Reports

FOOD FROM SEED-DISPERSAL MUTUALISM SHIFTS SEX RATIOS IN COLONIES OF THE ANT *APHAENOGASTER RUDIS*

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Abstract. Workers of *Aphaenogaster rudis* collect seeds of many species of spring-flowering, perennial herbs. This is part of a seed-dispersal system (myrmecochory) for which benefits to plants are documented, but consequences to ants have not been quantified. To test the predictions that colony size or reproductive output will be enhanced as a consequence of ants collecting seeds, we conducted a field experiment in a forest near Gambier, Ohio, in June and July 1993.

Experimental colonies receiving seeds of *Sanguinaria canadensis* ($N = 24$ colonies) had ~3.5 times as many gynes as control colonies ($N = 27$). Only 25% of control colonies produced any gynes, whereas 65% of experimental colonies did so. Control colonies produced as many males as experimental colonies. Access to seeds shifted the mass and numerical investment ratio in colony reproductive output toward female bias but did not affect the number of workers or queen size. These data support the hypothesis that myrmecochory is a true mutualism. Additionally, the experiment indicates that resource levels influence investment ratios in reproductive ants.

Key words: ants; Aphaenogaster rudis; *elaiosome as food source; field experiment; Formicidae; Hymenoptera; mutualism; myrmecochory; reproductive investment ratios; seed dispersal; sex ratios, ants; social insects.*

INTRODUCTION

Seed dispersal by ants (myrmecochory) is important to many species of plants, especially in eastern North America and Australia (Beattie 1985, Gunther and Lanza 1989). Ants respond to external cues on the seeds by carrying seeds to the nests, where ant larvae consume the lipid-rich external appendages (elaiosome). Plant embryos are usually undamaged and the seed is left to germinate, either within the nest or after being discarded to waste piles (Beattie 1985). Seed dispersal by ants can reduce parent–offspring or interspecific competition (Handel 1978, Pudlo et al. 1980), decrease seed predation (Heithaus 1981, 1986), move seeds to fertile micro-sites (Culver and Beattie 1980, Westoby et al. 1991) or protect seeds from fires (Berg 1975). However, we are unaware of any published data quantifying the ecological benefits for ants in systems of myrmecochory (Beattie 1991).

Although ants are obviously obtaining food for larvae, it is not known whether seed elaiosomes signifi-

cantly influence colony dynamics. Uncertain demographic consequences to ants calls into question whether this system of myrmecochory is actually a mutualism with reciprocal benefits as is usually assumed (sensu Cushman and Beattie 1991).

Untested assumptions of positive demographic effects for at least one partner are characteristic in studies of apparent mutualisms. The result is an incomplete view of the dynamics of these interactions (Cushman and Beattie 1991). Accordingly, increased attention must be paid to identifying benefits towards both participants in these interactions (Beattie 1991, Cushman and Beattie 1991). Although ants are a dominant partner in apparent mutualisms (Hölldobler and Wilson 1990, Cushman and Beattie 1991), only three studies (all ant-lycaenid interactions) have experimentally demonstrated benefits to ants (Nash 1989, Cushman et al. 1994, Fiedler et al. 1995). Much more work in many different systems needs to be done if ecologists are to understand the frequency and dynamics of mutualistic interactions (Cushman and Beattie 1991).

We tested for benefits to ants in a system of myrmecochory involving a forest-dwelling ant of eastern North America (*Aphaenogaster rudis*) and several species of perennial, spring-flowering herbs. Large vari-

ation in food supply can influence the demography of *A. rudis* (Southerland 1988), but several factors call into question whether the seed collecting associated with myrmecochory has demographic consequences. First, ants pick up seeds in response to a chemical stimulus (Marshall et al. 1979, Brew et al. 1989), making it possible that seed-dispersal behavior could be manipulated by plants irrespective of nutritional reward. Additionally, ants satiate to elaiosomes so that relatively few (often <40) seeds of all myrmecochorous species combined are carried into a single nest over an entire growing season (Smith et al. 1989, Cummings and Heithaus 1992, Liu and Heithaus 1993). Our experiment attempts to identify the demographic consequences of adding elaiosome-bearing seeds of *Sanguinaria canadensis* to the basic food supply of colonies of *A. rudis* in natural conditions.

