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PLANT ANIMAL INTERACTIONS

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Ant benefits in a seed dispersal mutualism

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Abstract Myrmecochorous plant seeds have nutrient rich appendages, elaiosomes, which induce some ant species to carry the seeds back to their nest where the elaiosome is consumed and the seed is discarded unharmed. The benefits to plants of dispersal of their seeds in this way have been well documented, but the benefits to the ants from consuming the elaiosomes have rarely been measured and are less clear. Ant benefits from myrmecochory were investigated in a laboratory experiment using the ant *Myrmica ruginodis* and seeds of *Ulex* species. To separate the effects of elaiosome consumption on the development of newly produced larvae versus existing larvae, ten 'Queenright' colonies containing a queen were compared to ten 'Queenless' colonies. Six measures of colony fitness over a complete annual cycle were taken: sexual production, larval weight and number, pupal weight and number, and worker survival. Queenless colonies fed with elaiosomes produced 100.0 ± 29.3 (mean ± SE) of larvae compared to non-elaiosome fed colonies which produced 49.6 ± 19.0; an increase of 102%. Larval weight increased in both Queenright and Queenless colonies. In colonies fed with elaiosomes, larvae weighed 1.02 ± 0.1 mg, but in non-elaiosome fed colonies larvae weighed 0.69 ± 0.1 mg; an increase of 48%. The food supplement provided by *Ulex* elaiosomes was trivial in energetic terms, under the conditions of an ample diet, suggesting that these effects might be due to the presence of essential nutrients. Chemical analysis of *Ulex* elaiosomes showed the presence of four essential fatty acids and four essential sterols for ants.

Keywords Elaiosome · Essential nutrients · Myrmecochory · Myrmica ruginodis · Ulex

Introduction

In recent years, positive species interactions, such as mutualisms, have received increasing attention, particularly because of their potential to maintain complex and diverse communities (Holbrook and Schmitt 2004; Rudgers and Gardener 2003). A mutualism is any interaction between individuals of two species, which confers a benefit (e.g. increased fitness) to both partners (Begon, Harper and Townsend 1996). Ants form diverse mutualisms with many taxa, particularly arthropods and plants (Hölldobler and Wilson 1990).

Myrmecochory, seed dispersal by ants, is described as another ant-plant mutualism (Handel and Beattie 1990). The seeds of myrmecochorous plants bear nutrient-rich appendages called elaiosomes. Certain species of ant are attracted by elaiosomes and will carry the whole diaspore (seed with elaiosome) back to the nest, where they will bite off the elaiosome and feed it to the larvae (Handel and Beattie 1990). The seed is then discarded unharmed and germinates either within the nest or on a waste pile outside the nest (Beattie 1985). Myrmecochory is found in a diverse range of habitats including arid, tropical and temperate regions. The number of plant species that are classified as myrmecochores is estimated to be between 2,000 and 3,000 (Brew et al. 1989; Hughes and Westoby 1992; Ohkawara et al. 1996; Gomez and Espadaler 1998) and four of the seven ant families disperse their seeds (Sheridan et al. 1996).

The benefits of myrmecochory to plants have been investigated more thoroughly than the ant benefits. The seeds are taken back to the ant nest which can prevent detection by predators and confer protection from fire

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(Handel and Beattie 1990; Bond et al. 1991; Ohkawara et al. 1996; Christian 2001). Ant nest soil has a higher nutrient content and increased aeration compared to the surrounding soil which increases germination rates, seedling survival and growth (Horvitz 1981; Beattie and Culver 1983; Zettler et al. 2001). Although seeds are taken only over short distances by ants in comparison to wind or vertebrate dispersal (Wilson 1993), this may be enough to remove the seed away from competition with the parent plant and other competitors (Andersen 1988; Bond et al. 1991; Espadeler and Gómez 1997).

The ant benefits from a myrmecochorous interaction are generally believed to be through the nutrient-rich elaiosome, which has high concentrations of proteins, lipids and carbohydrates compared to other plant tissues, acting simply as an extra food item (Marshall et al. 1979; Brew et al. 1989; Kusmenoglu et al. 1989; Handel and Beattie 1990). Alternatively, it has been suggested that the elaiosome is an extremely high quality food, and might contain essential nutrients which the ants cannot synthesise themselves (Brew et al. 1989). However there has been little study of this hypothesis. Morales and Heithaus (1998) tested the hypothesis that ant colony size or reproductive output would be enhanced by the consumption of seed elaiosomes in a field experiment in Ohio, USA. Sixty five percent of *Aphaenogaster rudis* colonies that were given seeds of *Sangunaria canadesis* produced gynes (female reproductives), whereas only 25% of control colonies did so. Other colony variables such as production of males, workers, larvae and pupae and queen size were not affected. Bono and Heithaus (2002) suggested such effects were due to the feeding of larvae with elaiosomes and found that radio-labelled fats from the elaiosomes were more abundant in gyne larvae than in worker larvae.

