Role of resource availability on sex, caste and reproductive allocation ratios in the Argentine ant *Linepithema humile*

S. Aron[1], L. Keller[2] and L. Passera [3]

[1] Free University of Brussels, Unit of Animal Communities, Brussels 1050, Belgium

[2] University of Lausanne, Institute of Ecology, Lausanne 1015, Switzerland

[3] University Paul-Sabatier, Toulouse Cedex 31062, France

Correspondance

S. Aron

Free University of Brussels (U.L.B.)

Department of Animal Biology

Unit of Animal Communities - CP 160/12

av. F.D. Roosevelt, 50

B - 1050 Brussels, Belgium

Tel: +32-(0)2-650-3037

Fax: +32-(0)2-650-2445

e-mail: **saron@ulb.ac.be**

Summary

Resource availability has long been recognized to influence reproductive decisions in eusocial Hymenoptera. We analyzed how protein availability affects the number and the weight of worker, male and queen pupae, as well as its effect on sex, caste and reproductive allocation ratios, in the Argentine ant *Linepithema humile* Mayr. Colonies were maintained on diets with three levels of access to proteins: no protein, intermediate and high levels of proteins.

1. There was no significant difference between the intermediate and high levels of protein in the number of queen, male and worker pupae produced. Similarly, the intermediate versus high levels of protein treatments did not differ with regard to the weight of pupae nor the relative investments between the three castes. This suggests that, in *L. humile*, there could be a threshold over which additional amounts of protein have no more effect on reproductive allocation or the size of the individuals produced.

2. The main effect of protein supplementation was to increase the number of sexual pupae (queens and males) produced. By contrast, the number of workers produced remained unaffected by the level of proteins. The higher investment in sexuals in nests supplemented with proteins resulted in a significantly higher proportion of queen pupae among females, as well as higher proportion of males among all individuals produced in these nests. However, the proportion of males among sexuals (numerical sex ratio) was not significantly different between supplemented and unsupplemented nests.

3. Workers reacted to a higher protein diet not only by rearing more sexuals, but also by producing larger individuals of the three castes.

4. The proportional investment allocated to queens among females was higher in the protein-supplemented treatments. Protein-supplemented nests also invested proportionally more into sexual production than unsupplemented nests. However, whereas increased sexual production is

generally associated with a more female-biased sex investment ratio in ants, sex allocation ratio was not significantly different between supplemented and unsupplemented nests.

5. Overall, our results support the view that protein availability influences the proportion of brood that is culled, with workers eliminating a significant proportion of both the male and female sexual brood in unsupplemented nests.

6. The increasing amount of experimental data showing that brood culling is an important part of the biology of ants and other social insects emphasis the need to combine kin selection and life-history approaches to study the outcome of queen-worker conflicts on the dynamics of colony growth and sex allocation.

Key words: allocation ratio, resource availability, Formicidae, *Linepithema humile*

Introduction

Sex ratio studies in eusocial Hymenoptera (ants, bees and wasps) have played a central role in testing key predictions of sex ratio (Fisher 1930), kin selection (Hamilton 1964) and parent-offspring conflict theories (Trivers 1974). So far, most theoretical and empirical studies have focussed on the effect of relatedness asymmetries on sex allocation. Asymmetries in relatedness arise from the haplodiploid sex determining system of Hymenoptera, which causes females to be more related to sisters (r=0.75) than to daughters (r=0.5), sons (r=0.5) and brothers (r=0.25). Kin selection combined with Fisher sex ratio theory provides a quantitative prediction linking sex investment ratios to genetic relatedness asymmetry at both the population and colony levels. If workers control sex allocation, female bias is predicted to be 3:1 at the population level when all colony members are the offspring of a single, once mated queen. Female bias is expected to decrease concomitantly with a decrease in relatedness asymmetry, both at the population and colony levels (Trivers & Hare 1976; Boomsma & Grafen 1991; Bourke & Franks 1995; Crozier & Pamilo 1996). The most important factors reducing relatedness asymmetry are i) the presence of more than one reproductive queen per colony when queens are related, ii) multiple mating by queens and iii) worker reproduction.

