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Author(s): Robert E. Clark and Joshua R. King

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The Ant, *Aphaenogaster picea*, Benefits From Plant Elaiosomes When Insect Prey is Scarce

ROBERT E. CLARK^{1,2} AND JOSHUA R. KING³

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ABSTRACT Myrmecochory is a facultative, mutualistic interaction in which ants receive a protein-rich food reward (elaiosome) in return for dispersing plant seeds. In North American northeastern hardwood forests, *Aphaenogaster* ants are the primary genus dispersing myrmecochorous plants. In these forests, myrmecochores occur in plant guilds of understory spring ephemerals or seasonal greens. This mutualism has been demonstrated for *Aphaenogaster rudis* (Emery) and individual plant species, but it has not been demonstrated for other *Aphaenogaster* species or guilds of myrmecochores as they naturally occur. *Aphaenogaster picea* (Wheeler) colonies were fed three treatments over 5 mo: 1) a mixture of only elaiosomes from an entire plant guild, 2) a diet of only insect protein and 3) a combination diet of both elaiosomes and insect protein. This experiment investigated two potential hypotheses through which elaiosomes can benefit ants: 1) elaiosome proteins can substitute for protein nutritional requirements when ants are prey-limited, and 2) elaiosome nutrition can supplement insect protein when prey is ample. First, a mixture of elaiosomes from four myrmecochorous plant species provided to *A. picea* colonies was sufficient to maintain worker production, larval growth, and fat stores when no other food was available. *A. picea* colonies consuming elaiosomes as their only protein source could be sustained for a growing season (5 mo). Second, colonies fed both elaiosomes and protein did not yield more productive colonies than a control diet of just insect protein. These results support the hypothesis that myrmecochory is indeed a facultative mutualism in which ants take advantage of the protein content of elaiosomes when it is favorable, but when they are not limited by insect prey they do not gain any additional benefit from elaiosomes.

KEY WORDS ant–plant interactions, myrmecochory, elaiosome, mutualism, trophic interactions

Myrmecochory is a mutualism where ants disperse plant seeds in return for a protein-rich reward called an elaiosome (Rico-Gray and Oliveira 2007). In North American northeastern hardwood forests *Aphaenogaster* ants are the primary dispersers of myrmecochorous plants (Ness et al. 2009). Generally speaking, the benefit of myrmecochorous seed dispersal is context specific: the plant may gain a nutrient rich microsite (Beattie and Culver 1982) or protection from predation (Heithaus 1981), competition (Handel 1976), or fire (Rico-Gray and Oliveira 2007).

Aphaenogaster rudis ants are shown to benefit from this association with myrmecochores (Heithaus et al. 2005). This is because elaiosomes are an important food source for *Aphaenogaster rudis* colonies (Morales and Heithaus 1998, Bono and Heithaus 2002, Fischer et al. 2008, Fokuhl et al. 2007), providing protein and some lipid content. Like other facultative insect mutualisms, this food reward does not make up the entire

diet of the ant mutualist: in addition to scavenging insect protein and elaiosomes, it has been shown that eastern *Aphaenogaster* species take insect prey as an important food source (unpublished data, Buczkowski and Bennett 2007).

The *Aphaenogaster fulva-rudis-texana* species complex (Umphrey 1996) is a group of abundant and widespread ants in North American northeastern forests. For this study, in Connecticut, U.S.A., the species *Aphaenogaster picea* (Buckley) is an abundant member of this species complex. Like other members of the *Aphaenogaster fulva-rudis-texana* complex, *A. picea* disperses myrmecochorous plant seeds (unpublished data). *A. picea* is a widespread ant of mesic deciduous forests in eastern North America. Umphrey (1996) described the range of *A. picea*, which typically is associated with inland, relatively higher elevations than the Atlantic coast and spans the range from Connecticut and Ontario, Canada; west to Pennsylvania, south into the southeastern United States along the Appalachian Mountains. To date no study has shown that members of this species complex, other than *Aphaenogaster rudis*, benefit from this mutualism.

A. picea co-occurs with several myrmecochorous species (ranges published by Umphrey 1996 and Kartesz 2011). Multiple species of myrmecochores

¹ Wesleyan University, Department of Biology, Middletown, CT 06457.

