manipulation	0.001	8	7.70	0.001

Table 4. Results of a three-way ANOVA to test for the effect of the treatment (dilute vs medium vs concentrated solution), the day that the measures were done (Monday to Friday) and the colony size (250 vs 500 individuals) on the volume of sucrose solution ingested per colony

Source of variation	Mean squares	d.f.	F	P
<b>Between colonies</b>				
Treatment (sucrose concentration)	0.007	2	6.22	0.005
Colony size	1.094	1	1043.7	0.001
Treatment × colony size	0.003	2	2.69	0.084
<b>Within colonies</b>				
Day	0.003	4	28.18	0.001
Day × treatment	0.004	8	36.70	0.001
Day × colony size	0.000	4	4.10	0.052
Day × treatment × colony size	0.000	8	1.85	0.175

accurately than when the number of adult workers was doubled, i.e. they ingested more of the diluted solution (Fig. 5). This result suggests that larvae play an important role in providing nutritional feedback to workers. Given that hungry larvae initiate a chain of demand that culminates in foragers collecting food (Cassill and Tschinkel, 1999), the question arises as to how information about larval nutritional state is transmitted to workers. Inspired by other animal species with parental care (e.g. Kilner, 1995; Mondloch, 1995; Price and Ydenberg, 1995; Smiseth and Lorentsen, 2001; Whittingham et al., 2003), the existence of some sort of begging signal has been hypothesized in ant larvae (Bourke and Franks, 1995; Bourke and Ratnieks, 1999; Nonacs and Tobin, 1992). Cassill and Tschinkel (Cassill and Tschinkel, 1995) suggested that non-volatile chemical cues signal hunger in fire ant larvae, *Solenopsis invicta*, whereas others authors described larvae flexing their head or whole

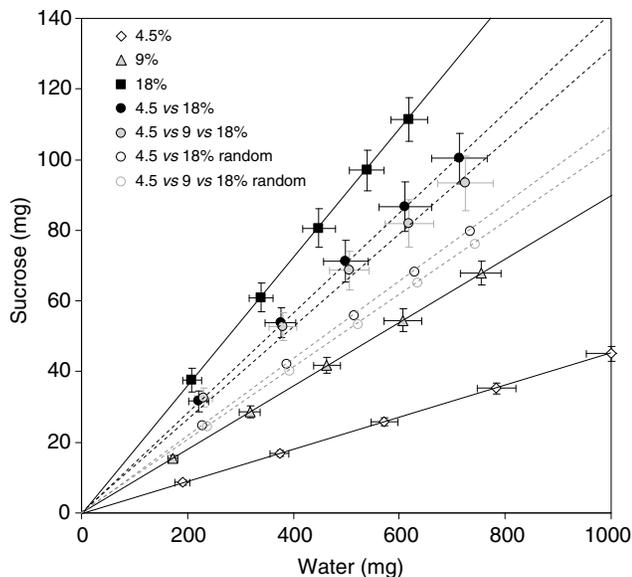


Fig. 6. Water and sucrose (4.5, 9 or 18% solutions) intake measured during choice experiment (measure of the intake target; mean  $\pm$  s.d.). Points along each trajectory represent the cumulative intake of water and carbohydrate over successive days, up to the last day of feeding. The 'random' outcomes indicate the expected carbohydrate intake trajectories if feeding had occurred indiscriminately between sucrose solutions.

body to attract workers in food-deprived colonies of *Myrmica* (Brian and Abbott, 1977; Creemers et al., 2003) and in the ponerine ant *Gnamptogenys striatula* (Kaptein et al., 2005). Whatever the cue employed, a larva attracts feeders at a rate regulated directly by its state of hunger (Cassill and Tschinkel, 1995; Cassill and Tschinkel, 1999; Kaptein et al., 2005) and ultimately by its size, such that when larvae were food-deprived, larger larvae were fed at significantly higher rates than smaller larvae (Cassill and Tschinkel, 1995; Cassill and Tschinkel, 1999). The pattern of worker feeding of larvae is that the large larvae are the first to be attended to, medium sized larvae are next and small larvae last (Markin, 1970). Larval soliciting has also been described in social wasps (Ishay and Landau, 1972) and bumblebees (den Boer and Duchateau, 2006). In bumblebees, several recent studies have challenged the prevalent view that foragers impose a feeding regime upon their larvae without any feedback (Plowright and Jay, 1977). In an experimental laboratory study, Pereboom et al. (Pereboom et al., 2003) showed that starved *B. terrestris* larvae are fed significantly sooner and more often than well fed larvae. Smeets and Duchateau (Smeets and Duchateau, 2001) simulated larval provisioning by manually feeding larvae in a laboratory colony with a micropipette, and showed that these larvae subsequently received fewer feedings from workers than unfed control larvae. In addition, hand-rearing experiments showed that larvae sometimes refuse food and thus cannot be forced to eat (Pereboom et al., 2003).

