

SHORT COMMUNICATION

Sodium-specific foraging by leafcutter ant workers (*Atta cephalotes*, Hymenoptera: Formicidae)

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Abstract. 1. Sodium is often a limiting nutrient for terrestrial animals, and may be especially sought by herbivores. Leafcutter ants are dominant herbivores in the Neotropics, and leafcutter foraging may be affected by nutritional demands of the colony and/or the demands of their symbiotic fungal mutualists. We hypothesized that leafcutter colonies are sodium limited, and that leafcutter ants will therefore forage specifically for sodium.

2. Previous studies demonstrated that leafcutter *Atta cephalotes* Linnaeus workers preferentially cut and remove paper baits treated with NaCl relative to water control baits. *Atta cephalotes* colonies in this study were presented with baits offering NaCl, Na₂SO₄, and KCl to test whether leafcutters forage specifically for sodium. Sucrose and water were used as positive and negative controls, respectively.

3. *Atta* foragers removed significantly more of the baits treated with NaCl and Na₂SO₄ than the KCl treatment, which did not differ from water. The NaCl and Na₂SO₄ treatments were collected at similar rates. We conclude *A. cephalotes* forage specifically for sodium rather than for anions (chloride) or solutes in general. This study supports the hypothesis that leafcutter ants are limited by, and preferentially forage for, sodium.

Key words. Costa Rica, geographical variation, herbivory, La Selva Biological Station, salt limitation, trophic level.

Introduction

The demand for sodium, a key animal nutrient, is expected to vary geographically and among trophic levels. Aerosol deposition of salt declines exponentially with distance from oceanic sources (Stallard & Edmond, 1981). Sodium concentration in consumers' tissues can be 1000 times higher than producers, so herbivores are expected to be more sodium deprived than carnivores (National Research Council, 2005; Kaspari *et al.*, 2008). Recent studies of salt foraging in ant communities

support these predictions. More herbivorous ant species at inland sites showed the strongest sodium preferences (Kaspari *et al.*, 2008).

Leafcutter ants (*Atta* and *Acromyrmex*) were poorly sampled by these studies that used vials as baits, but leafcutters are among the most herbivorous ants (Holldobler & Wilson, 2011). Leafcutter foraging preferences can be experimentally tested in the field by presenting paper baits at foraging trails. Leafcutters clear foraging trails to facilitate transport of leaf fragments to the nest (Kost *et al.*, 2005). Leaf fragments are fed to symbiotic fungi. Fungus is the primary larval food source, whereas adult workers subsist mainly on liquid leaf exudates (Richard *et al.*, 2005; Caldera *et al.*, 2009). Their diet suggests leafcutters should be among the most salt-seeking ant foragers (Kaspari *et al.*, 2008; Russell *et al.*, 2009).

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In previous studies, *Atta* foragers cut and removed fragments from papers treated with various chemical compounds (Costa *et al.*, 2008; O'Donnell *et al.*, 2010). In a previous experiment, sodium chloride-treated baits were preferred over water-treated baits by *Atta cephalotes* Linnaeus workers (O'Donnell *et al.*, 2010). This study did not determine whether the leafcutters were acquiring sodium (Na) or chlorine (Cl), and the study did not rule out a general response to solutes. To test whether *A. cephalotes* workers forage specifically for sodium we presented paper baits treated with aqueous solutions of sodium sulphate (Na_2SO_4), sodium chloride (NaCl), potassium chloride (KCl), and water-treated negative controls. We expected *Atta* foragers to harvest sodium chloride and sodium sulphate over potassium chloride and water.

Materials and methods

Data were collected between 22 and 23 March 2012 at La Selva Biological station ($10^\circ 26' \text{N}$, $83^\circ 59' \text{W}$; 50–150 m asl elevation). Proximity to the Caribbean coast (approximately 50 km) means aerosol sodium deposition is relatively high at La Selva (Kaspari *et al.*, 2008).

Five actively foraging *A. cephalotes* colonies were selected as subjects. All colonies had cleared foraging trunk trails at least 10 cm wide. Workers carrying leaf fragments were tracked to their nest mounds to confirm they came from different colonies. Subject colony nest mounds ranged from 100 to 600 m from each other. Each colony was used for one trial.

