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Collective choice in ants: The role of protein and carbohydrates ratios

S. Arganda [a], S.C. Nicolis [b], A. Perochain [a], C. Pechabadens [a], G. Latil [a], A. Dussutour [a,*]

[a] Centre de Recherches sur la Cognition animale, UMR 5169, CNRS, Université de Toulouse, France
[b] Mathematics Department, Uppsala University, Sweden

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In a foraging context, social insects make collective decisions from individuals responding to local information. When faced with foods varying in quality, ants are known to be able to select the best food source using pheromone trails. Until now, studies investigating collective decisions have focused on single nutrients, mostly carbohydrates. In the environment, the foods available are a complex mixture and are composed of various nutrients, available in different forms. In this paper, we explore the effect of protein to carbohydrate ratio on ants' ability to detect and choose between foods with different protein characteristics (free amino acids or whole proteins). In a two-choice set up, Argentine ants *Linepithema humile* were presented with two artificial foods containing either whole protein or amino acids in two different dietary conditions: high protein food or high carbohydrate food. At the collective level, when ants were faced with high carbohydrate foods, they did not show a preference between free amino acids or whole proteins, while a preference for free amino acids emerged when choosing between high protein foods. At the individual level, the probability of feeding was higher for high carbohydrates food and for foods containing free amino acids. Two mathematical models were developed to evaluate the importance of feeding probability in collective food selection. A first model in which a forager deposits pheromone only after feeding, and a second model in which a forager always deposits pheromone, but with greater intensity after feeding. Both models were able to predict free amino acid selection, however the second one was better able to reproduce the experimental results suggesting that modulating trail strength according to feeding probability must be the mechanism explaining amino acid preference at a collective level in Argentine ants.

1. Introduction

In social insect, in particular ants, foraging in group often leads to collective food selection, which relies on interactions between individuals that have access only to local information (Camazine et al., 2001). Collective food selection occurs when a group is faced with several food sources. Information about these food sources is gathered by individuals and relayed to nestmates. The by-product of this distributed information transfer is, most of the time, the collective choice of the nutritionally better food source, without any individual necessarily being aware of all the available opportunities (review in Detrain et al., 1999; Detrain and Deneubourg, 2008; Jeanson et al., 2012).

Until now, studies investigating collective decisions in a foraging context have focused on single nutrients, mostly carbohydrates (review in Detrain et al., 1999; Detrain and Deneubourg, 2008; Jeanson et al., 2012). It is true that ants require mainly carbohydrates to fuel metabolic requirements but they also require a source of nitrogen for enzyme synthesis, tissue repair, etc (Dussutour and Simpson, 2009). At the collective level, foraging patterns differ greatly according to the nutrient: colonies of *Lasius niger* strongly focus their activity on only one source when offered two identical sources of carbohydrates (Beckers et al., 1992; Portha et al., 2002; Dussutour et al., 2009), but show a rather homogeneous distribution of foragers between two identical protein sources (Portha et al., 2002). At the individual level, the decision to feed or not depends on the type of nutrient. A considerable proportion of individuals do not ingest proteins, whereas most ants feed on carbohydrates (Portha et al., 2004). Thus the proportion of individuals that recruit congeners is higher for a source of sugar than for a source of protein (Portha et al., 2004). Similarly, it has been shown that amino acid solutions are not attractive to many ants (Blüthgen and Fiedler, 2004).

In the environment, the foods available are a complex mixture and are rarely composed of only carbohydrates or only protein. For example, the sugary excreta of aphids, on which many ants feed, contains both carbohydrates and proteins in various

* Corresponding author. Tel.: +33 561556441.
E-mail address: audrey.dussutour@univ-tlse3.fr (A. Dussutour).

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concentrations (Blüthgen and Fiedler, 2004). Furthermore, in ants, foragers appear to prefer sugar solutions containing either protein or mixtures of amino acids over pure sugar solutions (Kay, 2004; Blüthgen and Fiedler, 2004). In this paper we investigate to what extent the balance of protein to carbohydrate as well as the protein characteristics of the food (whole proteins or amino acids) can influence the strategies used by a colony of ants to exploit food sources. We carried out a series of laboratory experiments with a simple experimental set-up consisting of a Y-shaped bridge offering the ants a choice between two food sources. At the collective level, in order to investigate the influence of the protein to carbohydrate ratio and the protein characteristics, we compared the recruitment dynamics and ants food choice when offered either two high carbohydrate foods differing in their protein characteristics or two high protein foods differing in their protein characteristics. Using measures of the different behaviors examined at the individual level (probability to contact the food, probability to feed, meal duration) we then propose a model to assess the mechanisms that account for the collective choice observed in the experiments.

