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# Current Biology

## Ant Foragers Compensate for the Nutritional Deficiencies in the Colony

### Highlights

- Ants overcome complex multidimensional nutritional constraints
- Collective homeostasis relies on individual feeding decisions
- Feeding decisions are flexible and rely on individual nutritional state
- Ants are able to solve nutritional conflicts within the colony

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### In Brief

Ants are able collectively to overcome complex nutritional challenges provided by their environment. Csata et al. show that ants deprived of carbohydrate, sterol, protein, a subset of amino acids, or a single amino acid are rapidly able to match their foraging decisions to their nutritional needs.

# Ant Foragers Compensate for the Nutritional Deficiencies in the Colony

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## SUMMARY

Achieving nutritional homeostasis is crucial for the fitness of all living organisms [1]. Using “collective wisdom,” ants have been shown to excel at making rapid and appropriate decisions under various contexts [2, 3], including foraging [4–7]. Ants often use pheromone trails to share information about food resources [8–10], a process allowing them to focus their foraging activity on the best food source available [7, 11–14]. However, what constitutes the best food source depends on the nutritional context of the colony in relation to its food environment [15]. In this study, we exposed ant colonies to various nutrient deficiencies and observed their compensatory nutritional responses. Ants were deprived of carbohydrate, sterol, protein, a subset of amino acids, or a single amino acid. We found that ants were rapidly able to match their foraging decisions to their nutritional needs, even if the deficiency concerned a single amino acid. An individual-based model demonstrates that these impressive feats of nutritional compensation can emerge from the iterative process of trail-laying behavior, which relies on a simple individual decision: to eat or not to eat. Our results show that, by adjusting their feeding behavior at the individual level, ants sustain homeostasis at the colony level.

## RESULTS

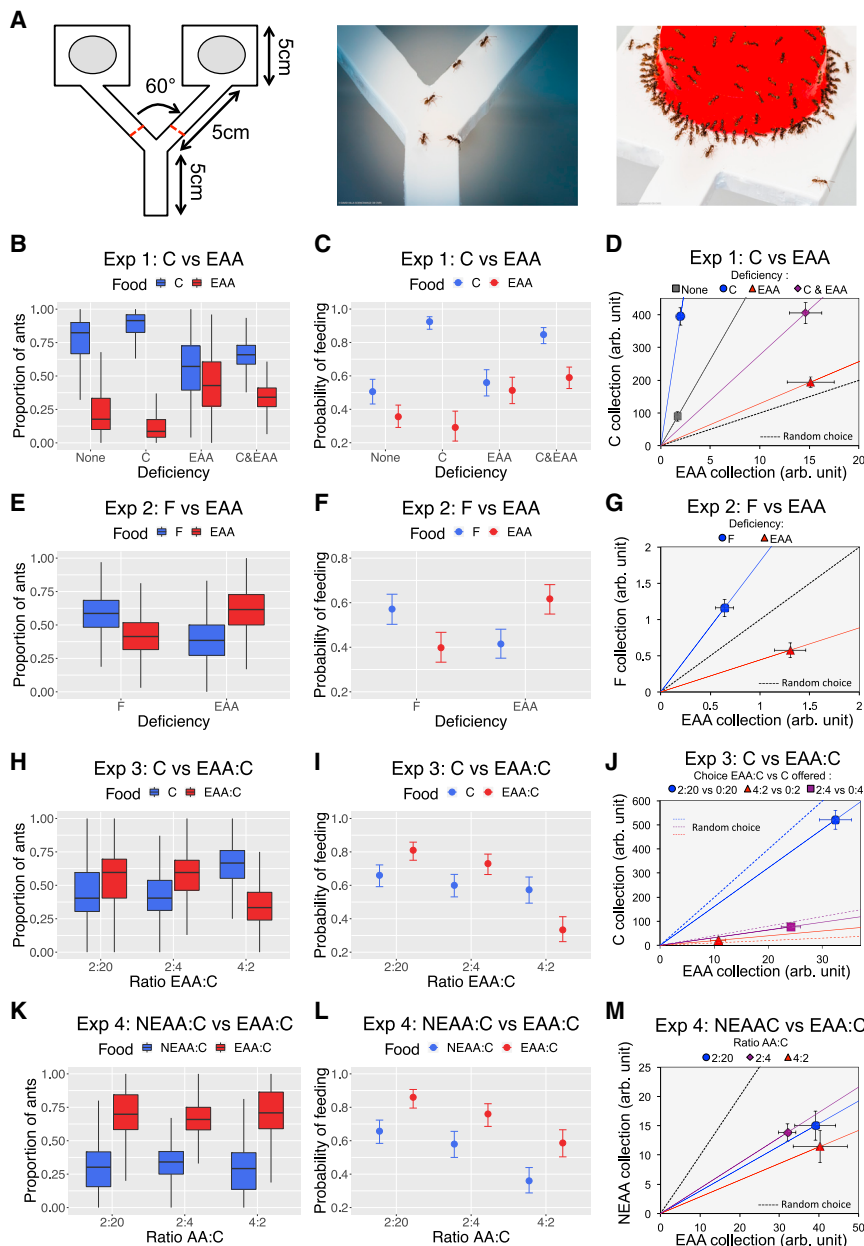
We aimed to establish whether Argentine ants, a major pest around the world [16], are able to adapt their foraging strategies depending on their nutritional state with a focus on essential amino acids. In each experiment, ant colonies were confined to a nutritionally defined diet (Data S1A), from which we removed one or more nutrients. The ants experienced this nutritional deprivation for 5–10 days, depending on the experiment (Data S1A). Ants were then provided with the opportunity to compensate for the deficiency incurred by allowing them to select

between two food solutions offering the missing nutrient or another non-missing nutrient in a 1 h choice assay (Figure 1A; Data S1B). The two solutions were placed on two platforms tied to a Y-shaped bridge, which had two branches (Figure 1A). To avoid familiarity effects [17], the dietary solutions received during the deprivation treatments and the solutions offered during the choice assay were different from one another (Data S1). Each nutritional deprivation was replicated at least 20 times using a minimum of 20 different colonies. This led to a total of 184 colonies tested and a total of 479 choice assays performed.