² Corresponding author: Robert E. Clark, Wesleyan University, Biology Department, 52 Lawn Ave., Middletown, CT 06457 (e-mail: rclark@wesleyan.edu).

³ University of Central Florida, Biology Department, Orlando, FL, 32816.

overlap within the same habitats at small spatial scales and make up an appreciable diversity and biomass of understory herbs (Handel et al. 1981, unpublished data, R.C.). Because of the frequent high density of both *Aphaenogaster* and myrmecochores and potentially spatially overlapping seed shadows, ant colonies may consume multiple plant species elaiosomes over the course of a season. For this reason, feeding experiments that use one plant's elaiosomes do not reflect the food rewards typically experienced by one ant colony. In these forests myrmecochory is a species-guild mutualism not a species-species mutualism. Therefore to determine the nutritional benefits of myrmecochory for ants it is realistic to provide a mixture of elaiosomes. In this experiment the mixtures chosen reflect the guild of myrmecochores *A. picea* interacts with.

Does a guild of myrmecochores provide a tangible benefit to ant colonies? We tested whether or not a mixture of elaiosomes provide enough nutrition to have a significant impact on worker production (by observing brood numbers and worker count) and energy (by observing lipid stores). This feeding treatment was achieved under conditions with strict diet control: testing for the benefit of elaiosomes when insect protein was limiting and the benefits of elaiosomes when insect protein was ample. *A. picea* colonies across different feeding treatments were reared under laboratory conditions.

Materials and Methods

From May to September of 2009, *A. picea* colonies were collected from secondary-growth, mesic deciduous forests of Connecticut, U.S.A. Colony rearing methods follow Lubertazzi and Adams 2010, who studied the role of nutrition in *Aphaenogaster rudis* colony growth and sex allocation. In this way, the ants were cultured into fluon-lined plastic boxes with moist dental plaster bottoms, and provided water, dead crickets, and 20% sucrose, *ad libitum*. Temperature was maintained at a constant 30°C. Variation in temperature can confound colony growth responses to food sources (Asano and Cassill 2012). Twenty complete colonies (with queens) were maintained under these conditions until the experimental treatments were started in December 2009. For each given colony, sexual castes other than the queen, larvae and visible eggs were stripped from colonies at the start of the experiment. This ensured that observed treatment effects were from diet and not from differences in energy stores between colonies—removing larvae prevents the particularly large energetic burden placed on colonies by the presence of sexual castes. The total number of adult workers varied between colonies from 15 to 450 workers.

For ant colonies the investment of resources in workers and sexual castes vary by seasonality (Tschinkel 1993). To observe the influence of food on colony growth accurately, it is necessary to control for temperature variation because of how temperature fluctuation impacts larval development (Asano and

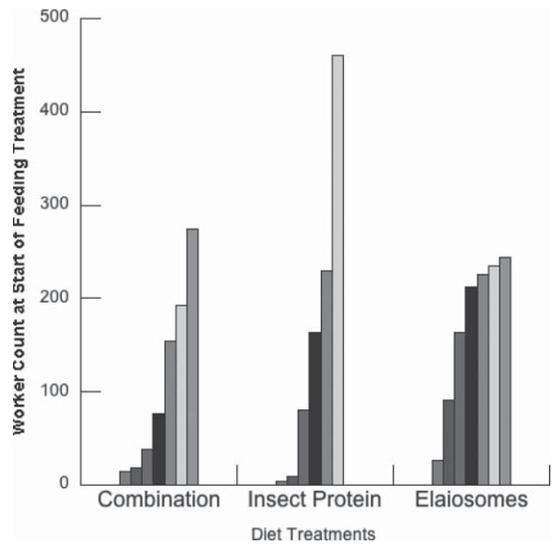


Fig. 1. Distribution of colony sizes in each experimental treatment. Total adult worker count in each *A. picea* colony varied from 15 to 450 workers. Colonies in the same treatment have connected bars. Ant colonies were stratified randomly between three treatments based on size.

