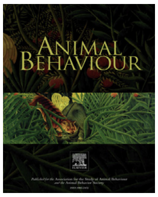
Animal Behaviour 117 (2016) 179–186

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Liquid foraging behaviour in leafcutting ants: the lunchbox hypothesis

Winnie Rytter [a, *], Jonathan Zvi Shik [a, b]

[a] *Centre for Social Evolution, Department of Biology, University of Copenhagen, Copenhagen, Denmark*
[b] *Smithsonian Tropical Research Institute, Ancon, Panama*

ARTICLE INFO

Article history:
Received 8 January 2016
Initial acceptance 5 February 2016
Final acceptance 7 April 2016

MS. number: 16-00026R

Keywords:
ant colony
attine
optimal foraging theory
proventriculus
social stomach

Optimal foraging theory makes clear predictions about the benefits of maximizing energetic returns per unit of foraging effort. However, predictions become less clear when animals belong to symbioses that would be destabilized by such foraging decisions. For instance, leafcutter ants are dominant herbivores in Neotropical ecosystems that harvest fresh vegetation and convert it into compost used to cultivate specialized fungus for food. Individual foragers have long been assumed to supplement their fungal diets by harvesting liquid nectar outside the symbiosis, although this has not been demonstrated in the field, and would probably destabilize the fine-tuned farming systems. By dissecting liquid storage organs in foragers of four sympatric Panamanian leafcutter ant species we found that liquid foraging is not a general strategy in the field. Moreover, while over 40% of these foragers returned to their nests without leaf fragments, these unladen ants were not more likely to carry liquids. Instead, we found support for a newly formulated ‘lunchbox hypothesis’ because most workers exited nests for foraging trips with midguts full of liquids that were depleted (assimilated and transferred to hindguts) if workers returned with a leaf fragment in the field or transported a load in laboratory experiments. Thus, in contrast to the destabilizing effects of external nectar foraging, these results provide a novel mechanism promoting symbiotic stability, as fungi provide fuel for foragers to harvest more substrate for fungal crop production.

Efficient food acquisition is a central challenge faced by organisms, and optimal foraging theory (OFT) explores the ways natural selection has fine-tuned foraging strategies to meet this challenge, maximizing food harvest while minimizing foraging time and exposure to predators (Stephens, Brown, & Ydenberg, 2007; Stephens & Krebs, 1986). OFT models typically weigh energetic dietary gains against energetic foraging costs and predict reduced fitness with excessively costly or unsuccessful foraging trips (Pyke, Pulliam, & Charnov, 1977). However, it is often not feasible to directly observe foraging dynamics including ecological processes related to ingestion (e.g. food capture and transport) and physiological processes related to digestion (e.g. food assimilation). This means that measuring foraging success, and thus testing OFT models, can be difficult even with inferential techniques (e.g. stable isotopes; Feldhaar, Gebauer, & Blüthgen, 2009).

Many animals are ‘central place foragers’ and a specific set of OFT models has helped researchers understand how they overcome issues such as local resource depletion when repeatedly returning harvested resources to a central nest or sleeping site (Orians & Pearson, 1979; Oster & Wilson, 1978; Stephens & Krebs, 1986). Ant colonies have provided model systems for testing OFT predictions because they are central place foragers that use diverse collective foraging behaviours to locate, defend and transport resources back to stationary nests (Lanan, 2014; Oster & Wilson, 1978; Roces & Núñez, 1993). Moreover, individual workers dynamically adjust foraging behaviours when harvesting resources with varied nutritional compositions (Dussutour & Simpson, 2008; Kay, 2002; Portha, Deneubourg, & Detrain, 2002) and physical properties (Robson & Traniello, 1998). For instance, many ants carry protein-rich insect prey in their mandibles and transport carbohydrate-rich liquids (e.g. nectar from plants or insect symbionts) within three connected specialized storage organs (crop, midgut, hindgut; Fig. 1; Engel, Fischer, Wäckers, & Völkl, 2001; Josens, Farina, & Roces, 1998).

In colonies where solid food is most commonly harvested, foragers returning full of liquids can appear ‘unladen’, or lacking harvested resources, and thus be erroneously considered energetic drains on their colonies. However, these liquid resources support an ant colony's ‘social stomach’, with a digestive adaptation called the proventriculus (Fig. 1c), enabling workers to regurgitate liquids and

---

* Correspondence: W. Rytter, Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark.
E-mail address: winnie.rn@hotmail.com (W. Rytter).

http://dx.doi.org/10.1016/j.anbehav.2016.04.022
0003-3472/© 2016 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

