# Proximate and ultimate control of sex ratios in *Myrmica brevispinosa* colonies

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The literature on sex ratio evolution in ant colonies is dominated by inclusive fitness arguments. In general, genetic theory makes good predictions about sexual investment in ant populations, but understanding colony-level variance in sex investment ratios has proven more difficult. Recently, however, more studies have addressed ecological factors that influence colony-level sex investment ratios. Food availability, in particular, has been manipulated because larval nutrition influences female caste determination, thus implying that resource availability should be of critical importance for colony sex investment ratios. However, results from food supplementation experiments are equivocal, and it is clear that ant colony response to food supplementation is dependent on the ecological background of the population. We presented field colonies of the ant *Myrmica brevispinosa* with two food types (proteins and carbohydrates), and assessed their relative impact on colony-level sex investment ratios. We show that colonies receiving carbohydrate enhancement invested in more female sexuals and produced more female-biased sex allocation ratios than protein-fed or control colonies. Thus, our study is the first, to our knowledge, to demonstrate that sex ratios in social insect colonies might be sensitive to resource quality. Male investment was not influenced by food treatment, but was positively correlated with colony size. Therefore, the shift in sex ratios in our study must have been mediated through nutritional influences on female caste determination rather than male brood elimination. We also used our data to evaluate evidence for sex ratio compensation by queen-right colonies in response to male production by workers from orphaned colonies.

**Keywords:** food supplementation; sex investment; sex ratio compensation; kin selection; *Myrmica*

## 1. INTRODUCTION

Hamilton's (1964) theory of kin selection successfully explains the evolution of social behaviour in Hymenoptera, and has also served as a framework to study population-level sex investment ratios (Trivers & Hare 1976). In general, genetic theory makes good predictions about sex investment ratios produced by populations (Bourke & Franks 1995; Griffin & West 2002). However, there is considerable variation in the sex investment ratios among colonies within a population that can reflect relatedness structure, queen–worker conflict and worker reproduction, *inter alia* (Crozier & Pamilo 1996). Yet the proximate mechanisms used by colonies to adjust sex investment ratios are poorly understood.

In hymenopteran societies, queens can control the primary sex ratio by varying the proportion of fertilized and unfertilized eggs laid; workers can influence the secondary sex investment ratio either by cannibalizing male larvae or by directing female larvae to develop into gynes (virgin queens) rather than into workers. Female caste determination is influenced by several factors including larval nutrition, with larvae receiving more food developing into gynes and starved larvae becoming workers (Wheeler 1986; Hölldobler & Wilson 1990). It is generally believed that the fate of female larvae is not fixed genetically, though recent evidence suggests that a genetic component is likely, at least in some species (Julian *et al.* 2002; Volny & Gordon 2002). Worker manipulation of sex ratios

occurs via male cannibalism in *Solenopsis invicta*, *Linepithema humile* and *Formica exsecta* (Aron *et al.* 1995; Passera & Aron 1996; Sundstrom *et al.* 1996) but by selective feeding in *Leptothorax acervorum* (Hammond *et al.* 2002). The ability to bias sex ratios has produced a rich literature on evolutionary strategies to be employed by queens versus workers (Crozier & Pamilo 1996; Queller & Strassmann 1998; Chapuisat & Keller 1999; Reuter & Keller 2001).

The literature on sex ratio evolution in social Hymenoptera is dominated by inclusive fitness arguments. Only recently, however, have studies addressed ecological factors that might influence colony-level sex investment ratios (Backus & Herbers 1992; Deslippe & Savolainen 1995; Herbers & Banschbach 1998, 1999; Morales & Heithaus 1998; Aron *et al.* 2001). Food availability, in particular, has been studied because it is easy to manipulate and its link to female caste development implies that it should be of critical importance to the proximate control of sex ratios (Hölldobler & Wilson 1990; Wheeler 1986). Thus, we expect colonies with more food resources to produce more female-biased sex investment ratios relative to food-stressed colonies. Similarly, colonies experiencing food stress should invest in males and workers that are typically smaller and less costly to produce than gynes (Nonacs 1986; Rosenheim *et al.* 1996).

