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NOTE AND COMMENT

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Colony growth in *Myrmica rubra* with supplementation of myrmecochorous seeds

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Abstract Myrmecochory is an important ant–plant relationship, which presumably has benefits for the reproductive success of ant colonies through the nutritional value of elaiosomes. In a feeding experiment, we provided elaiosomes of *Scilla bifolia* and *Corydalis cava* to colonies of *Myrmica rubra*. Seeds were transported by ant workers to their nests and the elaiosomes were removed afterwards. After 3 months, elaiosome-supplemented colonies contained significantly more worker pupae than control colonies, whereas the number of new female sexuals was reduced. This result is discussed as a possible long-term benefit for *M. rubra* colonies through myrmecochory by colony growth.

Keywords Ant–plant mutualism · Caste determination · Colony growth · Elaiosome · Myrmecochory · *Myrmica rubra*

Introduction

The ratio between workers, and male and female reproductives produced in ant societies is influenced by various parameters, such as the relatedness among colony members and different environmental factors (Hamilton 1964; Trivers and Hare 1976; Nonacs 1986). Food availability after hibernation may affect the number of virgin queens produced in a colony by increasing the weight of female larvae above a certain threshold (Brian 1956). For example, a higher amount of carbohydrates can lead to a more female-biased sex ratio in *Myrmica brevispinosa* (Bono and Herbers 2003).

The red ant *M. rubra* is common in deciduous woodlands, gardens, and grasslands of temperate regions throughout Eurasia. Its colonies consist of several hundred workers and may contain several queens ('facultative polygyny'; see Seppä and Walin 1996). *M. rubra* quickly exploits new food sources by recruiting foragers with scent trails (Cammaerts and Cammaerts 1980). Colonies produce two different types of broods: the slow brood (female sexuals, workers, and males) develops in autumn until larvae are in the third instar and pupates only after hibernation, whereas the rapid brood (only males and workers) develops within the same season without hibernation (Brian 1956, 1977).

Myrmica is involved in mutualistic relationships both with *Maculinea* butterflies (Elmes et al. 1998) and myrmecochorous plants (Sernander 1906; Kjellsson 1985), and *M. rubra* workers use elaiosomes for larval nutrition (Fischer et al. 2005). In an enclosed garden experiment, *M. ruginodis* workers dispersed *Scilla bifolia* seeds up to 322 cm from the mother plants (G. Fokuhl, unpublished data). Myrmecochory may be beneficial to ants in that elaiosome supplementation can lead to an enhanced proportion of female sexuals after hibernation, as shown in *Aphaenogaster rudis* (Morales and Heithaus 1998), or to increased production and weight of larvae, as in *M. ruginodis* (Gammans et al. 2005), probably because elaiosomes contain essential amino and fatty acids. Here, we investigate the influence of elaiosomes on the development of hibernated *M. rubra* larvae. Especially in spring after hibernation, the high availability of elaiosome-bearing seeds of spring geophytes may influence the larval development in ants.

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Methods

In early April 2005, 15 natural colonies of *M. rubra* were collected in a deciduous woodland area at the river Pfatter near Regensburg, Germany (48°56′N, 12°22′E). From every colony, haphazardly selected workers and larvae were taken and separated into two plastic boxes with 30–50 workers each and the same numbers of 'castelabile' third instar larvae, i.e., female larvae that can either develop into workers or into queens (Brian and Jones 1980). The plastic boxes (20 × 20 cm^2) contained a plaster floor and were filled with moss pads, moistened cotton wool, and an empty snail shell (*Helix pomatia*), which was offered as a new nest site. Snail shells have been observed to harbor *Myrmica* nests occasionally in the field. The colonies were set up as pair-wise replicates (elaiosome treatment and control) and kept in a climate chamber at 10°C at night and 18°C during the day. All nests received a drop of honey and three small cockroaches twice a week. With the beginning of fruit-set of native myrmecochorous plants, elaiosome-fed nests additionally received five seeds of *Scilla bifolia* (April–May) or *Corydalis cava* (May–June) twice a week. The elaiosomes of the supplemented plant species contain lipids and sugar, but no proteins (Bresinsky 1963).