These studies provide some evidence of a benefit to ants from consuming elaiosomes, although in a field experiment where alternative food sources are not quantified, it is hard to determine whether the elaiosomes are simply an additional food resource or whether they provide essential nutrients. *Myrmica ruginodis* is truly mutualistic, i.e. workers consume only the elaiosome and dispose of the remaining seed. *M. ruginodis* co-occurs with the myrmecochorous plants dwarf gorse, *Ulex minor*, and European gorse, *U. europaeus*, on heathland habitats in the UK and western Europe (Bullock 2000). Here we used laboratory colonies of *M. ruginodis*, which were fed with more than sufficient amounts of a balanced diet, to determine whether the addition of elaiosomes of the *Ulex* species would affect colony fitness. We tested the following non-exclusive hypotheses. The colonies fed with elaiosomes will: 1). show a higher ratio of gynes to males; 2). have enhanced colony fitness as measured by larval and pupal number and weight, and worker number and survival. We also studied the free carboxylic acid and cholesterol content of *Ulex* elaiosomes with particular reference to compounds that are recognised as insect essential nutrients.

Materials and methodology

Study species

In southern England *U. minor* produces seeds from the end of May for approximately two weeks and *U. europaeus* produces seeds from the middle of June until the end of July (Stokes et al. 2003). The seeds are explosively ejected from pods which contain 1–2 (*U. minor*) or 3–6 (*U. europaeus*) seeds. Ants will then collect the seeds where they have fallen. *U. minor* seed length without the elaiosome is 2.0 ± 0.1 mm (mean ± SE) and the elaiosome length is 1.2 ± 0.1 mm. For *U. europaeus*, the seed is slightly longer at 2.2 ± 0.1 mm and the elaiosome is 1.2 ± 0.01 mm.

Myrmica ruginodis is a red ant 3–6 mm in length which forms polygynous (multiple queen) colonies with between 18–6,560 workers (Wardlaw and Elmes 1996). *Myrmica* species are generally scavengers, with approximately 80–90% of their diet made up of homopterans, hemipterans, dipterans and arachnids, but they are also known to collect plant matter (Brian 1977). In heathland habitats, the nests of *M. ruginodis* are found under small heather (*Calluna*, *Erica*) or gorse (*Ulex*) bushes in vegetation with a large amount of bare ground and low vegetation height, and forage in close proximity to the *Ulex* species (Gammans unpublished data).

Myrmica colonies contain distinct castes. The workers are female and can produce haploid males from unfertilised eggs but only do so if all queens have died. Colony queens are responsible for laying the vast majority of eggs, which are diploid and develop into workers or female reproductives (gynes), and occasionally males (Brian 1977; Smeeton 1982). *Myrmica* species have a split brood rearing cycle on lowland heaths in Britain. Within the ‘rapid’ brood cycle, the eggs are laid by the queen in early spring and mature by the end of August, to become summer workers. Within the ‘over-wintered’ brood cycle, the eggs are laid also in the spring, but mature in August the following year (approximately 15 months later). Both queens and workers can lay eggs within the over-wintered cycle. It is thought that all sexual larvae are over-wintered, however male larvae occasionally develop rapidly within a season (Elmes 1991).

Feeding experiment

In April 2003, ten *M. ruginodis* colonies were collected from five heathland locations in Dorset, SW England. Nests were located by baiting with cake crumbs and then taken to the laboratory where they were extracted from their nest soil. Colony size varied from 200–2,000 workers. Two experimental colonies were derived from each field colony, except where one field colony was not big enough to be split and so another field colony was split into three. Experimental treatments were assigned randomly in relation to field colony identity which was included as a random factor in the analysis. Ten ‘Queenright’ colonies were set up, comprising one queen with 200 workers but no larvae, and ten ‘Queenless’ colonies were set up with 200 workers and 150 larvae. Half of each the Queenless and Queenright colonies were fed with elaiosomes. The Queenright treatment did not contain larvae to investigate how feeding with elaiosomes would affect the sex ratio of larvae derived from newly laid eggs. The Queenless colonies contained larvae to investigate how feeding with elaiosomes would affect the development of pre-existing larvae. If the colony does not contain a queen, the workers will begin very quickly to lay new larvae (all male) in a matter of weeks (Brian and Rigby 1978; Smeeton 1981).