Predictions from this resource-based model are straightforward, but experimental manipulations of food availability have produced contradictory results. Morales & Heithaus (1998) found that providing elaiosomes (a lipid-rich food gained from an ant–plant mutualism) to *Aphaenogaster rudis* colonies increased gyne investment but not

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male or worker investment. Deslippe & Savolainen (1995) also observed a shift toward gyne investment following food supplementation, but they did not report data on production of males or workers. Aron *et al.* (2001) reported an increase in the production of males and gynes following food supplementation, but they argued that these shifts were best explained by lower rates of fracticide in well-fed colonies, and not simply by nutritional influences on female caste determination. Herbers & Banschbach (1998) observed a shift toward gyne production in a study of *Leptothorax longispinosus*, but this contrasted with a shift toward male production in a previous, identical experiment on the same population. Furthermore, manipulations of food availability in two different populations of *Myrmica punctiventris* produced strong shifts toward male production and decreased nest-mate relatedness in one population, but produced no changes in the other population (DeHeer *et al.* 2001; Herbers & Banschbach 1999). This welter of results does not allow us to generalize about how ant colonies respond to resource enhancement.

Clearly, ecological context is a key determinant of ant colony response to food supplementation. The ecological background of a population can vary from year to year, and may differ even for species in the same community (DeHeer *et al.* 2001; Herbers & Banschbach 1999). Moreover, sexual investment strategies among colonies within a population can vary in response to other factors such as queen turnover and worker reproduction. We recognize the importance of ecological context, but we suggest that the confusion of results from previous investigations reflects another factor: investigators have confounded food quality with food quantity.

Differential nutrient availability could have strong consequences for sex investment in ant colonies, particularly if certain resources favour the production of one sex over the other (Grafen 1986; Boomsma 1993). In ant colonies, proteins are generally fed to larvae and are essential for the production of brood in some ant species (Brian 1956, 1973; Markin 1970; Porter 1989; Sorensen & Vinson 1981). Therefore, we expect protein supplementation to influence total sexual investment. By contrast, investment ratios would be changed only if proteins have differing effects on male and female production. Protein supplementation, potentially, could lead to increased gyne investment, mediated through influences on female caste determination. However, this could be balanced by reduced levels of male cannibalism in protein-supplemented colonies leading to increased male investment. Carbohydrates are the main source of energy for workers in most ant colonies (Brian 1956, 1973; Porter 1989), and also serve as a critical source for fat synthesis in gynes during the period between eclosion and nuptial flights (Passera & Keller 1990; Passera *et al.* 1990). Thus, we expect carbohydrate supplementation to increase adult gyne weight. Boomsma (1993) suggested that gynes are most easily produced when ample proteins are available early in larval development and carbohydrates are available after gynes have eclosed. Therefore, we might expect these nutrient types to act synergistically with higher gyne investment in colonies given both food types. Most food supplementation studies have used proteins (Aron *et al.* 2001) or a combination of proteins and carbohydrates (Backus & Herbers 1992; Deslippe & Savolainen 1995;

Herbers & Banschbach 1998, 1999). Our interest, then, was to separate out the effects of different food types for sex investment.

In this study, we manipulated both food quantity and food quality for the ant, *M. brevispinosa*. We relied on extensive information on *Myrmica* biology in the literature. Many species are polygynous, with the average queen number per nest ranging from less than 1 to greater than 15 (Elmes 1991). Queens produce male and female eggs throughout the active period, but female sexuals require two seasons to develop. Large larvae that overwinter maintain the capacity to develop into workers or gynes, depending on environmental rearing conditions in early spring (Elmes 1991). Workers are continually produced throughout spring and summer, but late season larvae may enter winter diapause. Thus, we designed an experiment to take advantage of this information and to separate out food quality from quantity. By presenting different food types (proteins and carbohydrates) to colonies, and assessing their relative impact on colony-level sex investment ratios, we show that different components of sex investment are sensitive to these two nutrient types.