2. Material and methods

2.1. Species studied and rearing conditions

We used the Argentine ants *Linepithema humile*, an invasive species that uses 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. Honeydew is rich in essential and non-essential amino acids (Woodring et al., 2004). Accordingly, these ants are confronted with foods varying widely in their ratio of protein to carbohydrate, from nearly pure sources to mixtures.

We collected the ants in Toulouse (France). Ants were subdivided into 38 experimental colonies each containing between 500 and 600 workers without brood (mean ± SD 535.4 ± 37.6). Thousands of workers were kept in a stock colony in order to maintain a stable number of ants in the experimental colonies throughout the duration of the experiment. For each experimental colony, ants were installed in 5 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) with a 14:10 L:D photoperiod. We supplied each colony with water and a mixed diet of vitamin-enriched food (Bhatkar and Whitcomb, 1970 modified after Dussutour and Simpson, 2008).

2.2. Experimental set-up and protocol

In the experiment, a colony starved for 5 days, was connected to a Y shaped bridge, with two branches of equal length ($L = 50$ mm, 60° angle between the two branches). At the end of each branch was a platform (50×50 mm) on which food sources could be placed.

Artificial foods were used because they enabled us to manipulate and standardize nutrient characteristics. The experimental foods were based on the Dussutour and Simpson diet (Dussutour and Simpson, 2008). In the experiment both carbohydrate (C) and protein characteristics (P) were manipulated. The two ratios of protein to digestible carbohydrates (P:C) used were 5:1 (high protein food) and 1:5 (high carbohydrate food), with a fixed total P + C of 124 g L^{-1}. Glucose was used as a digestible carbohydrate source for all foods while the protein characteristics of the foods consisted of either a mixture of whole proteins: casein, whey protein and egg protein (5:1p or 1:5p) or a corresponding mixture of free amino acids (5:1aa and 1:5aa, Supplementary Table 1). The quantity of fat, vitamins and minerals were kept constant in all foods. The foods were presented to the ants in a 1% agar solution. Further preparation details are given in Dussutour and Simpson, 2008.

The experiment consisted of two treatments. In the first treatment, ants had to choose between two high carbohydrate foods differing in their protein characteristics: choice 1:5p versus 1:5aa (high C treatment). In the second treatment, ants had to choose between two high protein foods differing in their protein characteristics: choice 5:1p versus 5:1aa (high P treatment).

We replicated each treatment 19 times using 19 different experimental colonies. Thus each colony was only used once. The replicate started when the ants were given access to the food sources placed on the two platforms. The whole experimental set-up was surrounded with white paper walls isolating it from any sources of disturbance. Throughout the experiments the traffic on the bridge was filmed from above for 40 min as soon as the first ant climbed onto the bridge.

2.3. Traffic flow according to P:C ratio

For both treatments (high C and high P), to assess traffic flow between the nest and the foods we measured the flow of outbound and inbound ants on each branch at a particular point (one centimeter from the choice point). Counting began as soon as the first ant climbed onto the bridge and lasted for 40 min. We repeated this procedure for the 19 replicates for each treatment, and used a two-way ANOVA with repeated measures on time to test for the effects of treatment and time interval on the flow of workers.

2.4. Collective choice between whole proteins and free amino acids according to P:C ratio

We tested whether ants preferred a food with free amino acid or the food with whole proteins (asymmetric distribution), or whether they showed no preference (symmetric distribution) using a binomial test on the number of ants choosing each branch in each replicate. The null hypothesis was that ants choose both foods with equal probability (Siegel and Castellan, 1988). We assumed that a food was selected when the binomial test showed a significantly higher number of foragers visiting this food.

2.5. Individual behavior according to whole proteins characteristics and P:C ratio

For the individual responses, we followed individuals chosen at random and observed them throughout a visit to the food i.e. from the moment they entered the platform to the moment they leaved the platform. Variables recorded for focal ants were the probability to contact the food, the probability to initiate a meal and the duration of feeding. All variables taken together reflect taste responses to the food (Simpson and Raubenheimer, 2000). Behavioral data were collected for 360 individual ants observed across six replicates (60 ants per replicate, 30 ants per food) for each treatment, leading to a total of 720 ants followed. All replicates chosen were characterized by the same volume of traffic, which allows us to isolate the effects of protein characteristics on individual behavior. The measures began twenty minutes after the beginning of the experiment, when the outbound and nestbound flow of ants were at equilibrium.

Both the probability to contact the food source and the probability to feed were compared across treatment (high C or high P) and protein characteristics (aa or p) using a generalized linear model for binary data. The time spent feeding was compared across treatments and protein characteristics using a two-way ANOVA.

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