Experimental colonies were set up in the following way. Two hundred workers were randomly selected from their original colony and transferred into an artificial nest. All of the brood from the original ten colonies were pooled and sorted into four size classes; eggs, first, second and third instar larvae. One hundred and fifty brood were then assigned to each Queenless colony with approximately equal numbers of each class (Elmes and Wardlaw 1983). Some of the original colonies contained a small amount of larvae, so larvae were pooled to ensure each colony would have an equal number; this method also reduced any difference among colonies. The larvae added produced an optimum worker to brood ratio of slightly greater than 1:1, which ensures there are enough workers to service the brood (Elmes and Wardlaw 1983). For the Queenright colonies, one queen and 200 workers were also randomly selected and moved into an artificial nest. The artificial nests were commercially available Ant World systems (Interplay UK Ltd), set up with a mixture of 1:3 *Myrmica* nest soil to sand. The nest soil was first sieved to remove any large debris and then sterilised by heating for three days at 100°C.

The experiment was started on the week commencing on the 14th May 2003. Each week, the colonies were fed with an ample standard *Myrmica* diet, of 200 mg white sucrose sugar and 250 mg *Drosophila* larvae (average weight of 200 larvae) (Brian and Abbott 1977; Elmes 1989). Elmes (1989) and Elmes et al. (2004) give detailed descriptions of amounts to use for an ample, adequate and low diet for *Myrmica* ants. An ample diet is more than the ants would need per week to survive and reproduce, and a low diet is associated with starvation. The colonies in this experiment were fed with an ample diet, comprised of 1 mg of sugar per worker and one *Drosophila* larva per worker, per week. The elaiosome treatment for both the Queenright and Queenless colonies consisted of 35 *Ulex* seeds added per week for a period of 10 weeks (*U. minor* the first two weeks and *U. europaeus* thereafter) corresponding with their natural availability in the field. Field observations showed that *M. ruginodis* colonies collect on average five seeds per day (Gammans unpublished data). The nests were kept in incubators at 20°C (9 am–7 pm) and 15°C (7 pm–9 am) and were exposed to natural light conditions (Elmes and Wardlaw 1983; Elmes 1989, Elmes et al. 2004).

All larvae from the rapid brood cycle and from the over-wintered brood cycle of the previous year (2002) would have emerged as adults by the end of August 2003. The nests were examined during the first week of September 2003 and the number of males, workers, the number and weight of pupae and overwintering larvae were assessed. All larvae are in the third instar by the time of overwintering. The nest waste piles were investigated each week for dead workers. The number of dead workers could have been used to estimate the number of workers that emerged during the experiment. However, many dead workers were dismembered and the counts from the waste piles were clearly underestimates, so these data were not used further.

After dismantling the nests, colonies were transferred into clear polyethene boxes, 33×19×20 cm, with fluon around the seal and a lid. Fluon forms a non-stick surface which prevents ants from escaping. The queens were removed from the Queenright colonies so that any gyne-potential larvae would not be suppressed by the ‘queen effect’ and thus become workers (Brian 1977). The incubator temperature was gradually reduced to 6°C in December and was then slowly increased back to the original temperatures used, by the end of April 2004. This is a standard procedure for over-wintering *Myrmica* colonies to ensure they experience a period of nest ‘diapause’ (Elmes and Wardlaw 1983). At the beginning of May 2004, the colonies were reopened and the number of workers, males and gyne pupae, and larvae were counted. The caste and sex of the pupae can be determined under a microscope (Elmes and Wardlaw 1983).

Chemical analyses of elaiosomes

Ripe seed pods of *U. minor* were collected from the end of May 2004, for a period of two weeks and *U. europaeus* seeds were collected from mid June until the end of July. They were stored in paper bags at 4°C until use. For each *Ulex* species elaiosomes were removed from 70 diaspores, slightly macerated, and submerged in 3 ml of chloroform for 24 h at 20°C. The chloroform extracts were then fractionated using solid phase extraction (SPE) following a protocol adapted from Kalunzy et al. (1985). After conditioning LC-NH_2 SPE-columns (Supelco) with hexane and applying the chloroform extract, the column was then washed with 3 ml 2:1 chloroform:isopropanol to remove neutral lipids, and a subsequent wash with 2% acetic acid in diethyl ether to obtain the fatty acid fraction and a wash with 5% ethyl acetate in hexane to obtain cholesterol (*U. minor* only). The fatty acid and sterol fractions were analysed using standard procedures by Mylnefield Research Services, Dundee.