## 2. MATERIAL AND METHODS

We found colonies of *M. brevispinosa* nesting under rocks in a spruce/fir forest located 26 km northwest of Fort Collins, CO, USA. The study site (elevation 2000 m) has steep rocky slopes where *M. brevispinosa* colonies excavate shallow nests under rocks. These ants are scavengers, and we observed foragers returning to nests with insect prey. Although nest chambers under rocks are often extensive and extend beyond the rock, colonies do not appear to occupy multiple nesting sites.

### (a) *Food supplementation experiment*

Ninety-six colonies were randomly assigned to four food treatment groups (24 per treatment): protein supplementation, carbohydrate supplementation, protein and carbohydrate supplementation, and no supplementation. Supplemented nests received 4.5 mg of tuna and/or access to a 20% sucrose feeder twice weekly (Tim Judd, personal communication). We positioned the feeding stations very close to the target nest's entrance to ensure that its foragers would find the resource and that other colonies would not. Indeed, foragers quickly found and recruited nest-mates to both types of food source. Colonies receiving both protein and carbohydrate supplementation were given access to only one food type per feeding session to prevent selective foraging for one food type. Thus, colonies in this treatment group received half of the amount of protein and carbohydrates given to colonies in the pure treatment regimes. We checked feeders periodically to ensure that only target colonies were using them.

We carried out food supplementation over the course of two active seasons running from 29 May–12 November 2000 and 6 May 2001 until nests were collected at the end of July 2001. By the end of the first field season a disproportionate number of colonies assigned to the protein treatment group appeared to have moved, as they no longer visited food sources. Therefore, to boost sample size, we found seven new colonies and assigned them to the protein treatment group prior to the second field season.

## (b) *Colony collection and demographic analysis*

Adult alates began to emerge in late July, and we collected colonies over a 3-day period (29–31 July 2001). We used hand trowels to shovel ants and soil into large plastic bags. Nest chambers were often extensive so care was taken to ensure that colonies were collected fully. We continued digging to a depth of several centimetres below where ants were sighted and we checked soil under nearby rocks for any sign of ants or brood. We feel confident that we collected all of the ants residing in one nest area.

We transported colonies to the laboratory where they were counted and sorted by caste. We dried up to 10 ants of each caste (except worker pupae) in an oven at 80 °C for at least 3 days. These were then weighed individually to the nearest 0.01 mg. Dry weight data were used to calculate the energetic cost ratio (ECR; Boomsma 1989), which was then used to compute sex-allocation ratios $(P_m) = [(\text{number of males})/(\text{number of males} + \text{ECR} \times \text{number females})]$. These ratios ranged from 0 (all female broods) to 1 (all male broods), and were arcsine-transformed for statistical analysis.

## (c) *Data analysis*

We used regression analysis to assess the effects of our food treatments on demographic and reproductive parameters. Data were first analysed using a full model including the two food types (carbohydrate and protein) with their three levels (none, half and full), and a carbohydrate–protein interaction term. We included worker number as a covariate in the analysis of reproductive parameters. All variables were log-transformed with the exception of $P_m$ (arcsine) and worker number (square root). In all cases the interaction term was not significant and was therefore eliminated from the model. Qualitative treatment effects were indicated when the effect of one or both food types was significant in the model. Protein and carbohydrate supplementation were not simultaneously significant in any of our models, which allowed us to further examine our data for quantitative effects; we used a simple regression across treatments, which coded as none, half (for colonies fed alternately with carbohydrate and protein) and full supplementations (for colonies fed only one type of nutrient).

We also tested for a general effect of food supplementation (pooling all supplemented colonies) with one-way ANOVAs. We used the *G*-test of independence to determine whether the likelihood of investing in males or gynes was affected by our food treatments or by the presence of a queen. There were no differences for any parameter between colonies receiving protein treatment for one season and those receiving the treatment for two seasons (ANOVAs, $p > 0.05$ for all tests), so all colonies receiving protein were combined for subsequent analyses.