First, we measured the ant flow on each branch of the Y-shaped bridge (termed “foraging effort”) to estimate the ants’ distribution between the two food solutions. We then tracked the behavior of the ants once they reached the platform housing the food and recorded whether they fed or not before returning to the nest (termed “probability of feeding”). An approximation of nutrient collection for the colony was derived from foraging effort multiplied by the nutrient concentration, multiplied by the probability of feeding (see STAR Methods section) [18].

We investigated compensatory feeding, first considering carbohydrates and essential amino acids (Figure 1B). A first treatment in which ants were fed *ad libitum* with a balanced food (termed “no deficiency”) indicated that, when well-fed ants were offered a choice between a sucrose solution (20%; C) and an EAA solution (2%; EAA), they focused most of their foraging effort on C (Figure S1A; Table S1; C was selected in 19 assays out of 20;  $p < 0.001$ ). C-deprived and C and EAA-deprived ants facing the same choice also selected C over EAA (Figure S1A; Table S1; C was selected in 19 assays out of 20;  $p < 0.001$  in each treatment). By contrast, EAA-deprived ants redistributed half of their foraging effort to EAA (Figure S1A; Table S1; EAA was selected in 10 assays out of 20,  $p = 0.412$ ; Data S2A, deficiency effect,  $p < 0.001$ ). C and EAA-deprived ants collected as much C and EAA as C-deprived ants and EAA-deprived ants, respectively, indicating that ants regulated intake of both EAA and C separately (Figure 1D).

In a second experiment, ants were deprived of EAA or the sterol, cholesterol. Because pure solutions of 0.2% cholesterol were unattractive, we offered a choice between a solution (F) containing cholesterol mixed with sucrose (F:C 0.2%:2% w/v) and a solution (EAA) containing EAA mixed with sucrose (EAA:C 0.2%:2% w/v; Figure 1E). Ants deprived of cholesterol



**Figure 1. Ants Compensate for Essential Amino Acid, Carbohydrate, and Sterol Deficiencies**

(A) Experimental set-up: colonies were confined to a single diet lacking one nutrient offered *ad libitum*. The nutritional treatments lasted for 5 days for experiments 1, 3, 4, and 8 and for 10 days for experiments 2, 5, 6, and 7. Each nutritional treatment was followed by a 1 h food choice assay, during which ants could compensate their nutrient deficiency. A colony was connected to a Y-shaped bridge, with two branches of equal length. At the end of each branch was a platform where each solution (5 mL) was poured on a piece of cotton in a plastic plate (diameter 4 cm). The ants had access to both food solutions for 1 h. To assess foraging activity, we counted the number of ants crossing a line drawn 1 cm from the choice point (red dotted line) every minute for 1 h in each assay. Each assay was replicated at least 20 times for each nutritional treatment. The behavior of at least 150 ants traveling to each solution for each nutritional treatment was tracked. For each ant, we recorded whether the ants fed for more than 5 s at the food source. The measures began 10 min after the beginning of an assay, when the outbound and nest-bound flows of ants were at equilibrium.

(B, E, H, and K) Ants distribution for experiment 1 (B), 2 (E), 3 (H), and 4 (K), respectively. Proportion of ants visiting the two solutions offered per minute is shown. The boxes extend from lower to upper quartile values, and horizontal black lines indicate median values. Vertical black lines extend to most extreme data point.

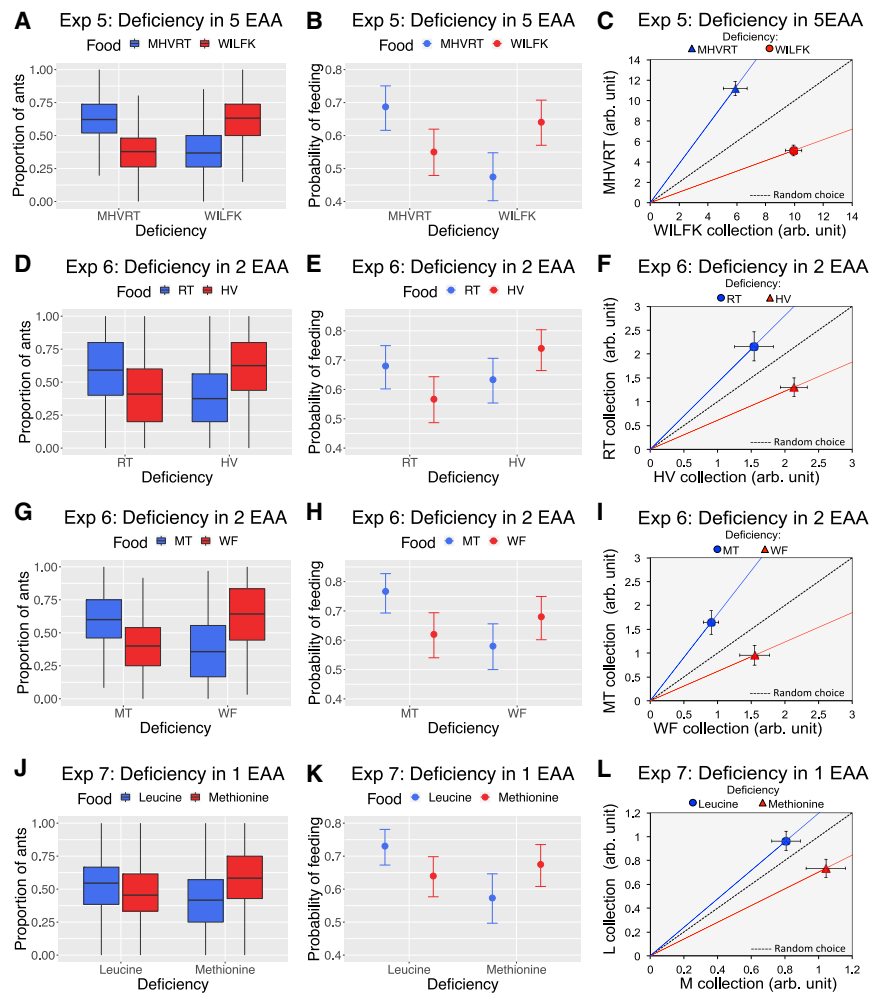
(C, F, I, and L) Probability of feeding computed as the number of ants that fed divided by the total number of ants tracked for experiment 1 (C), 2 (F), 3 (I), and 4 (L), respectively. Error bars are 95% confidence intervals.