Seed and elaiosome removal and the subsequent deposition of used seeds were noted during the study. All ants were frozen on 4 July 2005 before the first young queens had emerged. Individuals of all developmental stages were counted. For the statistical analysis, we used Friedman tests with a posteriori Wilcoxon–Wilcox test, pair-wise *t*-tests, and Wilcoxon tests with Bonferroni correction and sharpened FDR control following Benjamini and Hochberg (2000; see also Verhoeven et al. 2005) using the programs Microsoft Excel 2003 and SPSS for Windows 12.0. To compare investment in females, female sexual pupae were dried at 65°C and weighed separately to the nearest microgram on a Sartorius SC-2 scale.

Results

The offered *Helix* snail shells were readily accepted as nests. Summed over all *M. rubra* colonies, 82% of adult workers (37 ± 10 individuals; mean ± SD) survived the 10 weeks of experimentation, with no difference between treatments ($N_{\text{colonies per treatment}} = 15$; *t*-test; $P > 0.05$). As expected, most larvae developed into female sexuals or workers and only 1/10 of the brood developed into males (4 ± 6 individuals). From the original larvae in elaiosome-fed colonies, 97% survived as larvae, prepupae, pupae, or callow workers, compared to 88% in control colonies. Only the total number of new workers was significantly increased in the elaiosome treatment (28 ± 13 vs. 20 ± 13 in control nests; $N_{\text{colonies per treatment}} = 15$; see Table 1). In contrast, there was a trend for more new queens in the control group, which was not significant after Bonferroni correction but remained significant with sharpened FDR control following Benjamini and Hochberg (2000). Elaiosome-supplemented *M. rubra* colonies laid more new eggs, a finding that was also significant after sharpened Benjamini and Hochberg FDR control, whereas the dry weights of neither new workers nor new queens differed significantly between the feeding treatments.

Discussion

Our data suggest that elaiosome supplementation results in an increase in the number of surviving larvae by increasing the final number of worker pupae, whereas the number of new female sexuals was not significantly affected. This is the first study on myrmecochory suggesting that elaiosome nutrition leads to more colony growth through the development of more workers from the same number of larvae. Moreover, the slight decrease in the number of female sexuals could suggest that workers also prevented development into queens by equably feeding all larvae with elaiosomes.

Our study therefore complements the results of a recent study on *M. ruginodis* (Gammans et al. 2005), in which the number of larvae and larval weight in autumn were found to be increased in elaiosome-supplemented colonies. Increased colony growth provides a second possible benefit ants receive from myrmecochory in addition to previously reported elaiosome-induced shifts in sex allocation (Morales and Heithaus 1998). Bono and Herbers (2003) showed that carbohydrate supplementation resulted in a stronger queen-bias in the female brood

Table 1 Mean ± SD of individual numbers and dry weights in the feeding experiment with elaiosome supplementation and control

	Elaiosome	Control	*P*
N adult workers	35.33 ± 8.93	38.33 ± 11.24	0.278
N new workers	27.67 ± 12.77	20.00 ± 12.63	0.006[ab]
N new queens	7.00 ± 6.70	10.33 ± 9.04	0.027[b]
Dry-weight new workers (mg)	0.705 ± 0.097	0.711 ± 0.104	0.818
Dry-weight new queens (mg)	1.686 ± 0.152	1.723 ± 0.193	0.531
N new eggs and larvae	7.20 ± 8.19	3.47 ± 3.91	0.022[b]

P-values for pair-wise *t*-tests and Wilcoxon test (for new eggs) are given
[a] Value significant after Bonferroni correction
[b] Value significant after sharpened FDR correction

of queenright *M. brevispinosa* colonies, while protein supplementation did not have an effect. By including queenless colonies in their analysis, all treatment effects disappeared. Wardlaw and Elmes (1998) reported that *M. rubra* workers in queenless colonies frequently lay viable, male-destined eggs. In our experiment, the workers in elaiosome-fed nests laid more eggs. This indicates that, after most present larvae had pupated, workers had more resources left, which they then could invest in their own eggs. Depending on their life cycle, ants may generally follow two different strategies of investment under favorable conditions: species with the opportunity of colony budding may favor colony growth, while species with solitarily founding queens may invest more in new queens. The seed usage by *M. rubra* in our experiment also indicates a hygienic behavior: molding seeds were relocated to the farthest corners (garbage dumps; see Gorb et al. 2000) and clean seeds were left in the vicinity of the nest.

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