## 3. RESULTS

## (a) *Demographic analysis*

Colonies contained $445 \pm 50$ (mean ± s.e.; $n = 56$) workers, and only one was polygynous. The majority (78%), though, were queenless and presumably orphaned. To determine how long queenless colonies had been orphaned, we looked for female (diploid) pupae. The presence of worker and/or gyne pupae in a queenless colony implied a recent orphaning. Only 9 of the 44 queenless colonies had no female pupae, indicating that most orphaned colonies were still able to produce male and female broods. Out of the nine colonies that failed to

produce females, seven invested in males, presumably by worker reproduction. These data suggest that a high proportion of colonies lose their queens every year, and some of them persist for up to 2 years as congregations of workers that lay haploid eggs. Food type had no effect on the distribution of queens among nests (*G*-test, $p > 0.05$) or the number of queens per nest (regression, $p = 0.276$ for protein; $p = 0.960$ for carbohydrates). Moreover, food supplementation (regardless of food type) did not affect the likelihood of a colony containing a queen (*G*-test, $p > 0.05$). Food type had no effect on the number of workers per colony (regression $p = 0.617$ for protein; $p = 0.852$ for carbohydrates), and there was no difference in worker number between fed and unfed colonies (one-way ANOVA, $p = 0.932$). Furthermore, there were no differences in colony size among queenright colonies, colonies recently orphaned and colonies orphaned for longer periods of time.

Means and confidence intervals for weights are presented in table 1. Workers were smaller in colonies receiving protein supplementation (to be fully explored below) but we found no evidence that weights of reproductive pupae or adults were affected by food treatment. However, our failure to detect differences among treatments might be due to a lack of power from small sample sizes, as implied by the wide confidence intervals (table 1).

Sexual investment comparisons were complicated by the fact that sexual development times were not consistent across colonies, meaning that some colonies contained adult sexuals, while others contained sexual pupae. Male pupae were heavier than male adults (*t*-test, $p = 0.041$), so comparisons across nests would be problematic. Moreover, only a small number of colonies contained both sexual pupae and adults, so it was not possible to reliably predict adult weights with pupal weights. However, since average sexual adult weights did not differ by food treatment, we calculated sexual investment as the product of the number of male or female sexuals reared by a colony and the population-wide average weight of adults in that caste.

## (b) *Analysis of reproductive parameters*

Out of the 56 colonies used in this study, 41 invested in sexual reproductives, 13 reared only workers and two colonies did not produce brood of any kind. Most of the colonies that reared sexuals specialized in male production (29 total), while five colonies specialized in gyne production and seven colonies produced mixed sexual broods. Because queen presence/absence did not influence the likelihood of colonies investing in gynes or males (*G*-tests, $p > 0.05$), we considered colonies capable of producing both gynes and males if they reared any diploid (gyne or worker) brood at all; colonies not fitting that description were considered orphans. Seven colonies in the population were orphaned but still produced a substantial number of males (268, or 25% of the population total), prompting us to analyse our data for evidence of sex ratio compensation (Taylor 1981; Crozier & Pamilo 1996). Sex ratio compensation is expected in populations when orphaned colonies produce a preponderance of males, such that queenright colonies benefit by overproducing females. Consequently, queenright colonies produce a more female-biased sex ratio than otherwise expected and the populational sex ratio is more male-biased than

Table 1. Back-transformed means and confidence intervals (given in brackets) for dry weights (mg) of individuals reared by *M. brevispinosa* colonies.
(Colonies received different food treatments over the course of two active seasons.)

|  | carbohydrate | protein | mixed | control |
|---|---|---|---|---|
| workers | 0.53 [0.45, 0.62] | 0.43 [0.38, 0.49] | 0.48 [0.42, 0.54] | 0.51 [0.45, 0.58] |
| male pupae | 0.53 [0.36, 0.77] | 0.50 [0.39, 0.66] | 0.48 [0.36, 0.66] | 0.57 [0.43, 0.75] |
| male adults | 0.42 [0.33, 0.54] | 0.42 [0.33, 0.53] | 0.46 [0.35, 0.63] | 0.43 [0.30, 0.62] |
| gyne pupae | 3.24 [0.95, 11.01] | —[a] | 2.17 [1.07, 4.40] | 2.52 [0.74, 8.57] |
| gyne adults | 2.88 [1.77, 4.69] | 2.69 [1.84, 4.10] | 3.23 [1.98, 5.26] | 4.10 [2.05, 8.19] |

[a] Missing data.