On the first day of each experimental week, after a weekend without food, ants recruited more concentrated solution than dilute solution (Fig. 2C and Fig. 3) as reported in various earlier studies [*Solenopsis saevissima* (Wilson, 1962; Cassill and Tschinkel, 1999), *Solenopsis geminata* (Hangartner, 1969) *Lasius niger* (Beckers et al., 1990; Beckers et al., 1993), *Monomorium* and *Tapinoma* (Szlep and Jacobi, 1967), *Tetramorium impurum* (Verhaeghe, 1982), *Myrmica sabuletti* (de Biseau et al., 1991) (for a review, see Detrain et al., 1999)]. Consequently they collected more of the concentrated than of the dilute sugar solution (Fig. 2A). This pattern was seen to a lesser extent each morning during the experimental weeks, following the 19 h food deprivation period (Fig. 3). The number of ants feeding at the concentrated source increased exponentially during the first 2 h of the day, but reached a plateau thereafter, indicating that the colony reached satiety (Pasteels et al., 1987). By

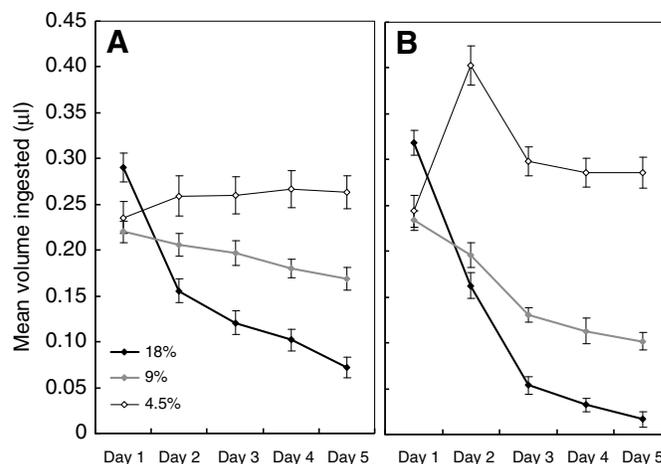


Fig. 7. Mean volume ( $\pm$  s.e.m.) of sucrose solution ingested per individual ant with the influence of the colony (A) and without the influence of the colony (B).

Table 5. Results of a three-way ANOVA to test for the effect of the treatment (dilute vs medium vs concentrated solution), the day that the measures were done (Monday to Friday) and the colony influence (ants isolated from the colony or not) on the volume of sucrose solution ingested per individual

Source of variation	Mean squares	d.f.	F	P
Between colonies				
Treatment (sucrose concentration)	2.894	2	134.28	0.001
Colony influence (ants isolated or not)	0.025	1	1.17	0.280
Treatment × colony influence	0.281	2	13.04	0.001
Within colonies				
Day	0.625	4	30.14	0.001
Day × treatment	0.369	8	17.80	0.001
Day × colony influence	0.109	4	5.24	0.001
Day × treatment × colony influence	0.029	8	1.38	0.202

contrast, the number of ants that fed at the diluted food source increased linearly throughout the 5 h during which food was available (Fig. 3). These results indicate (1) that the colonies with dilute sugar solution had not reached satiety even after 5 h; (2) that the switch from responding positively to sucrose concentration in response to prior deprivation, to exhibiting compensatory feeding (eating more of diluted than concentrated solution) occurred at around 2 h, and (3) that recruitment of foragers to feeding sites is homeostatic with respect to the colony's sugar nutrition.