(D, G, J, and M) Food collection for experiment 1 (D), 2 (G), 3 (J), and 4 (M), respectively. For each assay, the approximate nutrient collection for the colony was derived from the foraging effort (i.e., the number of ants visiting each food solution) multiplied by the nutrient concentration multiplied by the probability of feeding. Error bars are SEs. See also Table S1 and Figures S1, S2, and S3.

focused a significant amount of their foraging effort on F (Figure S1B; Table S1; F was selected in 17 assays out of 22;  $p = 0.002$ ), although they foraged mostly on EAA when deprived of EAA (Figure S1B; Table S1; EAA was selected in 18 assays out of 22;  $p < 0.001$ ; Data S2B, deficiency effect,  $p < 0.001$ ).

In their natural habitat, ants do not encounter pure solutions of EAA when foraging on prey, honeydew, or nectar. The third experiment explored the foraging strategies of colonies deficient in both C and EAA facing a choice between a solution offering both nutrients (EAA:C) against a sucrose-only solution (C). The EAA:C solutions differed in their ratio and concentration (2%:20%; 2%:4%, and 4%:2%), whereas the C solution varied only in concentration (20%, 4%, and 2%). We aimed to discover

whether foragers would rather focus their foraging effort on a solution offering the two missing nutrients, regardless of the EAA:C ratio, than on a solution presenting only C, as had been observed in experiment 1. When the food solutions were carbohydrate biased, ants foraged mostly on EAA:C (Figures 1H and S1C; Table S1; EAA:C was selected in 21 assays out of 30,  $p = 0.008$ , for EAA:C 2:20 and 2:4). When offered a high EAA:C ratio solution (4:2), ants foraged mostly on C (Table S1; Figure S1C; C was selected in 26 assays out of 30,  $p < 0.001$ ; Data S2C, ratio effect,  $p < 0.001$ ). Ants did not converge on a similar EAA:C ratio, as they were not able to maintain C intake constant across a 10-fold range of dilutions over a 1h assay (Figure 1I). This is unsurprising; compensatory feeding for C (eating more of a diluted



**Figure 2. Ants Compensate for Subset of Essential Amino Acids**

(A, D, G, and J) Ants distribution for experiment 5 (A), 6 (D and G), and 7 (J), respectively. Proportion of ants visiting the two solutions offered per minute is shown. The boxes extend from lower to upper quartile values, and horizontal black lines indicate median values. Vertical black lines extend to most extreme data point.

(B, E, H, and K) Probability of feeding computed as the number of ants that fed divided by the total number of ants tracked for experiment 5 (B), 6 (E and H), and 7 (K), respectively. Error bars are confidence interval 95%.

(C, F, I, and L) Food collection for experiment 5 (C), 6 (F and I), and 7 (L), respectively. For each assay, the approximate nutrient collection for the colony was derived from the foraging effort (i.e., the number of ants visiting each food solution) multiplied by the nutrient concentration multiplied by the probability of feeding. Error bars are SEs. See also Table S1 and Figures S1, S2, and S3.

colonies when deprived of a subset of EAA (Figure 2). When offered a choice between two mixes of 5 amino acids, WILFK and MHVRT, ant colonies deprived of WILFK focused their foraging effort on the WILFK solution (Figures 2A and S1E; Table S1; WILFK was selected in 22 assays out of 28;  $p = 0.001$ ), whereas the opposite was observed for ant colonies deprived of MHVRT (Table S1; MHVRT was selected in 16 assays out of 21,  $p = 0.004$ ; Data S2E, deficiency effect,  $p < 0.001$ ). When deprived of only two amino acids, HV, RT, MT, or WF, the ants' distribution between the two food sources matched their nutrient deficiency (Figures 2D and 2G; Data S2F, deficiency effect,  $p < 0.001$  for HV versus RT and MT versus WF). The number of assays in which ants significantly chose the missing EAA was significantly different from a random model (Table S1; the EAA duo missing was selected in 53 assays out of 80;  $p = 0.002$ ). When ants were deprived of a single amino acid, either methionine or leucine, we observed a significant difference in their distribution according to their state of deficiency (Figure 2J; Data S2G; deficiency effect;  $p = 0.035$ ), but a preference for the missing EAA was noticeable only when ants were deprived of methionine (Table S1; the EAA missing was selected in 14 assays out of 20;  $p = 0.021$ ). This might be explained by the fact that methionine is necessary for normal growth and development, as it is an initiator of protein synthesis and a donor of methyl groups.