Baits were single pieces of circular filter paper (Whatman no. 4 qualitative papers, 125 mm diameter) soaked in aqueous solutions. Bait papers were soaked to saturation in approximately 250 ml of one of five solutions: 1 M sucrose (positive control), three 1 M salt solutions (treatments: NaCl, KCl, and Na_2SO_4), and tap water (negative control). We then dried each paper for 2–4 h in a convection oven at 40°C . After drying an assistant labelled each paper in pencil with a letter code representing the treatment. Letter codes varied among trails and researchers were blind to the treatments. Bait papers were weighed to the nearest 0.01 g on an electronic balance for pre-trial weight. We used a Licor LI-3100C automated area meter to measure areas of bait papers. The areas of four pieces of filter paper were measured and the mean resulting area (121.08 cm^2) was used as the pre-trial area estimate for all baits.

For each trial, five baits, one per treatment, were placed along a single *A. cephalotes* foraging trunk trail. The first bait was placed 3 m from a nest entrance; baits were separated by a 3 m distance. The order of treatment placements was randomized. Baits were placed with the centre of the paper within 5 cm of the trail edge. We staked each bait paper to the ground with a metal surveyor flag. *Atta* foraging was strongest after dark, so baits were placed at approximately 22.00 hours local time and left overnight (O'Donnell *et al.*, 2010).

Bait papers were collected the next morning at approximately 08.00 hour local time, after approximately 10 h had elapsed. The remaining bait papers were dried for 1–2 h at 40°C and allowed to cool to ambient temperature. Papers were

reweighed on the same digital balance used before trials. We estimated the weight of bait paper removed by the ants as the difference between before and after weights. The area of the remaining paper was measured with the same area meter used before trials. We estimated the area of bait paper removed as the difference between before and after areas. We assumed all paper removed had been harvested by *Atta* workers because most bait papers showed cutting and fragment removal typical of *Atta* leaf harvesting, and *Atta* workers were observed cutting papers in all trials.

General linear models (GLM; SAS v. 9.2) (SAS Institute, Cary, NC, USA) were used to analyse the effect of colony identity and treatment on the log-transformed weight and area of paper removed by the ants. The data were log transformed to reduce inequality of variance among treatments. We did not measure forager traffic along the trails, and baits were not presented for identical time periods among colonies (times were constant within colonies). Uncontrolled colony differences were accounted for by including colony identity as a covariate first in the statistical model. The positive control sucrose treatment was used primarily to verify bait attraction and foraging activity. We excluded the sucrose data from the analyses because the relatively high removal of sucrose-treated paper could mask differences among the other treatments (O'Donnell *et al.*, 2010).

Results and discussion

The measures of weight removed and area removed were highly correlated within baits ($r = 0.96$, $n = 20$, $P < 0.0001$). In all colonies ants removed more of the sucrose positive control than any other bait (Fig. 1). There was no significant effect of colony on weight removed ($F_{4,12} = 1.88$, $P = 0.18$) or area removed ($F_{4,12} = 2.40$, $P = 0.11$). Treatments

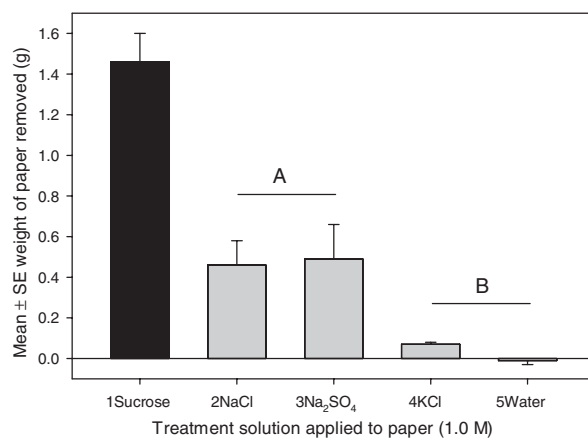


Fig. 1. Bar graph showing amounts of paper baits removed by *Atta cephalotes* foragers. Baits were treated with sucrose (black bar, positive control), water (open bar, negative control), and metallic salts (grey bars, treatments). Horizontal lines and letters above bars show the results of *post hoc* tests for pair-wise comparisons of means; treatments with the same letter were not significantly different.