Regarding the regulation of carbohydrates at an individual level, on day 1, workers feeding on dilute solutions returned to the nest with smaller crop loads than ants feeding on concentrated solution (Fig. 7). Smaller meal sizes on dilute than concentrated sugar solutions in food-deprived insects is well known (Dethier, 1976; Bernays and Simpson, 1982), including in social insects (Balderrama et al., 1992; Josens et al., 1998; Moffatt and Nunez, 1997; Nunez, 1966; Nunez and Giurfa, 1996; Pflumm, 1969). However, by the second day, colonies fed with dilute solution had not only increased the numbers recruited to the food site, but also individual ants were collecting larger loads: a result previously shown in *Formica aquilonia* ants (Cosens and Toussaint, 1986) and *Camponotus mus* (Josens and Rocas, 2000), as well as in blowflies (Simpson et al., 1989). By contrast, the crop loads of ants fed with concentrated solution declined significantly throughout the week (Fig. 7). Ants were therefore able to contribute to regulation of the colony at an individual level. However, individuals were constrained from meeting the intake target of the colony. The array of sugar vs water intake for week 1 in Fig. 4 implies that there was a volumetric limit to sugar collection (note the vertical intake array, with sugar intakes on the y-axis aligning along the x-axis at a near constant water intake). The only way to overcome this limitation was to begin to recruit more foragers; a response that became apparent as the weeks progressed.

There was an interesting interaction between crop loads carried by individuals and numbers recruited to the feeding source over successive weeks. The volume ingested by individuals as predicted from the number of ants feeding indicated that ants showed a more pronounced response to sugar concentration during the first week (i.e. in the absence of brood; Fig. 2D). As the weeks progressed, volumes collected by individuals declined, most notably of the dilute solution. This decline was compensated for at the colony level by substantially increased recruitment of foragers to the dilute solution (Fig. 3). The pronounced individual-level regulation observed during

week 1 seems close to that observed when ants had no contact with the colony (Fig. 2D and Fig. 7B). When ants were kept away from the colony, they could not regurgitate it to congeners and were presumably responding mainly to their own requirements. The results, therefore, imply that during weeks 1 and 2, in the absence of larvae, ants regulated their own intake but did not change their recruitment behaviour, which led to an underconsumption of carbohydrates when colonies were offered diluted solution (Fig. 2A). After week 2, with the emergence of larvae, ants managed to recruit more ants to the diluted food source, removing the necessity for individuals to collect greater volumes of dilute solution.

The present data show that, when confined to single foods of varying carbohydrate content, ants showed distinct regulatory responses. Social insect foraging behaviour has been discussed as resulting from a trade-off between maximizing individual carbohydrate intake and maximizing colony growth. Discovery of a food source and feeding are individual activities, whilst exploitation of the discovered food sources is a collective behaviour mediated through communication signals. In this sense, individual foraging behaviour is affected by colony needs, which regulate, via negative or positive feedback, food-source exploitation. For *R. metallica* workers, we have demonstrated that not only the dynamics of recruitment, but also individual decisions about crop load, are directly modulated by the nutritional state of the colony. However, the precise nature of feedback mechanisms acting to regulate foraging patterns at the colony level remain to be investigated.

A.D. was supported by a post-doctoral grant from The University of Sydney. S.J.S. was supported by an ARC Federation Fellowship.