Table 2. Values for $P_m$ (sex allocation ratio) predicted by a sex ratio compensation model (Crozier & Pamilo 1996) compared with mean values and pooled population values obtained from a *M. brevispinosa* population.
(Means are back-transformed.)

|  | mean predicted under queen control | mean predicted under worker control | actual mean values [95% confidence interval] | pooled $P_m$ for population |
|---|---|---|---|---|
| mean $P_m$ for queenright colonies | 0.46 | 0.14 | 0.88 [0.75, 0.97] | 0.49 |
| mean $P_m$ for entire population | 0.53 | 0.28 | 0.92 [0.82, 0.98] | 0.57 |

Table 3. Back-transformed means and confidence intervals (given in brackets) for reproductive parameters in *M. brevispinosa* colonies receiving food supplementation over the course of two active seasons.

|  | carbohydrate | protein | mixed | control | all fed colonies |
|---|---|---|---|---|---|
| with orphans: |  |  |  |  |  |
| total | 17.76 [4.69, 60.89] | 8.42 [3.26, 19.87] | 15.09 [5.82, 36.96] | 6.12 [2.01, 15.76] | 12.15 [6.86, 20.99] |
| male investement | 6.26 [1.64, 18.93] | 6.06 [2.60, 12.83] | 4.49 [1.65, 10.35] | 3.41 [1.13, 8.12] | 5.47 [3.21, 8.94] |
| gyne investment | 4.88 [0.38, 24.10] | 0.86 [−0.29, 3.89] | 3.41 [0.55, 11.53] | 0.78 [−0.37, 4.06] | 2.21 [0.71, 5.05] |
| $P_m$ | 0.85 [0.46, 1.00] | 0.97 [0.82, 0.99] | 0.80 [0.51, 0.97] | 0.96 [0.79, 0.99] | 0.89 [0.77, 0.98] |
| without orphans: |  |  |  |  |  |
| gyne investment | 40.16 [3.71, 358.63] | 0.97 [−0.27, 4.35] | 4.00 [0.67, 13.95] | 0.94 [−0.39, 5.18] | 2.83 [0.84, 6.98] |
| $P_m$ | 0.38 [−0.44, 0.95] | 0.96 [0.78, 0.99] | 0.77 [0.43, 0.96] | 0.95 [0.73, 0.99] | 0.86 [0.69, 0.98] |

would be expected without orphaned colonies (Crozier & Pamilo 1996, p. 151).

For queen control and 25% of all males produced by orphaned colonies, the mean male allocation ratio in queenright colonies should be 0.462, and that over the entire population (including orphans) should be 0.533. For worker control, the expectation for queenright colonies is 0.139, and for the entire population is 0.276 (table 2). In our population, the mean male allocation ratio for queenright colonies was 0.884 and the average value for the entire population was 0.920, both more male-biased than predicted under queen or worker control. We note the large discrepancy for queenright colonies between mean male allocation ratio (0.884) and pooled male allocation ratio (0.493), which indicates that most queenright colonies produced only a few males each, but some produced a large number of gynes.

Means and confidence intervals for reproductive parameters are presented in table 3. Food type and food supplementation (regardless of food type) did not affect the likelihood of colonies investing in reproduction, or of

producing either male or female reproductives (*G*-tests, $p > 0.05$ for all tests). Moreover, we found no evidence that food supplementation (regardless of food type) had an effect on total sexual investment, gyne production, male production or sex-allocation ratio ($P_m$) (one-way ANCOVA, $p > 0.05$ for all tests). However, we note again that low power, as evidenced by the wide confidence intervals, may have limited our ability to detect differences among treatments (table 3).