Numerical and investment sex ratios have been analyzed across a large range of eusocial Hymenoptera. Overall, investment ratios at the population level seem to lie between the queen and worker optima, suggesting that workers have at least partial control over sex ratio investments (reviews of Bourke & Franks 1995; Crozier & Pamilo 1996). Evidence that workers manipulate sex ratio so as to maximize their inclusive fitness also comes from intraspecific studies of sex ratio variation across colonies with variable relatedness asymmetry. As expected under worker control, colonies with high relatedness asymmetry tend to produce more females than colonies with lower relatedness asymmetry (reviews of Queller & Strassmann 1998; Chapuisat & Keller 1999). However, in many of these studies, relatedness asymmetry has been measured indirectly from information on the breeding structure and it has been shown that these

measures are not always good estimates of the actual relatedness asymmetry (Aron et al.1999a; Brown & Keller 2000). Moreover, in some ants workers do not bias colony sex ratios as predicted by split sex ratio theory (Pamilo & Seppä 1994; Brown & Keller 2000).

The emerging picture from these studies is that there is considerable variation within and among species in the extent to which workers are able to control sex ratio investments at both the colony and population levels. Differences in the relative control of queens and workers may stem from idiosyncratic differences among species in the relative powers of queens and workers to influence reproductive decisions. Queens are in control of the primary sex ratio (through the proportion of male and females eggs laid) but workers may influence sex ratio investment by selective elimination of one sex (e.g., males; Aron, Passera & Keller 1994, 1999b; Aron, Vargo & Passera 1995; Passera & Aron 1996; Sundström, Chapuisat & Keller 1996) or altering the proportion of female larvae developing into workers or new queens (i.e., controlling the process of caste determination). Hence, queens may potentially force workers to raise more males by limiting the number of females' eggs. But workers may react by raising more females as queens to bias investment ratios toward females. The outcome of this tug-of-war depends on how early workers can determine the sex of the developing brood and the extent to which workers can influence caste determination (Pamilo 1991; Bourke & Chan 1999; Bourke & Ratnieks 1999). In some species queens may also gain some control over sex ratio by influencing the caste of female eggs (Passera 1982; Helms 1999), for example by changing egg hormone level (JH, ecdysteroids) (Passera, Suzzoni & Strambi 1980). Queen could thus limit the number of reproductive females by primarily laying worker-destined eggs. Another important parameter is whether or not queens may effectively limit the number of female eggs (Reuter & Keller, in press), which depends on life-history traits such as whether males and new queens are raised together with workers or whether workers and sexuals develop from distinct batches of eggs.

Finally, a key factor, which has received relatively little attention, is the role of temporal and spatial variation in resources and the extent to which these variations affect the relative power of

queens and workers on resource allocation. For instance, if resources are abundant, workers may raise all the brood produced by queens, including males. Hence, workers may influence investment ratios only by altering the proportion of females developing in new queens. Alternatively, if resources become limited, workers may have to cull a fraction of the brood, e.g. males, therefore achieving a female-biased sex investment ratio (Chapuisat, Sundström & Keller 1997). Limited resources may also induce workers to decrease the proportion of female larvae that are directed to the queen developmental pathway. Thus, differences in resource abundance can have important effects on the number of males, queens and workers produced, hence on sex ratio investments and the relative amount of resources invested in colony maintenance (worker production) versus reproduction (male and queen production).

Several lines of evidence suggest that resource availability indeed influences sex allocation. For instance, there is a strong association between productivity and sex allocation, with more productive colonies investing proportionally more in queen production than smaller colonies that tend to produce only or mostly males. Large colonies having more resources would still raise male brood but direct most female larvae to the queen developmental pathway (Nonacs 1986a). The association between colony productivity and investment ratios may also stem from a shortage of brood, for example if large colonies headed by a single queen are egg limited (Rosenheim, Nonacs & Mangel 1996). As a result, workers would benefit to produce the more expensive sex, typically females, in larger colonies. A positive relationship between resource levels and colony sex ratio has been hypothesized in some ant species (Brian & Elmes 1974; Boomsma, Van der Lee & Van der Have 1982; Herbers 1984; Aron *et al*. 1999a; Foitzik & Heinze 2000). However, few studies have experimentally addressed this issue. In particular, the effect of resource levels on the relative investment in workers, males and queens has received little attention and, in some species, remains equivocal. In *Leptothorax longispinosus* Mayr Backus & Herbers (1992) found an increased investment in male, but not queens, in protein-supplemented plots. When the same experiment was repeated a few years later, however, queen