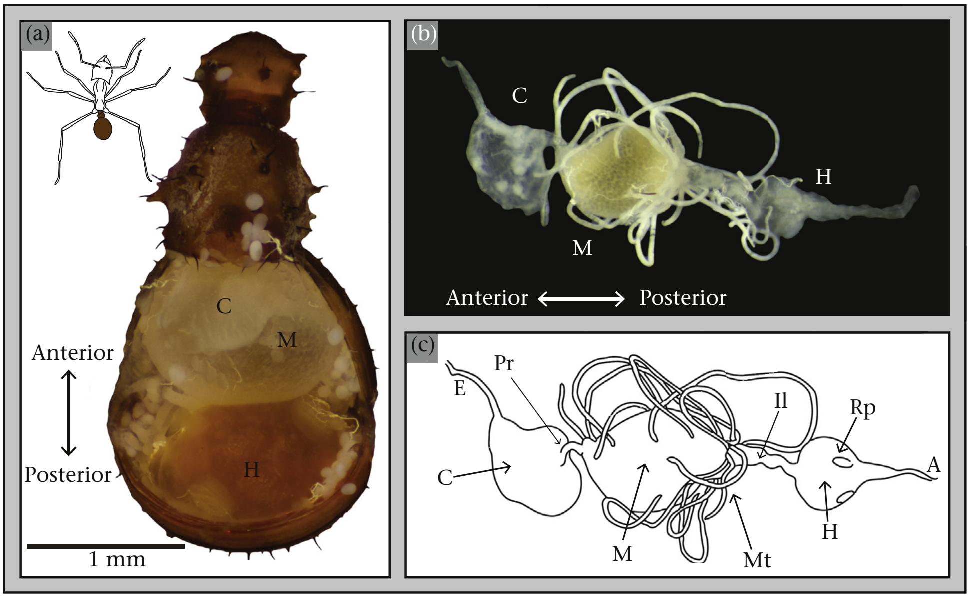


Figure 1. Leafcutter ant digestive system, highlighting the three connected storage organs (crop, midgut, hindgut) described in the text. (a) Dorsal view of storage organs in a partially dissected *Ac. echinatior* worker abdomen showing a full hindgut and midgut and empty crop (white spheres are fat body cells). (b) Lateral view of fully dissected *At. sexdens* digestive system showing a midgut full with the characteristic yellow fluid flanked by an empty crop and hindgut. (c) Schematic illustration of the digestive system highlighting key digestive organs: E, oesophagus; C, crop; Pr, proventriculus; M, midgut; Mt, Malpighian tubules; Il, ileum; H, hindgut; Rp, rectal pads; A, anus. Images viewed at 250X magnification.

share them with nestmates (Cook & Davidson, 2006; Eisner, 1957; Eisner & Brown, 1958). Leafcutter ants (Attini; genera *Atta* and *Acromyrmex*) are the most evolutionarily derived fungus-farming attine ants, cutting and transporting mostly fresh vegetation they prepare as compost to cultivate fungus crops for food in subterranean nests (Hölldobler & Wilson, 2010). Leafcutter foragers often transport loads optimized in size and shape to their individual foraging abilities (Lewis, Pollard, & Dibley, 1974; Wetterer, 1994, 1995). However, foraging trips also often appear unsuccessful as foragers return without carrying any vegetation (Araújo, Della Lucia, Lima, Souza, & Petternelli, 2002; Lewis et al., 1974, but see Kooij, Aanen, Schiott, Boomsa, 2014; Kooij, Rogowska-Wrzesinska et al., 2014). While these unladen foragers appear to present efficiency problems relative to OFT predictions, they have alternatively been hypothesized to lead nestmates to high-quality resources (Bollazzi & Roces, 2011; Jaffe & Howse, 1979; Roces & Núñez, 1993) or maintain foraging trails (Lewis et al., 1974).

Unladen leafcutter foragers have also frequently been assumed to transport, consume and assimilate liquid resources in the form of carbohydrate-rich plant nectar (Bass & Cherrett, 1995; Littledyke & Cherrett, 1976; Mueller, Schultz, Currie, Adams, & Malloch, 2001; Wirth, Herz, Ryel, Beyschlag, & Hölldobler, 2003), but no direct evidence has been provided that this happens routinely in the field. While many ant lineages are known to consume nectar or similar plant secretions (Hölldobler & Wilson, 1990), it has remained underappreciated that specialized fungivory may constrain opportunities to maintain a complementary generalist feeding strategy. Indeed, since the digestive system of *Acromyrmex* leafcutter ants appears to be specialized for vectoring fungal enzymes to new garden growth via faecal droplets (De Fine Licht et al., 2013; Kooij, Aanen et al., 2014; Kooij, Rogowska-Wrzesinska et al., 2014; Martin, 1970; Schiøtt, Rogowska-Wrzesinska, Roepstorff, & Boomsma, 2010), opportunistic foraging on other liquids would probably destabilize this fine-tuned system. Although it is possible that foragers do collect liquids, no study has involved dissections of ants to confirm their presence or absence in the storage organs of unladen workers returning to the nest (Fig. 1a).

We dissected individuals of four Panamanian rainforest leafcutter species to test the OFT prediction that unladen leafcutter ants actually represent successful foraging trips because they are more likely to harvest liquids. We initially established baseline levels of liquid storage in foragers collected as they left their nests, reasoning that if returning foragers had excess liquids above this baseline, they harvested them outside the nest. This led to the surprising observation that most foragers carried liquids in their midguts when exiting their nests, which, in turn led us to perform additional experiments testing a newly formalized ‘lunchbox hypothesis’. Below, we develop this hypothesis within an OFT framework, outlining how it integrates digestive physiology, energetic foraging costs and symbiotic stability.

The lunchbox hypothesis provides an OFT prediction that foraging leafcutter ants leave nests with full midguts, which they deplete to fuel energetically costly foraging activities. These energetic costs include the cutting of leaves, which requires extreme mandibular forces (Roces & Lighton, 1995), foraging trips extending >200 m from the nest (Lewis et al., 1974) and the transport of heavy loads (Lighton, Bartholomew, & Feener, 1987) weighing more than double the body mass of a forager (Wetterer, 1994). Whereas nectar foraging would appear at odds with the specialized interplay between ant farmers and fungal crops, lunchbox dynamics would provide a powerful nutritional mechanism integrating the performance of symbiotic partners, as fungi would fuel ant foragers to perform foraging tasks needed to harvest resources that fuel fungal growth. We tested the lunchbox prediction that liquid depletion reflects task performance using a series of laboratory experiments manipulating foraging distance, load mass and leaf-cutting activity.

METHODS

Liquid Transport in Field-Collected Foragers

We observed foraging behaviour in a rainforest within Soberania National Park, Panama (9.15451°N, 79.73583°W) in May 2015, during the start of the rainy season, a period of high leafcutter