Cassill 2012). Colonies were cooled and then kept in a temperature regulated to ensure this control. A cooling period, however, accounts for potential cold temperature triggers normally experienced by ants endemic to temperate regions. Before experimental treatments in December 2009, *A. picea* colonies were chilled in a 2°C lab refrigerator. For the first week, colonies were removed from the refrigerator every 2 d for 12 h. Colonies were not fed during this cooling cycle to mimic the natural nutritional limitations of winter.

All seeds, with elaiosomes, were gathered opportunistically as seed pods started to dehisce from May to August of 2009. These were collected from the same local Connecticut forests where colonies were collected. The feeding treatments represent a distribution of elaiosomes that would typically occur with *A. picea* and included the four most common myrmecochores in these habitats (unpublished data, R.C.). These elaiosome-bearing seeds were stored in a freezer in air tight plastic bags to be used later for treatments. To verify whether ants would consume elaiosomes after freezing, seeds of *Sanguinaria canadensis* L. were placed into colony trays. In this trial, workers manipulated the seeds with their mandibles and carried those seeds to their brood piles and larvae fed on the elaiosome portion.

Colonies were divided based on worker counts in a random stratified fashion with a distribution of colony sizes from very small to large (Fig. 1). Three treatments included the following: a diet of dead crickets; a diet of mixed elaiosomes; and finally a diet of mixed elaiosomes and dead crickets. *Aphaenogaster picea* colonies have been kept under the control conditions and these colonies maintained worker production for up to

a year (unpublished data, R.C.). Cultured ant colonies must be fed some form of insect protein to maintain brood production (Cassill and Tschinkel 1999) and crickets are a readily available form of insect protein. Colonies were provided sugar water (table sugar and distilled water) to maintain worker activity (Cassill and Tschinkel 1999) but not provide additional protein. All colonies also were provided water with cotton-plugged test tubes.

Any time that the colonies were fed elaiosomes, they were presented a mixture of seeds. This mixture included *Trillium erectum* L., *Erythronium americanum* Ker Gawl., *S. canadensis*, and *Dicentra cucullaria* (L.) Bernh., respectively. As many seeds were collected as possible in the field to provide ample elaiosomes to ant colonies. Past work studying the nutritional benefit of myrmecochory followed this same strategy of providing ample elaiosomes (Morales and Heithaus 1998). Elaiosomes were fed to each ant colony in the two experimental treatments once a week on a mixture containing 10 *Sanguinaria* seeds, 4 *Erythronium* seeds, 4 *Trillium* seeds, and 1 *Dicentra* seed. These ratios are because of differences in seed production per individual plant species. Effectively, ant colonies were fed more elaiosome-bearing seeds from abundant plants and fewer from less abundant plants. This diet regime occurred for 2 mo.

To observe colony productivity *in vitro*, larvae present in each nest tube were counted for a 3 wk period starting at week 6 of the experiment in March 2010.

At the conclusion of the experiment in May of 2010 all colony members were freeze-killed, dried in a drying oven at 65°C for 48 h, weighed, and fat extracted. Fat content of each ant colony was measured using 24-h minimum Soxhlet extraction upon all members (Smith and Tschinkel 2009). The difference in weight before and after extraction was a measure of the total lipid content of the colony.

Results and Discussion

Colony productivity was equal for all three treatments. For the three diet combinations there was no significant difference in fat content by weight (Fig. 2a; $F(2,18) = 0.0381$, $P = 0.9627$; $KW-H(2,21) = 0.0076$, $P = 0.9962$) or mean larval production (See Fig. 2b for statistics). Even though some colonies were fed a combination of elaiosomes and insect protein, they did not have greater energy stores (Fig. 2a) or produce more larvae (Fig. 2b) at the observed time points, than other colonies fed any protein source. In sum, elaiosomes provided sufficient nutrition when insect protein was not available but there was no observed additional benefit when insect protein was ample. To account for the worker variation between colonies, dry weight and fat content of five workers was recorded. No correlation was observed between colony size and worker fat content (linear regression in SAS 9.1, PROC GLM) illustrating that worker counts and percent fat content are independent of each other.