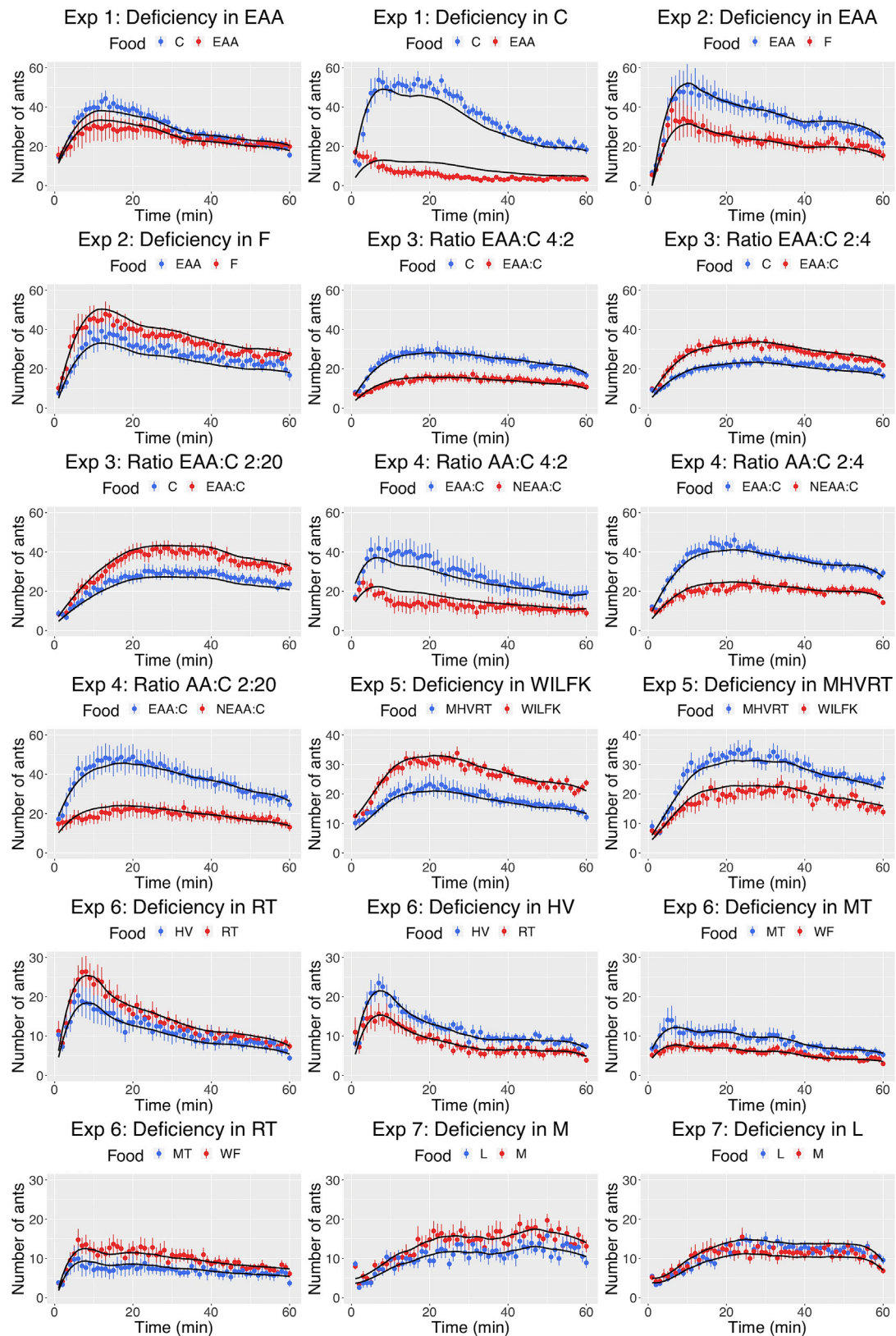
than a concentrated solution) across a 3-fold range of dilutions has been reported previously to only occur in ants after several hours or days [19–21].

Ants, like most animals, must ingest a full suite of essential amino acids to sustain their nutritional needs. Thus, the next question that arose was whether ants would be able to discriminate essential amino acids from non-essential ones and whether this ability depends on the amino acid to carbohydrate ratio (AA:C). When ants that were deficient in both C and EAA were offered a solution containing sucrose combined with essential amino acids (EAA:C) versus a solution with non-essential amino acids (NEAA:C), ants focused their foraging effort on EAA:C, regardless of the AA:C ratio tested (Figures 1K and S1D; Table S1; EAA:C was selected in 65 assays out of 72,  $p < 0.001$  for all ratios tested; Data S2D, ratio effect,  $p = 0.391$ ). Interestingly, ants appeared to regulate the amount of both EAA and NEAA collected to an intake target (Figure 1M). Some NEAA are important regulators of key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity in organisms. NEAA can be synthesized *de novo* by the organism, but the process is costly, so regulating their consumption might be adaptive.

Having demonstrated that ant colonies were able to distinguish EAA from NEAA, we next examined the responses of

After having assessed the foraging effort over the bridge, we next examined whether ants consumed the food. In all seven experiments, the probability of feeding was the highest on the food solution offering the missing nutrient (Figures 1 and 2; Data S3A–S3G), whereas the time spent feeding was not significantly different between the two solutions offered within an assay (Figure S2; Data S3A–S3G). Thus, the probability of feeding closely mirrored the ants' distribution over the bridge (Figures 1 and 2).





(legend on next page)

Finally, we modified Goss et al.'s mathematical model [22] to assess the link between the probability of feeding and the foraging effort. In many ant species, including Argentine ants, when an individual discovers a food source, it lays a pheromone trail on its way back to the nest. Recruited ants use the trail to find the food source and in turn reinforce it on their way back to the nest. This positive feedback mechanism often leads to the use of only one trail, because small initial differences in pheromone concentration between trails are amplified as greater numbers of ants choose the trail that was initially slightly stronger and hence reinforce it. Our model, as for most models investigating path or food selection in trail-laying ants (see e.g. [22–24]), reflects the change over time in pheromone concentration on the paths leading to both food solutions. An ant arrives at the bifurcation on the bridge and chooses branch 1 or 2 with probabilities  $P_1$  and  $P_2$  depending on the trail pheromone concentrations  $C_1$  and  $C_2$ .

$$P_1(C_1, C_2) = \frac{C_1^n}{C_1^n + C_2^n} = 1 - P_2(C_2, C_1),$$

where  $n$  determines the degree of nonlinearity of the choice. A high value of  $n$  means that, if the amount of pheromone is slightly higher on one branch, then the next ant that arrives at the branch fork will have a very high probability of choosing it. In Argentine ants, individuals not only deposit pheromone when they return from the food source but also when they leave the nest [25]. Authors consider that a single pheromone is used for both exploration and exploitation [25]. Hence, in our model, we assumed that all ants deposit an amount of pheromone  $q_0$  when they travel to the food and when they return to the nest loaded with food or not. An ant that fed once at the food source adds a quantity  $q_e$  to  $q_0$  when returning to the nest. Hence, it leaves a stronger trail than an ant going back unloaded. In the model, pheromone quantities are in arbitrary unit (a.u.), and we set  $q_0 = 1$ . Thus, our individual-based model can be written

$$\dot{C}_1(t) = \phi(t)(q_0 + P_1(C_1(t), C_2(t)) \cdot P_{e1} \cdot q_{e1})$$

$$\dot{C}_2(t) = \phi(t)(q_0 + P_2(C_2(t), C_1(t)) \cdot P_{e2} \cdot q_{e2}),$$

where  $P_e$  is the probability of feeding and  $\phi$  is the overall flow of foragers (i.e., the foraging effort). A question that arose is whether the quantity  $q_e$  added by an ant that fed is maintained constant or whether it is modulated according to food composition [10, 14, 26] or individual or collective nutritional state [24, 27]. The similarity in the overall flow between treatments within an experiment suggests that the trail-laying frequency did not depend on the food composition per se (Figure S3). Thus, we proposed that  $q_e$  remains constant regardless of food composition, as suggested in [28]. Thus, the decision to add  $q_e$  is an all-or-nothing response based on the decision to eat or not, which could depend on the colony's or/and the forager's nutritional state. We then explore the predicted performance of the model