Data analysis

Data from the feeding experiment were analysed by ANOVA using Minitab 13 statistical software (Ryan et al. 2000). Main treatment effects were 'Colony' (i.e. the field colony used as a source of adult ants) as a random factor, 'Queenright/Queenless' (i.e. Queenright versus Queenless treatments) and 'Elaiosome' (i.e. feeding with *Ulex* seeds vs. the negative control). The interaction between Queenright/Queenless and Elaiosome was tested. Residuals in all analyses were normally distributed. Colony was not significant in any analysis.

Results

Two significant effects of seed supplement were found on colony measures made at the end of September 2003 (Table 1). Elaiosome-fed colonies produced heavier overwintering larvae in both Queenright and Queenless colonies, plus Elaiosome 1.0 ± 0.1 mg (mean ± SE) and minus Elaiosome 0.7 ± 0.1 mg ($F_{1,6} = 7.43$, $P < 0.05$). The number of larvae showed a significant interaction between Queenright/Queenless and Elaiosome treatments; elaiosome-fed Queenless workers 100 ± 29.3 mg produced more overwintering larvae than control colonies 49.6 ± 19 mg ($F_{1,7} = 5.21$, $P < 0.05$) while this effect was reversed in the Queenright colonies 79.0 ± 22.4 mg versus 127.0 ± 21.3 mg. There were no seed supplement effects on the number of workers, males or weight of pupae. Queenright colonies produced more pupae 27.0 ± 5.6 than Queenless colonies 2.3 ± 0.7, ($F_{1,7} = 13.64$, $P < 0.001$), which was expected because workers usually can only produce male (over-wintered) brood. This also explains the higher number of males produced in Queenless colonies 11.6 ± 1.9 compared to Queenright 0.2 ± 0.1 ($F_{1,7} = 33.1$, $P < 0.001$).

The production of male and female sexual larvae would not have been affected by the availability of food in the experimental year but by the previous year's food (as they are over-wintered). During the second census we concentrated on effects on sexual production in the colonies. Elaiosome addition had no significant effect on male ($F_{1,16} = 0.22$, $P > 0.05$) or gyne production in April 2004 due to small numbers produced ($F_{1,14} = 0.66$, $P > 0.05$) (Table 1) .

Twenty free carboxylic acids were identified in the elaiosomes of *U. europaeus*. Elaiosomes from *U. minor* contained only 17 free carboxylic acids but these represent a subset of those found in *U. europaeus* (Table 2). The three carboxylic acids in question were rare even in the *U. europaeus* samples and it is possible that they were not detected in *U. minor* due to their low concentration. If so, the carboxylic acid compounds would be identical between the two species, and even the relative abundance of the compounds are very similar (92% correlation coefficient), where oleic-, palmitic-, and linoleic-acid showed the highest abundance (Table 2). Four sterols were identified in the elaiosomes of *U. minor*. The sterols present in order of abundance were, β-sitosterol 0.006 μg/elaiosome, cholesterol 0.002 μg/elaiosome, campesterol 0.002 μg/elaiosome and Δ-5,24-stigmastadienol 0.001 μg/elaiosome.

Discussion

At colony level there was a striking effect of elaiosome addition to the ants' diet. Particularly the Queenless workers produced 102% more larvae and both Queenless workers and Queenright queens laid eggs which developed into larvae 48% heavier than in control colonies. In *Myrmica* species survival of the over-wintering larvae is a function of larval weight at the onset of vernal growth. Elmes and Wardlaw (1981) found that larger over-wintered larvae of *Myrmica* species survived better than medium or smaller sized larvae. This suggests that feeding on elaiosomes will cause colonies to suffer less over-winter mortality contributing to their resilience and/or growth. Bono and Heithaus (2002) found *Sanguinaria canadensis* elaiosome fatty acids in all larvae within *Aphaenogaster rudis* colonies. In laboratory colonies, the *M. ruginodis* workers always took collected elaiosomes into the larval chamber. These results suggest elaiosomes are fed to larvae and so affect their development directly.