The ultimate measure of colony fitness is the number of reproductives, so we specifically asked whether colonies provided with seeds produced more reproductive individuals or more workers (which might contribute to added reproductive output in following years). In addition, because levels of resources available to ants might influence sex ratios (Deslippe and Savolainen 1995), our experiment included measuring the ratio of reproductive males to females.

METHODS

Our experiment was conducted June and July 1993 in a deciduous forest located 2 km northwest of Gambier, Ohio, USA. Canopy trees average around 1 m in diameter at breast height and were dominated by *Acer saccharum, Fraxinus americana, Fagus grandifolia,* and *Quercus* spp. Common understory herbs included the myrmecochorous species *Viola papilionacea, V. canadensis, Dentaria laciniata, Claytonia virginiana,* and *Trillium grandiflorum.*

Aphaenogaster rudis colonies include a single reproductive queen and up to 2000 workers (Headley 1949, Talbot 1951). At our study site, nests are usually tunnels and chambers dug into fallen branches and under bark of dead trees; colony density is 0.5 nests/m². Gynes and males develop from a subset of overwintered larvae (Headley 1949, Talbot 1951), and, in the Ohio region, pupate no earlier than late June (Headley 1951). Reproductive pupae emerge no earlier than late July and mating flights occur in late August (Headley 1951). Worker development is continuous, with a peak in numbers of larvae, pupae, and workers during early June, late July, and mid-August respectively (Headley 1951).

A. rudis colonies were located by baiting. To facilitate collecting full colonies at the end of the study, we used only colonies nesting in fallen branches with diameters <40 cm (56% of all nests located). Vouchers were collected and compared to reference specimens identified by D. R. Smith, Systematic Entomology Laboratory, United States Department of Agriculture (vouchers are located at the Center for Environmental Study, Kenyon College, Gambier, Ohio, USA).

Sanguinaria canadensis is a perennial ant-dispersed plant (myrmecochore) commonly found in low-lying woodland habitats. Seed pods mature in mid-June, with an average of 45 seeds per pod (R. Heithaus, *personal observation*). Seeds are commonly available to a given colony for no more than two weeks (Rice and Heithaus 1993).

Seeds of *S. canadensis* were collected at two sites within 5 km of our study site. This species was selected as a seed source because it was not located in the study area (so all colonies would lack previous experience collecting these seeds), and seeds have large elaiosomes. Seeds were kept frozen with BHT crystals until used; ants respond equally to freshly collected seeds and seeds stored in this way for several months (R. Heithaus, *personal observation*).

Nests were randomly assigned to either experimental or control treatments ($N = 31$ for each). Control colonies were prevented from collecting elaiosome-bearing seeds by removing seed-pods from any myrmecochorous plants in the study area. Experimental colonies were provided with intact diaspores (seed and elaiosome) of *S. canadensis* in two cycles of presentation, 1 wk apart, starting 14 June 1993.

Seed presentations consisted of providing seeds to satiation on two consecutive days, for a total of 4 d in which seeds were available. Seeds were deposited within 1 m of nests, and seed collection was monitored for 4 h after the first foraging worker encountered the seed depot (colonies ceased collecting seeds early in this interval). To ensure accuracy of counting, only three colonies per day were monitored, so the enrichment experiment required ~3 wk to complete.

Because colonies that encounter a seed pod under natural conditions will satiate to this resource before it is exhausted, providing seeds to satiation did not result in an unnatural level of seed availability for experimental colonies. For example, in this study experimental colonies had an unlimited access to seeds and collected 31.8 ± 3.0 seeds (mean $\pm$ 1 SE) per colony during the course of the experiment (with 25 ± 2.4 of these seeds collected in the first 2 d) whereas seed pods generally contain ~45 seeds per pod. In other words, seed collection is essentially all or nothing depending on whether any given colony has access to a seed pod.