Regressions were performed with four response variables: total sexual investment, male investment, gyne investment and $P_m$ (sex ratio). Since orphaned colonies were not capable of producing female sexuals, we also analysed gyne investment and $P_m$ with orphaned colonies excluded. When orphaned colonies were included in the analysis we did not detect an effect of either food type on total sexual investment, male investment or $P_m$. The data in table 3 strongly suggest that colonies receiving carbohydrate enhancement had increased total sexual investment and gyne investment, but again the wide confidence intervals imply low power for these comparisons.
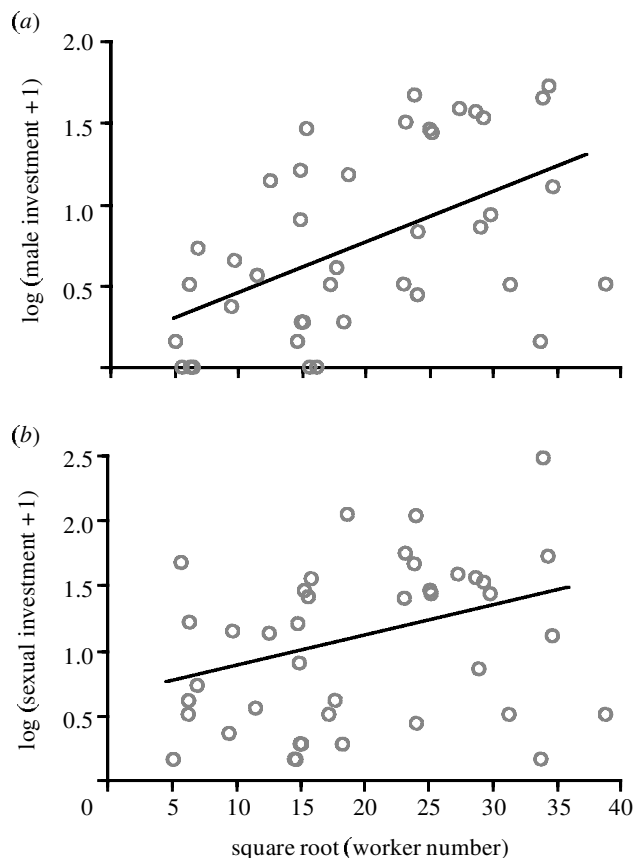
(a)



(b)



Figure 1. (*a*) Male production as a function of colony size in colonies of the ant *M. brevispinosa*. On average, large colonies invested more biomass in males than small colonies (regression, $p = 0.0005$ for worker number). (*b*) Total sexual investment as a function of colony size in colonies of the ant *M. brevispinosa*. There was a trend for larger colonies to invest more biomass in sexual individuals than small colonies (regression, $p = 0.103$ for worker number).

One clear result was that when we pooled our data across treatments, we found that colony size was positively correlated with male investment ($p = 0.0005$; figure 1*a*), an effect that drove a weaker, but similar, relationship between colony size and total investment in sexuals ($p = 0.103$; figure 1*b*) Thus, larger colonies put more energy into male reproduction, thereby increasing total sexual reproduction.

When orphaned colonies were excluded from the analysis, carbohydrate supplementation clearly influenced gyne investment, as carbohydrate-supplemented colonies produced more gynes (regression, $p = 0.009$ for carbohydrates; table 4). Moreover, the quantitative effect of this nutrient was additive, as the level of gyne investment for the mixed treatment group was intermediate to the other treatments (table 4). Colonies that received carbohydrate enhancement also produced lower sex-allocation ratios, and the quantitative effect of nutrient enhancement was additive as well (regression, $p = 0.020$ for carbohydrates; table 4). Figure 2 shows sex-allocation ratios for all colonies in the population, sorted by carbohydrate treatment. Out of the 29 colonies with a sex-allocation ratio of 1, seven were orphaned and therefore unable to produce female sexuals (three from full treatment, one from half treatment and three from no carbohydrate treatment). The only signifi-
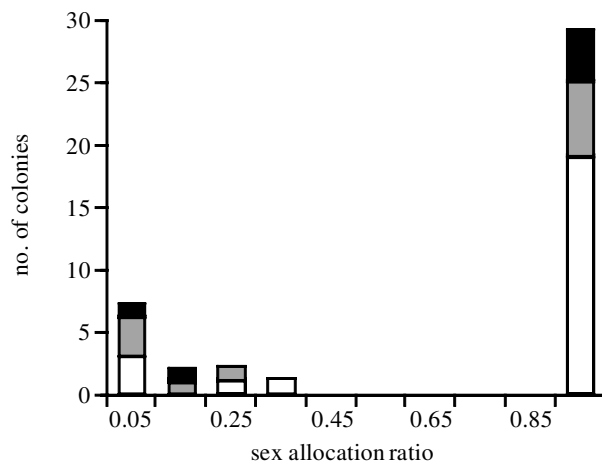