Table 1 Responses of *Myrmica ruginodis* colonies to experimental treatments; Queenright (+) versus Queenless (–) and Elaisome (+) versus no Elaisome (–), measures in mean ± SE

Response	+ Elaiosomes	–Elaiosomes	+ Elaiosomes	–Elaiosomes	*F* values		
	Queenless	Queenless	Queenright	Queenright	Elaiosome	Q+ versus Q–	Elaiosome *Q–/Q+
Number of workers (1)	179.4 ± 35.3	147.0 ± 27.2	103.6 ± 18.3	151.4 ± 10.2	0.30^{nsd}	3.49^{nsd}	3.12^{nsd}
Number of males (2)	12.8 ± 2.2	10.4 ± 3.1	0	0.4 ± 0.2	0.22^{nsd}	33.1***	0.54^{nsd}
Number of gynes (2)	2.0 ± 1.8	0.2 ± 0.2	0	0	0.66^{nsd}	1.30^{nsd}	0.66^{nsd}
Number of pupae (1)	2.6 ± 2.0	2.0 ± 1.0	21.2 ± 4.0	32.8 ± 10.5	0.39^{nsd}	13.64***	0.04^{nsd}
Weight of pupae (mg) (1)	3.2 ± 0.1	2.7 ± 0.7	3.2 ± 0.2	3.3 ± 0.2	0.37^{nsd}	0.05^{nsd}	0.47^{nsd}
Number of larvae (1)	100.0 ± 29.3	49.6 ± 19.0	79.0 ± 22.4	127.0 ± 21.3	0.49^{nsd}	4.05^{nsd}	5.21**
Weight of larvae (mg) (1)	1.0 ± 0.1	0.7 ± 0.2	1.1 ± 0.2	0.6 ± 0.1	7.43*	1.08^{nsd}	0.84^{nsd}

Censuses were in (1) September 2003 and (2) April 2004.
Data were analysed by ANOVA, $^{\mathrm{nsd}}P > 0.05$; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$

Table 2 The identity and quantities of free carboxylic acids identified in one elaiosome of *Ulex minor* and *U. europaeus*. Bold fatty acids are essential

Structure	Name	*U. minor* (μg/elaiosome)	*U. europaeus* (μg/elaiosome)
14:0	Myristic	0.006	0.011
14:1	Myristoleic	0.002	0.002
15:0	Pentadecanoic	0.002	0.002
16:0	Palmitic	0.031	0.030
16:1	Hexadecenoic	0.005	0.010
16:2	Hexadecadienoic	0.007	0.004
16:3	Hexadecatrienoic	–	0.009
17:0	Heptadecanoic	0.006	0.002
18:0	Stearic	0.020	0.013
18:2 ($n-6$)	**Oleic**	0.035	0.046
18:3 ($n-6$)	**Linoleic**	0.027	0.016
18:3 ($n-3$)	**γ-Linolenic**	0.004	0.001
20:0	**α-Linolenic**	0.004	0.003
20:1	Icosanoic	0.002	0.002
20:2 ($n-9$)	Icosenoic	0.002	0.001
22:0	Icosadienoic	0.003	0.002
22:1	Docosanoic	0.007	0.009
24:0	Docosenoic	–	0.001
24:1	Teracosanoic	0.005	0.002
24:1	Tetracosenoic	–	0.009

All ant colonies were kept in a controlled environment with a standard temperature, natural light conditions, the same ample standard diet and regular watering. In general, not all of the food was eaten each week, suggesting that the colonies had more than required. Insect larvae and sugar is a common diet used for captive insectivorous ant species, as it provides proteins and fats from the larvae and sucrose from the sugar (Brian 1977). Colonies will eventually begin to decrease in size, depending on its fitness, over time so it is possible that this basic diet may lack in some nutrients. Therefore, it seems that the increases in colony production caused by elaiosome addition were not simply a quantitative effect of increasing the amount of food. Rather elaiosomes increased the quality of the diet substantially, possibly by providing essential nutrients.

Analysis of the fatty acids in the elaiosomes of both *Ulex* species identified 'essential' fatty acids linoleic and linolenic acids, which cannot be synthesised by animals, and are dietary requirements for ants (Hagen et al. 1984; Barbehenn et al. 1999; Heil et al. 2004). These fats are polyunsaturated and are required for normal growth, reproduction and healthy development (Hagen et al. 1984; Barbehenn et al. 1999; Christie 2003). Oleic acid (present in both *Ulex* elaiosomes) also has great importance in the recovery of animals suffering from essential fatty acid deficiency (Christie 2003). Rock (1985) found that supplementary feeding with linoleic and linolenic acids accelerated larval development and increased the body weight of pupae in the tufted apple budmoth, *Platynota idaeusalis*, and Ferkovich et al. (2000) found that an artificial diet with added γ-linoleic acid increased the average pupal weight of females of the hymenoptera ectoparasitod *Diapetimorpha introita*.