For each experimental nest, we collected the entire colony one month after the first day of seed presentation. Control colonies were collected at the same rate, in random order. The timing of collection allowed time for reproductives to eclose or to pupate (Headley 1949,

TABLE 1. Responses of colony structure with addition of myrmecochorous seeds to *Aphaenogaster rudis* colonies. Analysis of covariance was used, with access to elaiosomes as the treatment effect and the number of workers in the colony (colony size) as the covariate. Data are means ± 1 SE. There were no significant interactions between treatment and the covariate. Adjusted and transformed means are given where indicated. Degrees of freedom = 1 for treatment effect; error df are given in the table (which vary for variables involving reproductives because not all nests successfully produced reproductives).

	Error df	Colony		Treatment effect		Colony size	
Dependent variable		Control	Experimental	F	P	F	P
No. larvae†,‡	48	1.63 ± 0.09	1.64 ± 0.09	0.01	0.930	3.86	0.055
No. pupae‡	48	184.14 ± 15.39	215.55 ± 16.34	1.93	0.172	91.79	<0.001
Alate/(pupae + alate)♂§	27	0.43 ± 0.13	0.40 ± 0.15	0.08	0.778	0.13	0.721
Alate/(pupae + alate)♀§	19	0.67 ± 0.29	0.95 ± 0.17	0.97	0.337	0.47	0.503
No. males (all colonies)†,‡	48	0.71 ± 0.18	0.62 ± 0.19	0.12	0.729	15.22	<0.001
No. gynes (all colonies)†,‡	48	0.12 ± 0.12	0.61 ± 0.13	7.26	0.010	19.69	<0.001
No. males (successful)†,‖	27	1.31 ± 0.24	1.28 ± 0.23	0.49	0.492	2.74	0.109
No. gynes (successful)†,‡,‖	19	1.13 ± 0.12	1.22 ± 0.08	0.37	0.548	15.68	0.001
Proportion gynes¶ (count)	30	0.16 ± 0.07	0.49 ± 0.09	9.09	0.005	0.90	0.350
Proportion gynes¶ (mass)	30	0.28 ± 0.09	0.69 ± 0.08	9.32	0.005	0.13	0.726

† (Log_{10} + ½)-transformed to stabilize variances.
‡ Means adjusted for colony size.
§ Arcsine-transformed to stabilize variances.
‖ Successful = reproductively successful colonies.
¶ Proportion = gyne output/total reproductive output.

Talbot 1951), but not so much time that reproductive mating flights would be initiated. To collect a colony, the branch containing the nest was cut, then placed in a Fluon-coated, plastic tray; ants outside the nest were collected with aspirators and placed in the same tray. Finally, the soil under the branch was scraped in layers to a depth of 10 cm to ensure that nests did not have chambers underground. Seven experimental and five control nests moved from their original locations between the time of seed presentation and collection, reducing the sample size for analysis.

After collection, we counted pupae and adults for workers, males, and female reproductives (gynes), and we counted larvae (for which caste could not be determined). We could not reliably collect and count eggs. Dry masses for each colony were measured to the nearest 0.1 mg for all castes.

Statistical analyses were conducted using Statistica for Windows Release 5.0 (StatSoft 1995). Analyses involving reproductives were performed separately for males and gynes, because the two sexes have different colony life histories. Experimental and control colonies were compared for effects of elaiosomes on numbers and masses of the colony segments, the proportion of workers to other colony segments, and the relative investment in gyne reproductives. Reproductive biomass was calculated as the total number of reproductives for each sex (pupae and alates) times the average dry mass of alates for that sex. Proportional investment in gynes was calculated as the number or biomass of gynes divided by total reproductive number or biomass, respectively.

RESULTS

The number of workers in a colony (colony size) significantly influenced several components of colony social structure, including numbers of larvae, worker pupae, and gynes (Table 1), so the influence of elaiosomes was analyzed using ANCOVA to control for colony size. To detect any differences between treatments in the developmental rates of reproductives, the proportion of reproductive ants that had eclosed was compared for experimental and control colonies. Proportion of reproductives that had emerged was not influenced by elaiosomes for either males or females (Table 1), so all other analyses with reproductives were performed with pupae and alates combined to give total production.