for various  $(n; q_e)$  pairs, which were the only parameters in the model not measured experimentally. The combination  $(n = 1.7; q_e = 2)$  gave the lowest error (Figure S4). Our model was able to reproduce accurately the results observed in all our experiments (Figure 3). In our model, the ant chooses the amount of pheromone to put down on the way back from food depending on whether it fed. This implies that trail-laying modulation according to food composition is not required to select the most adequate food, as had been suggested by previous models [26]. Our model suggests that slight differences in ants' feeding behavior coupled to amplification processes through communication lead to effective compensatory foraging strategies. This model could also be applied to species of ants in which the foragers pick up food items (rather than imbibe liquids), such as leaves, which they do not consume themselves, as they still evaluate food quality on site [29].

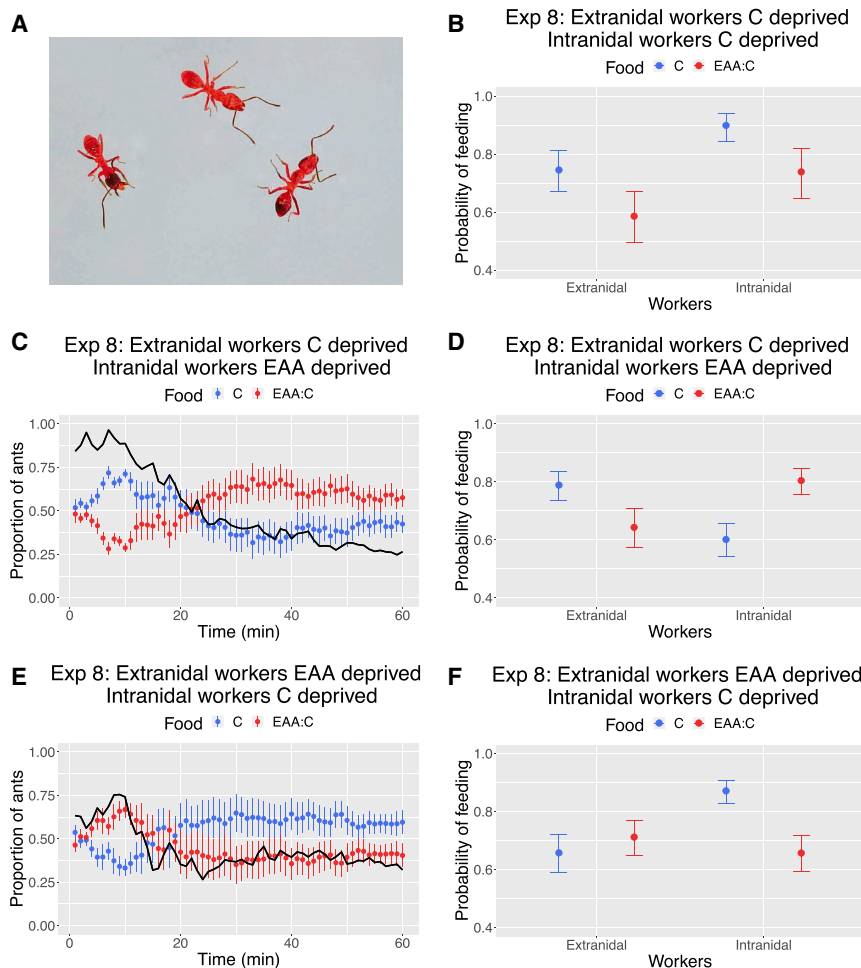
In an ant colony, during the recruitment process, workers found outside exploring (extranidal workers) recruit workers remaining within the nest (intranidal workers) once they have found a food source [27]. Extranidal workers returning to the nest hold information about the food, whereas recruited intranidal workers leaving the nest hold information about the colony's nutritional state due to their proximity with nurses, larvae, and queens. What happens, however, if intranidal workers differ in their nutritional needs to extranidal workers? In a final experiment, we generated nutritional conflicts within the colony by swapping extranidal and intranidal workers between 20 different colonies that experienced either a C deficiency or an EAA deficiency. Workers were tracked using powder paint (Figure 4A). First, we ran four control assays where no conflict was induced and showed that intranidal and extranidal workers both had a higher probability of consuming the food containing the missing nutrient (Figure 4B; Data S3H). When conflicting requirements were induced, however, the collective initially followed the nutritional state of the extranidal workers but then changed to satisfy the intranidal workers' needs (Figures 4C and 4E; Data S2H). As expected [27], extranidal workers were the first to forage, especially when they were deprived of carbohydrate, whereas the intranidal workers were recruited later in the assay. The probability of feeding depended on the worker's own nutritional state, especially for intranidal workers (Figures 4D and 4F; Data S3H).

## DISCUSSION

In all organisms, food intake needs to be adjusted over time to maintain nutritional homeostasis. In solitary animals, circulating signals inform the nervous system about the nutritional state of the individual and in turn dictate feeding behavior [15, 31–36]. In social insects, colony nutritional homeostasis relies entirely on a minority of workers, because any food entering the colony is brought by the foragers (reviewed in [33–37]). Hence, compensation for nutrient deficiencies to maintain nutritional homeostasis at the colony level [18, 38, 39] requires some form of signal to

### Figure 3. Model

Average number of ants per minute reaching each nutrient for each experiment. Experimental values are means  $\pm$  SEM. The black line shows the outcome of the model for  $q_e = 2$  and  $n = 1.7$ . We used the total flow  $\phi$  and the probability of feeding  $P_e$  observed in experiments to run the individual based model. Numerical simulations were performed in Python 3, using Jupyter Notebook on MacOS 10.11.6. The raw data and the averaged data are made available here [30]. The runnable Jupyter Notebook containing the python code is made available here [30]. See also Figure S4.