## REFERENCES

- Abbott, A. (1978). Nutrient dynamics of ants. In *Production Ecology of Ants and Termites* (ed. M. V. Brian), pp. 233-244. London: Cambridge University Press.
- Balderrama, N. M., Almeida De Balderrama, L. O. and Nunez, J. A. (1992). Metabolic rate during foraging in the honey bee. *J. Comp. Physiol. B* **162**, 440-447.
- Beckers, R., Deneubourg, J. L., Goss, S. and Pasteels, J. M. (1990). Collective decision making through food recruitment. *Insectes Soc.* **37**, 258-267.
- Beckers, R., Deneubourg, J. L. and Goss, S. (1993). Modulation of trail laying in the ant *Lasius niger* (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *J. Insect Behav.* **6**, 751-759.
- Bernays, E. A. and Simpson, S. J. (1982). Control of food intake. *Adv. Insect Physiol.* **16**, 59-118.
- Bhatkar, A. P. and Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *Fla. Entomol.* **53**, 229-232.
- Bignell, D. E. (1978). Effects of cellulose in the diets of cockroaches. *Entomol. Exp. Appl.* **24**, 254-257.
- Bonser, R., Wright, P. J., Bament, S. and Chukwu, U. O. (1998). Optimal patch use by foraging workers of *Lasius fuliginosus*, *L. niger* and *Myrmica ruginodis*. *Ecol. Entomol.* **23**, 15-21.
- Bourke, A. F. G. and Franks, N. R. (1995). *Social Evolution in Ants*. Princeton: Princeton University Press.
- Bourke, A. F. G. and Ratnieks, F. L. W. (1999). Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol.* **46**, 287-297.
- Brian, M. V. (1972). Population turnover in wild colonies of the ant *Myrmica*. *Ekol. Pol.* **20**, 43-53.
- Brian, M. V. and Abbott, A. (1977). The control of food flow in a society of the ant *Myrmica rubra* L. *Anim. Behav.* **25**, 1047-1055.
- Cassill, D. L. and Tschinkel, W. R. (1995). Allocation of liquid food to larvae via trophallaxis in colonies of the fire ant, *Solenopsis invicta*. *Anim. Behav.* **50**, 801-813.
- Cassill, D. L. and Tschinkel, W. R. (1999). Regulation of diet in the fire ant, *Solenopsis invicta*. *J. Insect Behav.* **12**, 307-328.
- Cosens, D. and Toussaint, N. (1986). The dynamic nature of the activities of the wood ant *Formica aquilonia* foraging to static food resources within a laboratory habitat. *Physiol. Entomol.* **11**, 383-395.
- Creemers, B., Billen, J. and Gobin, B. (2003). Larval begging behaviour in the ant *Myrmica rubra*. *Ethol. Ecol. Evol.* **15**, 261-272.
- de Biseau, J. C., Deneubourg, J. L. and Pasteels, J. M. (1991). Collective flexibility during food recruitment in the ant *Myrmica sabuleti* (Hymenoptera: Formicidae). *Psyche* **98**, 323-336.
- den Boer, S. P. A. and Duchateau, M. J. H. M. (2006). A larval hunger signal in the bumblebee *Bombus terrestris*. *Insectes Soc.* **53**, 369-373.
- Dethier, V. G. (1976). *The Hungry Fly*. Cambridge, MA: Harvard University Press.
- Detrain, C., Deneubourg, J. L. and Pasteels, J. (1999). Decision-making in foraging by social insects. In *Information Processing in Social Insects* (ed. C. Detrain, J. L. Deneubourg and J. Pasteels), pp. 331-352. Basel: Birkhauser Verlag.
- Gunn, F. J., Howard, D. F. and Tschinkel, W. R. (1981). Food preference in colonies of the fire ant *Solenopsis invicta*. *Insectes Soc.* **28**, 217-222.