Colonies with access to elaiosomes ($N = 24$) produced significantly more gynes than did control colonies ($N = 27$) (Table 1; untransformed, unadjusted $\bar{X}$ ± 1 SE = 14.71 ± 3.47 vs. 4.26 ± 1.82 gynes, respectively). In contrast, production of males was not significantly affected by elaiosomes (Table 1; untransformed, unadjusted means [±1 SE]: control colonies = 39.75 ± 12.41 males, experimental colonies = 44.26 ± 18.37 males). The overall increase in the number of gynes could result from having more gynes produced per worker for all enriched colonies, from a greater number of enriched colonies producing some gynes, or a combination of these responses. Elaiosomes increased the frequency of colonies that successfully produced gynes (25.9% controls vs. 62.5% experimental, Fisher's exact test, $P = 0.012$), but did not significantly

affect the ability to produce males (55.5% controls vs. 62.5% experimental, Fisher's exact test, $P = 0.777$).

To test the hypothesis that elaiosomes enhance production of gynes in those colonies that were able to produce gynes, we conducted a second analysis using only those colonies that demonstrated some reproductive success. Given that a colony could produce at least one reproductive offspring, elaiosomes did not increase the number of males or gynes that were produced (Table 1). We conclude that colonies that can feed elaiosomes to offspring are more likely to produce at least some gynes, with the number of gynes being most influenced by colony size.

Sex ratios are likely to shift with access to elaiosomes because female but not male production was affected. This was confirmed statistically by a significant shift toward female bias in both the numerical and biomass measures of sex ratio (Table 1). The number of workers in a colony, however, did not influence sex ratio (Table 1).

Future reproductive success might be influenced by colony size or increased mass of the queen. Colony size (measured by the number of workers) did not respond to enhanced seed availability. Colonies with access to elaiosomes were not significantly larger than control colonies as measured by adult workers (457 ± 59 vs. 360 ± 46 workers ($\bar{X} \pm 1$ SE), $t = 1.50$, df $= 44$, $P = 0.20$) or pupal workers (Table 1). Queen mass (3.5 ± 0.48 mg, $\bar{X} \pm 1$ SE) was not significantly influenced by access to elaiosomes ($t = 0.96$, df $= 17$, $P = 0.36$).

DISCUSSION

Is this myrmecochory a mutualism?

We provide the first experimental evidence that myrmecochory substantially benefits ants in a natural context. Recent work demonstrates shifts in the reproductive output of ant colonies for *Formica podzolica* after large increases in food supply (Deslippe and Savolainen 1995). In a different system, Davidson et al. (1984, 1985) found an effect of seeds on the colony density of granivorous ants for one of two desert systems examined (although reproductive output was not considered). In our system, whether elaiosome-bearing seeds provide sufficient food to enhance production of gynes might depend on levels of local seed production, availability of other foods, or the density of competing colonies. Our presentation of seeds was deliberately short term and matched to coincide with the timing of seed availability in nature. In this way, the results of our study should be indicative of colony response under natural conditions. For the year of our study, realistic levels of seed collection (see comments in *Methods,* above) significantly enhanced the production of female reproductives compared to colonies without access to seeds.

Several recent studies have identified benefits to worker ants in ant–lycaenid mutualisms (Nash 1989, Cushman et al. 1994, Fiedler and Saam 1995). However, the critical measure of colony success in ants is the number of reproductive ants that are produced. While an increase in the number of workers may ultimately influence reproductive output, such an effect would be indirect. Our study provides the first experimental evidence for direct benefits to ant colonies arising from a mutualism.

Differences in male and female production with enhancement

The basis for sex determination in ants, as in other hymenopterans, is haplodiploidy. One consequence of haplodiploidy is that queens are equally related to sons and daughters, but sisters are three times more related to each other as compared to brothers from the same mating. The mechanism for a facultative influence on sex ratios is feasible because workers control the production of gynes through environmental factors, while workers and queens control the production of males through selective culling and fertilization of eggs, respectively. Because workers are responsible for brood care, Trivers and Hare (1976) applied the theory of kin selection to predict that monogynous colonies, such as found for *Aphaenogaster rudis* (Crozier 1973), should have a female bias in reproductive biomass investment of 3:1.