**Figure 4. Nutritional Conflicts**

(A) Extranidal workers colored with powder paint. (B) Probability of feeding in absence of nutritional conflict. All ants were deprived of carbohydrate. (C) Ants' distribution between the two food sources when extranidal workers were C deprived and the intranidal workers were EAA deprived. (D) Probability of feeding when extranidal workers were C deprived and the intranidal workers were EAA deprived. (E) Ants' distribution between the two food sources when extranidal workers were EAA deprived and the intranidal workers were C deprived. (F) Probability of feeding when extranidal workers were EAA deprived and the intranidal workers were C deprived. The black line in (C) and (E) indicates the proportion of extranidal workers engaged in foraging behavior. Error bars in (C) and (E) are SE. Error bars in (D) and (F) are 95% confidence interval.

extranidal worker deprived of EAA discovering the EAA solution may decide to collect EAA to satisfy its own needs. On its way back to the nest, it may communicate about its discovery by laying a stronger chemical trail and therefore encourage intranidal workers to visit the same food. Extranidal workers may continue foraging, depending on their ability to transfer their load to other congeners [43, 44, 47].

Our model suggests that the collective foraging strategies adopted in response to nutritional deficiencies originated from the trail-laying behavior of workers return-

ing to the nest loaded with food. Owing to amplification processes, slight differences in the feeding behavior of workers due to their differing nutritional state led to flexible, robust, and efficient foraging strategies. Our final experiment demonstrated that, even if the collective decision depended initially on the extranidal workers' own nutritional state, this decision could be strengthened or reversed depending on the nutritional state of the recruited intranidal workers. Recently, it has been shown that heterogeneity in crop contents can be relatively high within a colony, even if the foods collected by each forager are distributed via repeated mouth-to-mouth exchange to almost every ant in the colony [47]. It follows that individuals have the potential to reach their individual intake targets by soliciting food from a range of ants in the colony [47].

inform the foragers about the colony's nutritional state and to dictate their foraging behavior. In ants, this signal comprises a chain of demand [37, 40–42]. Larvae solicit food from nurses, which in turn solicit food from foragers. As a result, foragers leave the nest to seek food. Then, in response to feedbacks emanating from the nest, e.g., the rate of food acceptance [43] or the rate of unloading [44], foragers adjust their subsequent foraging behavior to satisfy the current demands for nutrients within the nest (reviewed in [33, 36]) and consequently compensate for nutritional deficiencies [18].

A striking finding from the present study was that ants compensated for any deficiency in less than 10 min, likely precluding any learning [45] or feedback effect, as it is highly unlikely that an ant visited a food source, ingested, and unloaded the food more than once during this short time period. In experiments 1–7, given that they experienced a similar nutrient deficiency, the foragers and the colony may have required the same nutrient, even if the foragers' requirements for this particular nutrient might have been comparatively lower or higher than that of other colony members. For instance, larvae and queens have an intake target EAA:C ratio more biased toward EAA than do adult workers [37, 40], but all members of the colony still require C and EAA [37, 40, 46]. Hence, by responding to satisfy their own nutritional needs, foragers could still have fulfilled the needs of the colony. For example, an

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In conclusion, our results reveal how ants are able collectively to overcome complex nutritional challenges provided by their environment. We demonstrated that adaptive foraging responses emerged from a simple decision made at the individual level—to eat or not to eat. This in turn can drive collective exploitation of the appropriate foods through pheromonally mediated communication. The next questions to answer are how ants sense the need for individual nutrients, such as EAA, and what are the mechanisms directing their feeding decision at the individual level.



## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
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  - Experiment 2: Deficiency in EAA or Sterol
  - Experiment 3: Deficiency in Carbohydrate and Amino Acids
  - Experiment 4: Discrimination between EAA and NEAA
  - Experiment 5: Deficiency in 5 EAA
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  - Food collection
  - Statistical analyses
- **DATA AND CODE AVAILABILITY**

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2019.11.019>.

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## AUTHOR CONTRIBUTIONS

A.D. designed the experiment and analyzed the data. A.D. and S.J.S. wrote the manuscript. E.C., T.L., A.B., J.B., J.F., and F.F. performed the experiments and collected the data. J.G. wrote and ran the theoretical model.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Glucose	Sigma-Aldrich	CAS#50-99-7
Saccharose	Sigma-Aldrich	CAS#57-50-1
Ethanol	Fisher scientific	CAS#64-17-5
Cholesterol	Sigma-Aldrich	CAS#57-88-5
Choline Chloride	Sigma-Aldrich	CAS#67-48-1
Acid Ascorbic	Sigma-Aldrich	CAS#50-81-7
Vanderzant Vitamin Mix	Fisher scientific	CAT#903244
Wesson Salt Mix	Fisher scientific	CAT#902851
Inositol	Sigma-Aldrich	CAS#87-89-8
Methionine	Sigma-Aldrich	CAS#63-68-3
Histidine	Sigma Aldrich	CAS#71-00-1
Valine	Sigma-Aldrich	CAS#72-18-4
Threonine	Sigma-Aldrich	CAS#72-19-5
Arginine	Sigma-Aldrich	CAS#74-79-3
Tryptophan	Sigma-Aldrich	CAS#73-22-3
Phenylalanine	Sigma-Aldrich	CAS#63-91-2
Leucine	Sigma-Aldrich	CAS#61-90-5
Isoleucine	Sigma-Aldrich	CAS#73-32-5
Lysine	Sigma-Aldrich	CAS#56-87-1
Alanine	Sigma-Aldrich	CAS#56-41-7
Asparagine	Sigma-Aldrich	CAS#70-47-3
Aspartic Acid	Sigma-Aldrich	CAS#56-84-8
Cysteine	Sigma-Aldrich	CAS#52-90-4
Glutamic Acid	Sigma-Aldrich	CAS#56-86-0
Glutamine	Sigma-Aldrich	CAS #56-85-9
Glycine	Sigma-Aldrich	CAS#56-40-6
Proline	Sigma-Aldrich	CAS#47-85-3
Serine	Sigma-Aldrich	CAS#56-45-1
Tyrosine	Sigma-Aldrich	CAS#60-18-4
Paint powder	BioQuip Products	1166R
Fluon	Whitford	U-22101540
Deposited Data		
All Data	Supplementary Material	<a href="#">Data S1</a>
Code	Supplementary Material	Code S1
Experimental Models: Organisms/Strains		
Argentine Ants <i>Linepithema humile</i>	Université Paul Sabatier - Toulouse	N/A
Software and Algorithms		
R lme4	[48]	<a href="https://cran.r-project.org/web/packages/lme4/index.html">https://cran.r-project.org/web/packages/lme4/index.html</a>
Python 3 Jupyter Notebook on MacOS 10.11.6		<a href="https://jupyter.org/install">https://jupyter.org/install</a>
Antethoc	Author: Maud Combe <a href="mailto:maud.combe@univ-tlse3.fr">maud.combe@univ-tlse3.fr</a>	CRCA / CNRS

## LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Audrey Dussutour ([audrey.dussutour@univ-tlse3.fr](mailto:audrey.dussutour@univ-tlse3.fr)).