differed highly significantly in the weight of paper removed ($F_{3,12} = 10.05$, $P = 0.0014$) and area removed ($F_{3,12} = 5.68$, $P = 0.012$). *Post hoc* pair-wise means comparisons (Ryan–Einot–Gabriel–Welsch multiple range test, $\alpha = 0.05$) indicated both weight and area removal of NaCl and Na₂SO₄ treatments were not significantly different, and more paper was removed from each of these treatments than the KCl and water (negative control) treatments (Fig. 1). The KCl treatments did not significantly differ from the water treatments.

The foraging behaviour of leafcutters is of particular interest because these ants are dominant primary consumers in the Neotropics (Herz *et al.*, 2007; Costa *et al.*, 2008). We extend previous findings on *Atta* salt attraction (O'Donnell *et al.*, 2010) and demonstrate that *Atta* forage specifically for sodium. Similar sodium-specific foraging has been documented for ant communities in general (Kaspari *et al.*, 2009). Sodium sulphate was as attractive to *Atta* foragers as sodium chloride. In contrast, potassium chloride was not significantly more attractive than water. *Atta* workers invest time and energy into cutting and carrying materials offering only sodium rewards, supporting the hypothesis that a sodium-limited diet drives specific foraging for this resource. Although potassium chloride bait removal did not differ significantly from the negative control (water), the slightly higher removal of KCl bait paper could indicate a weak attraction to potassium, or a weak general response to solutes or electrolytes.

The fact that foragers both cut and carry off fragments of bait papers suggest their responses were similar to those towards living leaf tissue. We do not know how the bait paper fragments were treated after removal. Discarded paper fragments were not seen near the nest entrances, suggesting the fragments were carried into the nests. It remains unknown whether the *Atta* foragers gather the sodium for their own consumption or for the benefit of their symbiotic fungus. Fungi may also forage for salt: tropical leaf litter fungi harvest sodium, and medium for growing *Atta*'s symbiotic *Leucocoprinia* fungi must include sodium for successful fungal growth (Cromack *et al.*, 1977; Silva-Pinhati *et al.*, 2005). Leafcutter workers can alter their long-term foraging preferences to fit the requirements of the fungal garden (North *et al.*, 1999; Herz *et al.*, 2008). In the short term, leafcutter foragers sometimes harvest plant matter for their own consumption (Seal & Tschinkel, 2007; Herz *et al.*, 2008). Moreover, the fungal garden houses a complex community of other microbes, which could also play a role in the foraging decisions of the *Atta* workers (Caldera *et al.*, 2009). Observations on captive *Atta* colonies may indicate how salt-treated baits are handled inside the nest (Dussutour *et al.*, 2009). It may also be possible to track sodium flow in *Atta* colonies and their symbionts using sodium radioisotopes (Fassbender *et al.*, 2010).

Sodium is a critical but often limiting nutrient to animals in tropical rain forests because it is rapidly leached away by rain water. Ant communities generally respond relatively weakly to sodium baits in coastal areas with high aerosol salt deposition (Kaspari *et al.*, 2009; Dudley *et al.*, 2012). Sodium was a highly attractive resource to *Atta* at our site approximately 50 km inland on a narrow oceanic isthmus. Our data suggest the extreme herbivorous leafcutter ant diet

promotes sodium-specific foraging even in areas of relatively high sodium availability.