Although the "genetic-relatedness theory" has received strong support at the population level, there is still question about the proximate mechanisms governing sex ratios within colonies (Nonacs 1986*a*, *b*, Bourke and Franks 1995). A hypothesis for colony-level variation in reproductive investment in ants is that the resource-allocation function for reproductives is determined by food resources, with food-deprived colonies (which often are smaller and younger) producing fewer females (Brian et al. 1981, Nonacs 1986*a*, *b*).

While a number of studies have suggested a link between resource levels and sex ratios (reviewed in Bourke and Franks [1995]), only two other studies have experimentally addressed this issue. Backus and Herbers (1992) found an increase in total reproductive production but no change in sex ratio, while Deslippe and Savolainen (1995) found a shift toward female bias following food supplementation. Our study is consistent with the "resource-dependent theory." Production of reproductive females is strongly affected by seed availability.

Finally, we observed an effect of resource level on the sex ratio, but worker number did not influence sex ratios. The last observation allows us to test an addi-

tional hypothesis to explain colony-level variability in sex ratio. In this hypothesis, as worker number increases, queen–worker conflict shifts toward worker control with a resultant shift in investment ratio toward females (Herbers 1984). This prediction is inconsistent with our observation that worker number did not explain variation in sex ratios. This finding is consistent with a number of studies showing no correlation between worker number and sex ratio (Nonacs 1986a).

The colony structure of *A. rudis* provides a simple system to test the effect of resource levels on sex ratios. With a single queen fertilized by a single mating (Crozier 1973) this species is free of complications created by colonies having multiple queens and multiple queen matings (Nonacs 1986a, Sundström 1994, Bourke and Franks 1995). Sex ratios were influenced by resource levels, but we do not attempt to extrapolate to ants with more complex social structures.

Literature Cited

Backus, V. L., and J. M. Herbers. 1992. Sexual allocation ratios in forest ants: food limitation does not explain observed patterns. Behavioral Ecology and Sociobiology **30**:425–429.

Beattie, A. J. 1985. The evolutionary ecology of ant–plant mutualisms. Cambridge University Press, Cambridge, UK.

———. 1991. Problems outstanding in ant–plant interaction research. Pages 559–576 *in* C. R. Huxley and D. F. Cutler, editors. Ant–plant interactions. Oxford University Press, Oxford, UK.

Berg, R. Y. 1975. Myrmecochorous plants in Australia and their dispersal by ants. Australian Journal of Botany **62**:714–722.

Bourke, A. F. G., and N. R. Franks. 1995. Social evolution in ants. Princeton University Press, Princeton, New Jersey, USA.

Brew, C. R., D. J. O'Dowd, and I. D. Rae. 1989. Seed dispersal by ants: behavior-releasing compounds in elaiosomes. Oecologia **80**:490–497.

Brian, M. V., R. T. Clarke, and R. M. Jones. 1981. A numerical model of an ant society. Journal of Animal Ecology **50**:387–405.

Crozier, R. H. 1973. Apparent differential selection at an isozyme locus between queens and workers of the ant *Aphaenogaster rudis*. Genetics **73**:313–318.

Culver, D. C., and A. J. Beattie. 1980. The fate of *Viola* seeds dispersed by ants. American Journal of Botany **67**: 710–714.

Cummings, K., and E. R. Heithaus. 1992. Changing response of ants to myrmecochorous seeds. Ohio Journal of Science **92**:59.

Cushman, J. H., and A. J. Beattie. 1991. Mutualisms: assessing the benefits to hosts and visitors. Trends in Ecology and Evolution **6**:193–195.

Cushman, J. H., V. K. Rashbrook, and A. J. Beattie. 1994. Assessing benefits to both participants in a lycaenid–ant association. Ecology **75**:1031–1041.

Davidson, D. W., R. S. Inouye, and J. H. Brown. 1984. Granivory in a desert ecosystem: experimental evidence for indirect facilitation of ants by rodents. Ecology **65**: 1780–1786.