- Hangartner, W. (1969). Structure and variability of the individual odor trail in *Solenopsis geminata* Fabr. (Hymenoptera, Formicidae). *Z. Vgl. Physiol.* **62**, 111-120.
- Haskins, C. P. and Haskins, E. F. (1979). Worker compatibilities within and between populations of *Rhytidoponera metallica*. *Psyche* **86**, 299-312.
- Haskins, C. P. and Haskins, E. F. (1983). Situation and location-specific factors in the compatibility response in *Rhytidoponera metallica* (Hymenoptera: Formicidae: Ponerinae). *Psyche* **90**, 163-174.
- Howard, D. F. and Tschinkel, W. R. (1980). The effect of colony size and starvation on food flow in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **7**, 293-300.
- Howard, D. F. and Tschinkel, W. R. (1981a). The flow of food in colonies of the fire ant, *Solenopsis invicta*: a multifactorial study. *Physiol. Entomol.* **6**, 297-306.
- Howard, D. F. and Tschinkel, W. R. (1981b). Internal distribution of liquid foods in isolated workers of the fire ant, *Solenopsis invicta*. *J. Insect Physiol.* **27**, 67-74.
- Ishay, J. and Landau, E. M. (1972). Vespa larvae send out rhythmic hunger signals. *Nature* **273**, 286-287.
- Jones, S. A. and Raubenheimer, D. (2001). Nutritional regulation in nymphs of the German cockroach, *Blattella germanica*. *J. Insect Physiol.* **47**, 1169-1180.
- Josens, R. B. and Rocas, F. (2000). Foraging in the ant *Camponotus mus*: nectar-intake rate and crop filling depend on colony starvation. *J. Insect Physiol.* **46**, 1103-1110.
- Josens, R. B., Farina, W. M. and Rocas, F. (1998). Nectar feeding by the ant *Camponotus mus*: intake rate and crop filling as a function of sucrose concentration. *J. Insect Physiol.* **44**, 579-585.
- Kaptein, N., Billen, J. and Gobin, B. (2005). Larval begging for food enhances reproductive options in the ponerine ant *Gnamptogenys striatula*. *Anim. Behav.* **69**, 292-299.
- Kay, A. (2004). The relative availabilities of complementary resources affect the feeding preferences of ant colonies. *Behav. Ecol.* **15**, 63-70.
- Kilner, R. (1995). When do canary parents respond to nestling signals of need? *Proc. R. Soc. Lond. B Biol. Sci.* **260**, 342-348.
- Lee, K. P., Raubenheimer, D. and Simpson, S. J. (2004). The effect of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiol. Entomol.* **29**, 108-117.
- Mailleux, A. C., Deneubourg, J. L. and Detrain, C. (2000). How do ants assess food volume? *Anim. Behav.* **59**, 1061-1069.
- Mailleux, A. C., Detrain, C. and Deneubourg, J. L. (2006). Starvation drives a threshold triggering communication. *J. Exp. Biol.* **209**, 4224-4229.
- Markin, G. P. (1970). Food distribution within laboratory colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr). *Insectes Soc.* **17**, 127-158.
- McGinnis, A. J. and Kasting, R. (1967). Dietary cellulose: effect on food consumption and growth of a grasshopper. *Can. J. Zool.* **45**, 365-367.
- Moffatt, L. and Nunez, J. A. (1997). Oxygen consumption in the foraging honeybee depends on the reward rate at the food source. *J. Comp. Physiol. B* **167**, 36-42.
- Mondloch, C. J. (1995). Chick hunger and begging affect parental allocation of feedings in pigeons. *Anim. Behav.* **49**, 601-613.
- Nonacs, P. and Tobin, J. E. (1992). Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. *Evolution* **46**, 1605-1620.
- Nunez, J. A. (1966). Quantitative Beziehungen zwischen den Eigenschaften von Futterquellen und dem Verhalten von Sammelbienen. *Z. Vgl. Physiol.* **52**, 142-164.
- Nunez, J. A. and Giurfa, M. (1996). Motivation and regulation of honey bee foraging. *Bee World* **77**, 182-196.
- Pasteels, J. M., Deneubourg, J. L. and Goss, S. (1987). Self-organization mechanisms in ant societies (I): trail recruitment to newly discovered food sources. In *From Individual to Collective Behavior in Social Insects: les Treilles Workshop* (ed. J. M. Pasteels and J. L. Deneubourg), pp. 155-175. Basel: Birkhauser.
- Pereboom, J. J. M., Velthuis, H. H. W. and Duchateau, M. J. (2003). The organisation of larval feeding in bumble bees (Hymenoptera, Apidae) and its significance to caste differentiation. *Insectes Soc.* **50**, 127-133.
- Pflumm, W. (1969). Beziehungen zwischen Putzverhalten und Sammelbereitschaft bei der Honigbiene. *Z. Vgl. Physiol.* **64**, 1-36.
- Plowright, R. C. and Jay, S. C. (1977). On the size determination of bumblebee castes (Hymenoptera: Apidae). *Can. J. Zool.* **55**, 1133-1138.