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References

- Caldera, E.J., Poulsen, M., Suen, G. & Currie, C.R. (2009) Insect symbioses: a case study of past, present, and future fungus-growing ant research. *Environmental Entomology*, **38**, 78–92.
- Costa, A.N., Vasconcelos, H.L., Vieira-Neto, E.H.M. & Bruna, E.M. (2008) Do herbivores exert top-down effects in Neotropical savannas? Estimates of biomass consumption by leaf-cutter ants. *Journal of Vegetation Science*, **19**, 849–854.
- Cromack, F.J., Sollins, P., Todd, R.L., Crossley, D.A.J., Fender, W.M., Fogel, R. *et al.* (1977) Soil microorganism–arthropod interactions: fungi as major calcium and sodium sources. *The Role of Arthropods in Forest Ecosystems* (ed. by W. J. Mattson), pp. 78–84. Springer-Verlag, New York, New York.
- Dudley, R., Kaspari, M. & Yanoviak, S.P. (2012) Lust for salt in the western Amazon. *Biotropica*, **44**, 6–9.
- Dussutour, A., Beshers, S., Deneubourg, J.L. & Fourcassie, V. (2009) Priority rules govern the organization of traffic on foraging trails under crowding conditions in the leaf-cutting ant *Atta colombica*. *Journal of Experimental Biology*, **212**, 499–505.
- Fassbender, M., Bach, H., Kitten, J., Nortier, F.M. & Taylor, W. (2010) Radioisotope production at the Los Alamos National Laboratory. *Journal of Labelled Compounds and Radiopharmaceuticals*, **53**, 332–335.
- Herz, H., Beyschlag, W. & Holldobler, B. (2007) Herbivory rate of leaf-cutting ants in a tropical moist forest in Panama at the population and ecosystem scales. *Biotropica*, **39**, 482–488.
- Herz, H., Holldobler, B. & Roces, F. (2008) Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behavioral Ecology*, **19**, 575–582.
- Holldobler, B. & Wilson, E.O. (2011) *The Leafcutter Ants*. W.W. Norton & Co., New York, New York.
- Kaspari, M., Yanoviak, S.P. & Dudley, R. (2008) On the biogeography of salt limitation: a study of ant communities. *Proceedings of the National Academy of Sciences*, **105**, 17848–17851.
- Kaspari, M., Yanoviak, S.P., Dudley, R., Yuan, M. & Clay, N.A. (2009) Sodium shortage as a constraint on the carbon cycle in an inland tropical rainforest. *Proceedings of the National Academy of Sciences*, **106**, 19405–19409.
- Kost, C., de Oliveira, E.G., Knoch, T.A. & Wirth, R. (2005) Spatio-temporal permanence and plasticity of foraging trails in young and mature leaf-cutting ant colonies (*Atta* spp.). *Journal of Tropical Ecology*, **21**, 677–688.

- National Research Council (2005) *Mineral Tolerances of Animals*. National Academies Press, Washington, District of Columbia.
- North, R.D., Jackson, C.W. & Howse, P.E. (1999) Communication between the fungus garden and workers of the leaf-cutting ant, *Atta sexdens rubropilosa*, regarding choice of substrate for the fungus. *Physiological Entomology*, **24**, 127–133.
- O'Donnell, S., Garcia-C, J.M., Beard, J., Chiwocha, T., Lewis, D., Liu, C. *et al.* (2010) Leaf cutter ants (*Atta cephalotes*) harvest baits offering sodium chloride rewards. *Insectes Sociaux*, **57**, 205–208.
- Richard, F.-J., Mora, P., Errard, C. & Rouland, C. (2005) Digestive capacities of leaf-cutting ants and the contribution of their fungal cultivar to the degradation of plant material. *Journal of Comparative Physiology B*, **175**, 297–303.
- Russell, J.A., Moreau, C.S., Goldman-Huertasa, B., Fujiwaraa, M., Lohman, D.J. & Pierce, N.E. (2009) Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. *Proceedings of the National Academy of Sciences*, **106**, 21236–21241.
- Seal, J.N. & Tschinkel, W.R. (2007) Co-evolution and the superorganism: switching cultivars does not alter the performance of fungus-gardening ant colonies. *Functional Ecology*, **21**, 988–997.
- Silva-Pinhati, A.C.O., Bacci, M., Siqueira, C.G., Silva, A., Pagnocca, F.C., Bueno, O.C. *et al.* (2005) Isolation and maintenance of symbiotic fungi of ants in the tribe Attini (Hymenoptera: Formicidae). *Neotropical Entomology*, **34**, 1–5.
- Stallard, R.F. & Edmond, J.M. (1981) Geochemistry of the Amazon. 1. Precipitation chemistry and the marine contribution to the dissolved-load at the time of peak discharge. *Journal of Geophysical Research*, **86**, 9844–9858.

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