Figure 2. Sex allocation ratios for *M. brevispinosa* colonies receiving three levels of carbohydrate supplementation. When orphans were excluded from the analysis, colonies receiving carbohydrates had more female-biased sex allocation ratios (regression, $p = 0.009$). Seven colonies with a sex allocation ratio = 1 were orphans (three from full treatment (black), one from half treatment (grey) and three from no carbohydrate treatment (white)).

cant effect of protein supplementation was to produce smaller workers, an effect that was also additive (table 4). That is, we found quantitative effects of carbohydrates on sexual reproduction, but of proteins on worker weight.

## 4. DISCUSSION

Other studies have shown that food supplementation can affect sex investment ratios produced by ant colonies (Backus & Herbers 1992; Deslippe & Savolainen 1995; Herbers & Banschbach 1998, 1999; Morales & Heithaus 1998), but our study is the first, to our knowledge, to demonstrate that colony sex investment ratios are sensitive to the type of food used for supplementation. When we examined the responses of colonies that were supplemented with carbohydrates and/or proteins versus controls, only negative results were obtained. By contrast, when we separated out the effects of carbohydrate and protein supplementation, important patterns emerged; overall, the protein supplementation had no impact on sex allocation in our ant colonies, but carbohydrate supplementation produced a positive linear response in gyne production. Thus, it is important to distinguish food quantity from food quality in order to understand proximate mechanisms underlying conflict over sex allocation.

Moreover, our approach provides an insight into the mechanisms used by *M. brevispinosa* colonies to adjust sex investment ratios. Since gyne production but not male production was influenced by food type, manipulation of sex investment ratios must have occurred by differential determination of female larvae rather than elimination of male brood. Our results therefore corroborate Hammond *et al.* (2002), who showed that this mechanism is important for the ant *Leptothorax acervorum*. The resource-based model (Rosenheim *et al.* 1996) predicts that increased gyne investment should lead to a decrease in worker production. Unfortunately, we were unable to collect reliable data on the number of new workers pro-

Table 4. Back-transformed means and confidence intervals (given in brackets) for the response variables that were significantly influenced by food type.
(Data for log worker weight and gyne investment are given in milligrams. $p$ values were obtained by regression analysis.)

|  | significant factor | full | half | none | $p$ value |
|---|---|---|---|---|---|
| log worker weight | protein | 0.43 [0.38, 0.49] | 0.48 [0.42, 0.54] | 0.52 [0.47, 0.57] | 0.024 |
| gyne investment | carbohydrate | 40.14 [3.90, 344.48] | 4.00 [0.71, 13.66] | 0.96 [−0.07, 3.13] | 0.009 |
| $P_m$ | carbohydrate | 0.38 [−0.43, 0.94] | 0.77 [0.44, 0.96] | 0.96 [0.84, 1.00] | 0.020 |

duced by *M. brevispinosa* colonies, so we do not know whether more gynes were produced at the expense of new workers, as predicted by the resource-based model (Rosenheim *et al.* 1996). Yet, we saw no corroborating evidence of that effect when we compared colony size: worker number did not vary between treatments, implying that differentials in gyne production that we observed did not occur at the expense of new workers, but were in addition to worker production. Thus, it is possible that the increased gyne investment could reflect a higher diploid egg-laying rate by queens in carbohydrate-supplemented colonies.