This study did not generate new unique reagents

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

We used the Argentine ants *Linepithema humile*, an invasive species that deploys mass recruitment through pheromone trails to exploit abundant food sources. In the field, Argentine ants scavenge for dead insects and in addition collect honeydew from sap-feeding Homoptera [49, 50]. Honeydew is rich in both essential and non-essential amino acids [51]. Accordingly, these ants are confronted with foods varying widely in their ratio of amino acids to carbohydrates, from nearly pure sources to mixtures. Ant colonies were collected from a super-colony in Toulouse (France) in April and May every year from 2015 to 2019. Ant individuals were subdivided into 184 experimental colonies, each containing about 1000 workers with brood and queens (up to 10 queens). The number of workers was estimated by weight. A few thousand workers were kept in stock colonies to maintain a stable number of ants in the experimental colonies throughout the duration of an experiment.

For each experimental colony, ants were installed in 6 test-tube nests (15 cm length, 1.3 cm in diameter). These tubes were placed in a rearing box (20 × 10 × 10 cm) with walls coated with Fluon to prevent ants from escaping. Colonies were kept at room temperature (25 ± 1°C) under a 14:10 L:D photoperiod. When not being used in experiments, we supplied each colony with water and a mixed diet of vitamin-enriched food [52].

## METHOD DETAILS

We ran a total of 8 experiments consisting of 2 to 4 nutritional treatments that induced specific nutrient deficiencies (Data S1). To create a deficiency we removed one or two nutrients from a fully balanced diet [20] (glucose 20%, essential amino-acids: Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine, 0.2% each, sterol 0.1%, Vanderzant Vitamins mix 0.2%, Wesson salt mix 0.2%, ascorbic acid 0.2%, inositol 0.2%, and choline chloride 0.2%). Nutrient concentrations used were based on a diet formulation for Argentine ants found in [20]. Following each nutritional treatment, colonies were offered a food choice assay lasting one hour, allowing them to compensate for their experimentally imposed nutrient deficiency (Figure 1).

During a food choice assay, two solutions were placed on two platforms (5 cm × 5 cm) tied to a Y shaped bridge with two branches of equal length (L = 5 cm, 60° angle between the two branches) connected to the colony (Figure 1). For each assay, we added a colorant randomly to each solution and each examiner was blinded to the identity of the solution tested. The ants had access to both solutions for one hour. Each choice assay was replicated 20 to 30 times for each treatment, using 20 to 30 different colonies. For each experiment, treatments were run at random for each colony. Between each treatment, we supplied each colony with water and a mixed diet of vitamin-enriched food [52] for at least 10 days. Colonies were only used in a single experiment. We used a total of 184 colonies, and ran a total of 479 assays.

### Experiment 1: Deficiency in Carbohydrate or Amino Acids

Ants were confined to a diet either fully balanced [20] (*no deficiency treatment*), lacking carbohydrate (*C deficiency treatment*), lacking essential amino acids (*EAA deficiency treatment*) or lacking both carbohydrate and essential amino acids (*C&EAA deficiency treatment*) for 5 days. The deprivation lasted 5 days as longer periods of deprivation in carbohydrate resulted in rapidly increased mortality [20]. After 5 days, ants were offered a choice between two solutions: one containing only essential amino acids (EAA 2%) the other only sucrose (C 20%). The concentration of amino acids offered was lower than the concentration of carbohydrates. This was because amino acids are toxic at higher concentrations, and also because the intake target of *Linepithema humile* lies between 1:10 and 1:25 EEA:C [20]. We ran a total of 20 assays for each nutritional treatment, using 20 different colonies.

### Experiment 2: Deficiency in EAA or Sterol

Ants were confined to a diet lacking either sterol (*F deficiency treatment*) or essential amino acids (*EAA deficiency treatment*). After 10 days, ants were offered a choice between two solutions: one contained essential amino acids (EAA 0.2% mixed with ethanol 0.5%) combined with sucrose (2%) while the second contained cholesterol (F 0.2% mixed with ethanol 0.5%) combined with sucrose (2%). Sucrose was added to both solutions, as a solution presenting only sterols was not fed upon. We ran a total of 22 assays for each nutritional treatment using 22 different colonies.

### Experiment 3: Deficiency in Carbohydrate and Amino Acids

Ants were confined to a diet lacking both carbohydrate and essential amino acids for five days. Afterward, ants were given a choice between a solution that offered only carbohydrate (C) and a solution offering a mix of essential amino acids and carbohydrate (EAA:C). We tested different EEA:C ratios: 2%: 20% (*low EAA:C ratio treatment*), 2%: 4% (*Intermediate EAA:C ratio treatment*)

and 4%: 2% (*high EAA:C ratio treatment*). For each treatment, the C solution contained the same concentration of carbohydrate as the EAA:C solution. We ran a total of 30 assays for each nutritional treatment, using 30 different colonies.

#### Experiment 4: Discrimination between EAA and NEAA

Ants were confined to a diet lacking both carbohydrate and essential amino acids for five days. Next, ants were given a choice between a solution containing essential amino acids and carbohydrate (EAA:C) and a solution containing non-essential amino acids (Alanine, Asparagine, Aspartic acids, Cysteine, Glutamic acids, Glutamine, Glycine, Proline, Serine, Tyrosine) and carbohydrate (NEAA:C). We tested different AA:C ratios: 2%: 20% (*low AA:C ratio treatment*), 2%: 4% (*Intermediate AA:C ratio treatment*) and 4%: 2% (*high AA:C ratio treatment*). We ran a total of 24 assays for each nutritional treatment, using 24 different colonies.