Past work has shown that plant elaiosomes actually can contain the same ratios of amino acids as insect

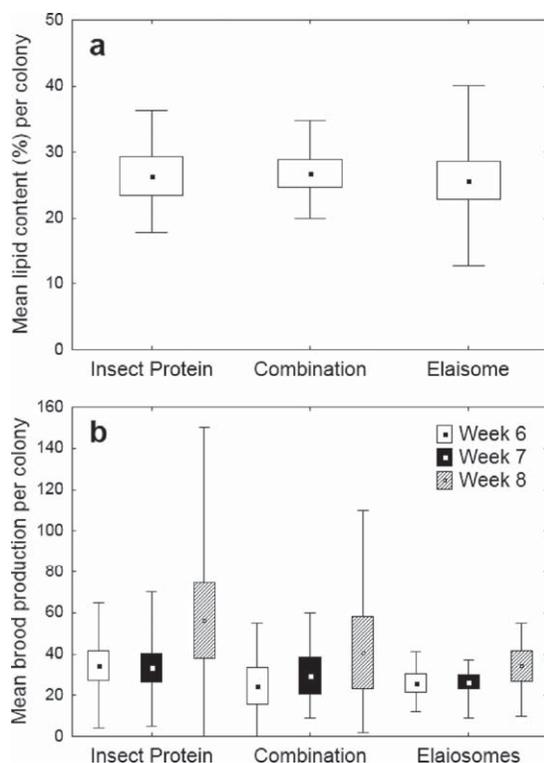


Fig. 2. (a) Mean lipid content of workers, larvae, and queens in *A. picea* colonies by percent. Boxes represent standard error of the mean, bars represent range. $F(2,18) = 0.0381$, $P = 0.9627$; $KW-H(2,21) = 0.0076$, $P = 0.9962$; (b) Mean brood production by *A. picea* colonies during weeks 6 through 8. Boxes represent standard error of the mean, bars represent range. Week 6: $F(2,18) = 0.6222$, $P = 0.5479$; $KW-H(2,21) = 1.15$, $P = 0.5627$; Week 7: $F(2,18) = 0.2891$, $P = 0.7523$; $KW-H(2,21) = 0.6017$, $P = 0.7402$; Week 8: $F(2,18) = 0.5766$, $P = 0.5718$; $KW-H(2,21) = 0.2422$, $P = 0.8859$.

prey (Hughes et al. 1994). Our results show that there is a functional consequence of these similar nutritional profiles. *A. picea* colonies that were stripped of all larvae, then starved and chilled under wintering conditions were cultured without any source of protein other than elaiosomes that persisted for 5 mo. This plant-derived diet replaced insect protein and allowed the colony to maintain worker production and keep up its fat stores. Our results suggest that myrmecochory between this plant guild and *A. picea* is indeed a mutualism. This is also indeed a facultative mutualism in which ants benefit only when the nutrients provided by elaiosomes are limiting. Experimentally this has been verified—only colonies lacking insect protein benefited from having elaiosomes in their diet.

This is also the first experiment testing the benefit of elaiosomes for *Aphaenogaster* ants with a controlled diet of laboratory raised *A. picea* colonies. The benefits that a nutrient, like protein, can confer to an ant colony are variable because of the complexity of a colony life cycle (Tschinkel 1993). There are many ways in which a nutrient can be beneficial to an ant

colony, other than reproduction. Previous supplementation experiments illustrated that providing elaiosomes of bloodroot, *S. canadensis*, to *A. rudis* colonies in the field did alter sex ratios and increased the size of young queens in *Aphaenogaster rudis* (Morales and Heithaus 1998, Bono and Heithaus 2002). Although the production of sexual castes was not observed in the course of these experiments (because sex brood was removed at the start of the experiment) the observation of maintained colony growth in protein starved colonies reveals the substitutive quality of elaiosomes. Hypothetically, the benefit of myrmecochory for the ants is tied to conditions in which the elaiosomes are actually beneficial. The highly controlled environment additionally reveals that the correlation observed, of a mutual benefit of myrmecochory, may be caused by the supplemental role elaiosomes play. For this reason future work should investigate whether or not *Aphaenogaster* are naturally protein limited, or if elaiosomes are timed to release when insect prey is scarce for *Aphaenogaster* colonies.

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