We did not expect to see an effect of carbohydrate enhancement on reproductive parameters, since carbohydrates are generally retained by ant workers (Markin 1970; Sorensen & Vinson 1981) and are accumulated by sexuals after pupal emergence (Passera & Keller 1990; Passera *et al.* 1990). Yet our data showed no differences in alate size between carbohydrate treatments. Rather, our results imply that gyne–worker development is influenced directly by carbohydrates, an effect that must occur during larval development. Alternatively, carbohydrate enhancement may have increased female investment indirectly: since carbohydrates serve as fuel for workers (Brian 1956, 1973; Porter 1989), our treatment may have released workers from food stress, allowing them to provide more prey items to developing larvae. Our study species is a generalist scavenger, for which carbohydrates may be more limiting than protein. Certainly laboratory colonies of *Leptothorax curvispinosus* and *Solenopsis invicta*, fed sugar water and insects, grew at faster rates than colonies fed only insects (Evans & Pierce 1995; Porter 1989). Repeating our experiments with a species that tends homopterans and thus are not carbohydrate limited would allow us to test this alternative.

We were also able to use our data to examine ultimate control over sex ratios in this species. Specifically, the high rate of orphaning in this population allowed us to test for sex ratio compensation by queenright colonies (Crozier & Pamilo 1996; Taylor 1981). Our mean male allocation ratios for queenright colonies and for the population are much more male biased than the optimal values derived from a model of sex ratio compensation. However, this model assumes mongynous colony structure and no worker reproduction in queenright colonies, assumptions that may have been violated for this population. While monogyny seems to predominate in our study system, queen supercedure may be important; if unrelated queens take over orphaned colonies then queenright colonies comprise a mix of family groups (Evans 1996). Similarly, we do not know whether workers of this species produce males in queenright colonies, as has been reported for

other *Myrmica* species (Evans 1998; Herbers & Mouser 1998). Given these complications, we cannot predict with precision the optimal ratio for queenright colonies in our population.

A comparison of mean sex allocation ratios to the pooled population sex allocation ratio does, however, imply sex ratio compensation from some queenright colonies. Despite the ambiguities of relatedness and worker laying, an unbiased allocation ratio over the entire population coupled with a highly male-biased mean male allocation ratio means that colonies investing in gynes produced a large number of them, just as predicted by the sex ratio compensation model. This effect was particularly evident for colonies receiving carbohydrate supplementation (figure 2), implying that the availability of this nutrient might constrain a queenright colony's ability to adjust its sex ratio. With high queen mortality and most colonies producing only males, colonies with the necessary resources (carbohydrates for example) gain more fitness by producing a large number of gynes. Thus, support for the sex ratio compensation hypothesis is mixed, and the possibility deserves further study.

Colony size strongly influenced the production of males (figure 1) but not gynes. A positive relationship between worker number and male production is inconsistent with a resource-based model if small colonies have low foraging success relative to larger colonies. Under those conditions, we expect small colonies to specialize in producing less costly males. It is possible that the positive relationship reflects enhanced worker reproduction in larger colonies but the effect would have to be much stronger than heretofore observed for any ant colony (Crozier & Pamilo 1996). The relationship could also be explained by the effect of local resource competition (Bourke & Franks 1995; Crozier & Pamilo 1996), but the extent to which females compete for resources in this population is unknown. Rather, we suspect that larger and therefore older colonies have experienced more queen turnover. If true, then larger colonies have lower levels of within-colony relatedness than smaller colonies, leading to increased male investment as predicted by genetic models of sexual investment in ants (Trivers & Hare 1976). Clearly, detailed genetic work to uncover family structure is needed to differentiate these possibilities.

Our study provides important insight not only into factors that influence ratios in *M. brevispinosa* colonies, but also into the mechanisms used by colonies to adjust them. Ours is the first study, to our knowledge, to demonstrate that food quality is at least as important as food quantity. Colony nutritional status therefore emerges as an important additional variable for interpreting sex allocation data.

Elmes (1991) suggested that the production of gynes and males in *Myrmica* colonies are controlled through separate mechanisms. Our data lend support to his hypothesis: gyne production was sensitive to nutritional status but male production responded to colony size. Investigations of sex allocation in social insects must therefore disentangle factors affecting investment in gynes versus males. Furthermore, future work on proximate mechanisms of control within those colonies must be careful to ensure that food quality is not confounded with food quantity.

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