#### Experiment 5: Deficiency in 5 EAA

Ants were confined to a diet either lacking Methionine, Histidine, Valine, Arginine and Threonine (*MHVRT deficiency treatment*) or lacking Tryptophan, Isoleucine, Leucine, Phenylalanine and Lysine (*WILFK deficiency treatment*). After 10 days, ants were offered a choice between two solutions: one contained 5 essential amino acids (M, H, V, R and T, 0.2% each) combined with sucrose (20%) while the second solution contained the other 5 amino acids (W, I, L, F and K, 0.2% each) also combined with sucrose (20%). We replicated this first nutritional treatment 21 times and the second 28 times, using 28 different colonies.

#### Experiment 6: Deficiency in 2 EAA

Ants were confined to a diet either lacking Histidine and Valine, (*HV deficiency treatment*) or lacking Arginine and Threonine (*RT deficiency treatment*). After 10 days, ants were offered a choice between two solutions: one contained H and V (0.2% each) combined with sucrose (20%) while the second contained R and T (0.2% each) also combined with sucrose (20%). We then ran the same experiment with new pairs of amino acids: Methionine and Threonine (MT) and Tryptophan and Phenylalanine (WF). All nutritional treatments were replicated 20 times, using 20 colonies.

#### Experiment 7: Deficiency in 1 EAA

Ants were confined to a diet either lacking Methionine (*M deficiency treatment*) or Leucine (*L deficiency treatment*). After 10 days, ants were offered a choice between two solutions: one contained Methionine (0.1%) combined with sucrose (20%), while the second contained Leucine (0.1%) combined with sucrose (20%). Each nutritional treatment was replicated 20 times, using 20 colonies.

#### Experiment 8: Nutritional conflicts

Ants were confined for 5 days to either 1) an imbalanced diet lacking carbohydrate (*C deficiency treatment*), or 2) an imbalanced diet lacking essential amino acids (*EAA deficiency treatment*). Once the nutrient deficiency was established, we collected all ants found exploring the foraging arena during a 1-hour period - the “extranidal workers.” These ants were colored red or yellow with powder paint (BioQuip Products, Inc.) (color assigned randomly). We did not color the intranidal workers, to avoid nest disturbance. After half an hour, we introduced these workers to a nest that experienced the alternative nutrient deficiency. Extranidal workers that experienced the *C deficiency treatment* were mixed with intranidal workers that experienced the *EAA deficiency treatment* and vice-versa. The proportion of extranidal workers in each newly formed colony was  $0.40 \pm 0.03$  (mean  $\pm$  CI95). After allowing the ants to settle for 15 min, we started the food choice assay. All newly formed colonies were offered a choice between an EAA:C solution (2%:20%) and a C solution (0%:20%) for one hour. We used a total of 20 colonies. We also ran 4 extra control assays where extranidal workers were painted and added to colonies sharing the same nutritional state. Impact of the colorant on survival was estimated after 4 hours (7 colored ants out of 320 died and 6 uncolored ants out of 320 died) and after 24 hours (34 colored ants out of 313 died and 5 uncolored ants out of 314 died).

### QUANTIFICATION AND STATISTICAL ANALYSIS

#### Collective behavior

The ant traffic on the two branches was recorded by a video camera (Canon LEGRIA HF G30) for one hour. To assess foraging effort, for all 479 choice assays we counted the number of ants traveling on each branch at a particular point (one centimeter from the choice point; Figure 1A) every minute for one hour (ant flow  $\phi$ ). Counting began as soon as the first ant climbed onto the bridge and lasted for 60 min.

#### Individual behavior

To assess feeding effort we tracked the behavior of at least 150 ants traveling to each solution for each choice offered on a subset of 4 to 10 assays for each nutritional treatment (at least 25 ants per assay,  $N = 9,710$  ants in total). Every ant was tracked from the moment it entered the platform housing the food to the moment it returned to the nest. We recorded if each ant fed for more than 5 s once at the food source and computed the probability of feeding  $P_e$  as the number of ants that fed divided by the total number of ants tracked. We also measured the time spent feeding on a subset of 4 to 10 assays for each nutritional treatment (at least 10 ants per assay,  $N = 3,175$  ants in total). The measures began 10 min after the beginning of the assay when the outbound and nest-bound flows of ants were at equilibrium.



### Food collection

The approximate nutrient collection for the colony was derived from the foraging effort (i.e ant flow) and the probability of feeding. It was calculated using the following equation inspired from [18]

$$\text{Nutrient} - X - \text{collection} = \phi_1 \times P_{e1} \times [X]_1 + \phi_2 \times P_{e2} \times [X]_2$$

Where  $\phi_1$  ( $\phi_2$ ) is the ant flow on the branch leading to the food 1(2),  $P_{e1}$  ( $P_{e2}$ ) is the probability of feeding on the food 1(2) and  $[X]_1$  ( $[X]_2$ ) is the concentration in nutrient X in the food 1(2).

### Statistical analyses

Generalized linear mixed models were used to assess the difference in general foraging activity (number of ants foraging) and the ants' distribution between the two treatments. The model was fitted by specifying the fixed effects (treatment, time and the order in which the treatments were offered), the random effect (assay), and the error family (Poisson and binomial, respectively, for the general foraging activity and the distribution of ants) (Data S2) [48]. We compared the probability of feeding between treatments and food solutions using a generalized linear model (error family: binomial) with treatment, order in which the treatments were performed and nutrient as fixed factors (Data S3). We compared the time spent feeding between treatments and food sources using a general linear model (error family: Gaussian) with treatment, order in which the treatments were performed, and nutrient as fixed factors (Data S3). The time spent feeding was square-root transformed to fit a Gaussian distribution. For all models, we used either the function *glmer* of *glm* from the R package *lme4* [53].

Within an assay, we tested whether ants preferred the solution presenting the nutrient that was deficient or not (asymmetric distribution), or whether they showed no preference (symmetric distribution) using a binomial test on the number of ants choosing each branch. We assumed that a food was selected when the binomial test showed a significantly higher number of foragers visiting this food. Once we determined this collective decision at the assay level, we then ran a binomial test to see if one collective decision (selection of the nutrient that was deficient) was observed more than the other (selection of the nutrient that was not deficient) (Table S1).

### DATA AND CODE AVAILABILITY

The published article includes all datasets and codes generated and analyzed during this study (see [30]).