A number of sterols were also found in the elaiosomes, including cholesterol and β-sitosterol. Insects are unable to synthesis sterols and must obtain these essential nutrients from their food (Hagen et al. 1984; Behmer et al. 1999). Cholesterol is important for cellular membranes, transporting lipoproteins and is required as a precursor of steroid hormones (Hagen et al. 1984). Cholesterol supports normal development in most insects but is rarely found above the trace amounts in plant material (Dadd 1977). Dadd (1977) found female houseflies must obtain cholesterol either during their adult or larval feeding in order to produce eggs. Behmer et al. (1999) showed that diets containing sitosterol increased survival and egg number, and decreased larval and pupal duration, of the moth *Plutella xylostella* over two generations.

Linoleic acid was also found in *Trillium* elaiosomes by Lanza et al. (1992). *Trillium* and *Ulex* are phylogentically distinct groups in the Monocotyledones and the Dicotyledones respectively. This suggests that elaiosomes contain essential nutrients for ants (including certain fatty acids and sterols) and distinct plant species have evolved elaiosomes with similar chemical composition. Linoleic acid has also been found in Beltian bodies (Heil et al. 2004). However it should be noted that this paper concentrates on fatty acids and sterols; more detailed analysis of plant elaiosomes may reveal other essential nutrients.

In this study, we examined one side of the mutualism between *Ulex* and their seed-dispersing ants. While there is a host of literature focused on benefits to plants of ant-aided seed dispersal, any benefits to the ants have been demonstrated rarely. In myrmecochory as well as other mutualisms involving ants, such as ant-tended insects (aphids or Lycaenid butterflies) or ant-guarding of acacia trees, the ants receive food from their mutualist. Many studies concentrate on the composition of the food received, such as sugars and amino acids in honeydew or Lycaenid secretions (Bluthgen et al. 2004), or fatty acids and amino acids in Beltian bodies found on ant-guarded new world acacias (Heil et al. 2004). Yet, the effect of these nutrients on the fitness of the recipient ant colonies is poorly studied. We know of only one experiment, other than Morales and Heithaus (1998) that has done so. Fiedler and Samm (1995) investigated the effect of Lycaenid butterfly secretion on three ant species, *Lasius niger*, *L. flavus* and *Myrmica rubra*. Mortality was 3–5 times lower in ant workers fed with the secretions. However, Fielder and Samm (1995) conducted their experiments on individual workers which were not in a colony setting. Our experiment was designed to investigate fitness at the colony level because this is the level at which natural selection acts in ants (Heinze et al. 1994).

This study provides evidence that elaiosome-supplemented diets affect colony parameters so as to increase colony fitness, but does not support the hypothesis that colonies shift sex ratios to produce more female reproductives (gynes), as suggested by Morales and Heithaus

(1998). This experiment used a controlled food intake to all colonies, whereas in a field experiment, the food intake cannot be quantified and it is possible that the effect in the Morales and Heithaus (1998) study was simply due to increased quantity of food. Although food resource may affect the level of gyne production, other factors are also important, such as the age of queen and the colony, and the health of the queen and the colony (Hölldobler and Wilson 1990; Brown et al. 2002).

The Queenright colonies fed with elaiosomes did not produce more larvae in their over-wintered brood cycle than the control. In fact there was a strong trend for control colonies to produce more larvae. This may be because the Queenright colonies fed with elaiosomes were able to give their larvae a better quality of food, so that the larvae could enter into the rapid brood cycle. This would produce more workers for summer foraging, which would increase food intake into the colony. In the control colonies, the lower food quality may have slowed larval development, resulting in more being over-wintered.

The offer of food to ants to manipulate them into protection, dispersal, pollination and other beneficial activities is seen in most, if not all, ant-mutualisms. The sugary solutions that ants take from extra-floral nectaries and aphid honeydew contain amino acids and proteins. However, few experiments have analysed components of food bodies such as Beltian bodies, Müllerian bodies and seed elaiosomes, which form the main food reward for mutualistic ants (Fischer et al. 2002; Raine et al. 2004). Some authors have suggested that the Beltian bodies are protein rich but until recently no one had analysed them (Heil et al. 2004). It would be interesting to use similar approaches to those in this experiment to investigate whether nutrient rewards received in mutualisms increase colony fitness in ants in a wide range of mutualisms.

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