Davidson, D. W., D. A. Samson, and R. S. Inouye. 1985. Granivory in the Chihuahuan Desert: interactions within and between trophic levels. Ecology **66**:486–502.

Deslippe, R. J., and R. Savolainen. 1995. Sex investment in a social insect: the proximate role of food. Ecology **76**:375–382.

Fiedler, K., and C. Saam. 1995. Ants benefit from attending facultatively myrmecophilous Lycaenidae caterpillars: evidence from a survival study. Oecologia **104**: 316–322.

Gunther, R. W., and J. Lanza. 1989. Variation in attractiveness of *Trillium* diaspores to a seed-dispersing ant. American Midland Naturalist **122**:321–328.

Handel, S. N. 1978. The competitive relationship of three woodland sedges and its bearing on the evolution of ant-dispersal of *Carex pedunculata.* Evolution **32**:151–163.

Headley, A. E. 1949. A population study of the ant *Aphaenogaster fulva* ssp. *aquia* Buckley (Hymenoptera, Formicidae). Annals of the Entomological Society of America **42**:265–272.

Herbers, J. M. 1984. Queen–worker conflict and eusocial evolution in a polygynous ant species. Evolution **38**: 631–643.

Heithaus, E. R. 1981. Seed predation by rodents on three ant-dispersed plants. Ecology **62**:136–145.

———. 1986. Seed dispersal mutualism and the population density of *Asarum canadense,* an ant-dispersed plant. Pages 199–210 *in* A. Estrada, and E. A. Fleming, editors. Frugivores and seed dispersal. Dr W. Junk, Dordrecht, The Netherlands.

Hölldobler, B., and E. O. Wilson. 1990. The ants. Belknap Press, Cambridge, Massachusetts, USA.

Liu, S. Y., and E. R. Heithaus. 1993. Carrying response and satiation of ants to seeds and insects. Ohio Journal of Science **93**:46.

Marshall, D. L., A. J. Beattie, and W. E. Bollenbacher. 1979. Evidence for diglycerides as attractants in an ant–seed interaction. Journal of Chemical Ecology **5**:335–344.

Nash, D. R. 1989. Cost–benefit analysis of a mutualism between lycaenid butterflies and ants. Dissertation. University of Oxford, Oxford, UK.

Nonacs, P. 1986a. Sex-ratio determination within colonies of ants. Evolution **40**:199–204.

———. 1986b. Ant reproductive strategies and sex allocation theory. Quarterly Review of Biology. **61**:1–21.

Pudlo, R. J., A. J. Beattie, and D. C. Culver. 1980. Population consequences of changes in an ant–seed mutualism in *Sanguinaria canadensis.* Oecologia **146**:32–37.

Rice, S. D., and E. R. Heithaus. 1993. *Aphaenogaster rudis* response to oleic acid and an evaluation of satiation effect on seed dispersal. Ohio Journal of Science **93**:46.

Smith, B. H., C. E. deRivera, L. Bridgman, and J. J. Woida. 1989. Frequency-dependent seed dispersal by ants of two deciduous forest herbs. Ecology **70**:1645–1648.

Southerland, M. T. 1988. The effects of temperature and food on the growth of laboratory colonies of *Aphaeno-*

gaster rudis Emery (Hymenoptera: Formicidae). Insectes Sociaux **35**:305–309.

StatSoft 1995. STATISTICA for Windows Release 5.0. StatSoft, Tulsa, Oklahoma, USA.

Sundström, L. 1994. Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. Nature **367**: 266–268.

Talbot, M. 1951. Populations and hibernating conditions of the ant *Aphaenogaster* (*Attomyrma*) *rudis* Emery (Hymenoptera: Formicidae). Annals of the Entomological Society of America **44**:302–307.

Trivers, R. L., and H. Hare. 1976. Haplodiploidy and the evolution of the social insects. Science **191**:249–263.

Westoby, M., L. Hughes, and B. L. Rice. 1991. Seed dispersal by ants; comparing infertile with fertile soils. Pages 434–447 *in* C. R. Huxley and D. F. Cutler, editors. Ant–plant interactions. Oxford University Press, Oxford, UK.