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

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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 myrmecochores (Ness et al. 2009). Generally speaking, the benefit of myrmecochores 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and elaiosomes, it has been shown that eastern *Aphaenogaster* species take insect prey as an important food source (unpublished data, Buczkowski and Bennett 2007).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the *Aphaenogaster fulva–rudis–texana* complex, *A. picea* disperses myrmecochores and takes anelaiosome plant seeds (unpublished data).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 myrmecochores (ranges published by Umphrey 1996 and Kartesz 2011). Multiple species of myrmecochores...
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 exper-
iments 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 mix-
ture 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 en-
ergy (by observing lipid stores). This feeding treat-
ment was achieved under conditions with strict diet
control: testing for the benefit of elaiosomes when
insect protein was limiting and the benefits of elai-
somes when insect protein was ample. A. picea col-
onies 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 decid-
uous forests of Connecticut, U.S.A. Colony rearing
methods follow Lubertazzi and Adams 2010, who stud-
ied the role of nutrition in Aphaenogaster rudis colony
growth and sex allocation. In this way, the ants were
cultured into flouon-lined plastic boxes with moist den-
tal plaster bottoms, and provided water, dead crickets,
and 20% sucrose, ad libitum. Temperature was main-
tained 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 condi-
tions until the experimental treatments were started 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 opportu-
nistically as seed pods started to dehisce from May to
August of 2009. These were collected from the same
local Connecticut forests where colonies were col-
lected. The feeding treatments represent a distribu-
tion of elaiosomes that would typically occur with A.
picea and included the four most common mymrem-
cochores 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 ca-
nadensis 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 treat-
ments 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 col-
onies have been kept under the control conditions and
these colonies maintained worker production for up to

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 treat-
ment have connected bars. Ant colonies were stratified ran-
domly between three treatments based on size.
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 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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References Cited


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