- Portha, S., Deneubourg, J.-L. and Detrain, C. (2002). Self-organized asymmetries in ant foraging: a functional response to food type and colony needs. *Behav. Ecol.* **13**, 776-781.
- Portha, S., Deneubourg, J.-L. and Detrain, C. (2004). How food type and brood influence foraging decisions of *Lasius niger* scouts. *Anim. Behav.* **68**, 115-122.
- Price, K. and Ydenberg, R. (1995). Begging and provisioning in broods of asynchronously-hatched yellow-headed blackbird nestlings. *Behav. Ecol. Sociobiol.* **37**, 201-208.
- Raubenheimer, D. (1992). Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in locusts. *Ecology* **73**, 1012-1027.
- Raubenheimer, D. and Simpson, S. J. (1993). The geometry of compensatory feeding in the locust. *Anim. Behav.* **45**, 953-964.
- Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67-82.
- Schneider, P. (1972). Versuche zur Frage der individuellen Futterverteilung bei der kleinen roten Waldameise (*Formica polyctena* Foerst.). *Insectes Soc.* **19**, 279-299.
- Simpson, S. J. and Raubenheimer, D. (1995). The geometric analysis of feeding and nutrition: a user's guide. *J. Insect Physiol.* **41**, 545-553.
- Simpson, S. J. and Raubenheimer, D. (2000). The hungry locust. *Adv. Stud. Behav.* **29**, 1-44.
- Simpson, S. J., Barton Browne, L. and van Gerwen, A. C. M. (1989). The patterning of compensatory sugar feeding in the Australian sheep blowfly. *Physiol. Entomol.* **14**, 91-105.
- Simpson, S. J., Sibly, R. M., Lee, K. P., Behmer, S. T. and Raubenheimer, D. (2004). Optimal foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**, 1299-1311.
- Slansky, F. (1993). Nutritional ecology: the fundamental quest for nutrients. In *Caterpillars: Ecological and Evolutionary Constraints on Foraging* (ed. N. E. Stamp and T. M. Casey), pp. 29-91. New York: Chapman & Hall.
- Slansky, F. and Wheeler, G. S. (1989). Compensatory increases in food consumption and utilization efficiencies by velvetbean caterpillars mitigate impact of diluted diets on growth. *Entomol. Exp. Appl.* **51**, 175-187.
- Smeets, P. A. M. and Duchateau, M. J. (2001). Feeding behaviour in the bumble bee *Bombus terrestris*. *Belg. J. Zool.* **131**, 11-18.
- Smiseth, P. T. and Lorentsen, S.-H. (2001). Begging and parent-offspring conflict in grey seals. *Anim. Behav.* **62**, 273-279.
- Sorensen, A. A. and Vinson, S. B. (1981). Quantitative food distribution studies within laboratory colonies of the imported fire ant, *Solenopsis invicta* Buren. *Insectes Soc.* **28**, 129-160.
- Sorensen, A. A., Mirenda, J. T. and Vinson, S. B. (1981). Food exchange and distribution by three functional worker groups of the imported fire ant *Solenopsis invicta* Buren. *Insectes Soc.* **28**, 383-394.
- Sorensen, A. A., Busch, T. M. and Vinson, S. B. (1985). Control of food influx by temporal subcastes in the fire ant, *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **17**, 191-198.
- Sudd, J. H. (1967). *An Introduction to the Behaviour of Ants*. London: E. Arnold.
- Szlep, R. and Jacobi, T. (1967). The mechanism of recruitment to mass foraging in colonies of *Monomorium venustum* Smith, *M. subopacum* ssp. *phoenicium* Em., *Tapinoma israelis* For. and *T. simrothi* v. *phoenicium* Em. *Insectes Soc.* **14**, 25-40.
- Timmins, W. A., Bellward, K., Stamp, A. J. and Reynolds, S. E. (1988). Food intake, conversion efficiency, and feeding behaviour of tobacco hornworm caterpillars given artificial diet of varying nutrient and water content. *Physiol. Entomol.* **13**, 303-314.
- Verhaeghe, J. C. (1982). Food recruitment in *Tetramorium impurum* (Hymenoptera: Formicidae). *Insectes Soc.* **29**, 67-85.
- Ward, P. S. (1986). Functional queens in the Australian greenhead ant, *Rhytidoponera metallica* (Hymenoptera: Formicidae). *Psyche* **93**, 1-12.
- Wheeler, G. S. and Slansky, F. (1991). Compensatory response of the fall armyworm (*Spodoptera frugiperda*) when fed water- and cellulose-diluted diets. *Physiol. Entomol.* **16**, 361-374.
- Whittingham, L. A., Dunn, P. O. and Clotfelter, E. D. (2003). Parental allocation of food to nestling tree swallows: the influence of nestling behaviour, sex and paternity. *Anim. Behav.* **65**, 1203-1210.
- Wilson, E. O. (1962). Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). 1. The organization of mass-foraging. *Anim. Behav.* **10**, 134-147.
- Wilson, E. O. (1971). *The Insect Societies*. Cambridge, MA: Harvard University Press.
- Wilson, E. O. and Eisner, T. (1957). Quantitative studies of liquid food transmission in ants. *Insectes Soc.* **4**, 157-166.