production was increased in protein-supplemented plots (Herbers & Banschbach 1998). In *Myrmica punctiventris* Roger, food supplementation increased total investment in males whereas female production was not affected by resource level (Herbers & Banschbach 1999). By contrast, food supplementation increased total sexual productivity and female bias in *Formica podzolica* Francoeur (Deslippe & Savolainen 1995) and *Aphaenogaster rudis* Emery (Morales & Heithaus 1998).

The effect of resource availability on the relative investment in workers versus sexuals (males and new queens) remains poorly studied. Backus (1995) did not find any effect of food supplementation on allocation to workers versus sexuals in *Leptothorax longispinosus*. Herbers & Bansbach (1998) obtained different results in the same species. In food supplemented nests, a higher proportion of resources was invested into sexual production. Moreover, dry weights of new queens, but not males and workers, was higher in supplemented nests. In *Myrmica punctiventris*, dry weights of workers, males and new queens did not vary with variation of food treatment (Herbers & Banschbach, 1999).

Altogether, these studies show that differences in resource availability can influence the number of males and queens produced, the relative allocation to worker and sexual production, as well as the size of individuals produced. However, the effect varies among studies, even when these are performed on the same species by the same authors. One possible explanation for these discrepancies is that the effect of food supplementation is confounded with the influence of other ecological variables in the field (Herbers & Banschbach 1999). To date, the few studies having dealt with the role of resource availability on allocation ratios in ants involved mainly species forming relatively small colonies (e.g., *Leptothorax, Myrmica*). Here, we examine the effect of protein availability on reproductive decisions under controlled laboratory conditions in the Argentine ant *Linepithema humile*, a species forming large colonies. We determined how protein affects the number and the weight (both fresh and dry weights) of worker, male and queen

pupae. We also studied the effect of protein supply on the patterns of investment by analyzing sex allocation, caste allocation and reproductive allocation ratios.

Materials and methods

Native from South-America, the Argentine ant *Linepithema humile* has world-wide spread over large areas with a Mediterranean climate (Newell & Barber 1913; Passera 1994). In its introduced range, this species forms highly polygynous (multiple queen) colonies (Newell & Barber 1913; Markin 1968). Workers are completely sterile. Sexual brood is reared in spring, after the workers have killed most of the mated queens (Keller, Passera & Suzzoni 1989). Queens do not depart on mating flights and they mate within the nest a few days after eclosion from the pupae (Markin 1968; Passera & Keller 1994). The Argentine ant is well suited for standardized laboratory experiments on resource allocation, because it can be easily reared in the laboratory and because natural conditions eliciting sexual production can be easily created in the laboratory (Passera, Keller & Suzzoni 1988a), in contrast to the vast majority of ant species.

Large number of *L. humile* ants were collected within a 800 x 300 m area, in Port-Leucate (southern France), in January 1999 to establish five large stock laboratory colonies. From these, 45 experimental nests, each consisting of 20 queens and 3000 workers were constituted a few days after collection. All the brood in the colonies was carefully removed. The nests were maintained at 25±2 °C and fed *ad libitum* on sugar water and maggots.

After three weeks, the experimental nests were orphaned in order to stimulate the rearing of sexual brood from the eggs laid by the queens. At this time, the brood consisted mostly of eggs and a few young larvae. Orphaning mimics the queen execution process that occurs in spring and precedes the rearing of a new cohort of sexuals under natural conditions (Keller *et al*. 1989). Nests were kept orphaned until the end of the experiment, i.e. when there was no more brood left to rear. After queen removal, the 45 experimental nests were randomly assigned to one of three feeding treatments: 15 nests were fed with sugar water only (sucrose 8%), 15 were fed with

sugar water and 1 maggot (1 blowfly cut into two pieces; weight per maggot: 78.0±12.3 mg; n=20) per week, and 15 were given sugar water and five maggots (also cut into two pieces) per week (one every single day, 5 days/week). Argentine ant workers are unable to feed on dried material so that they could feed on the maggots only during 1-2 hours. The amount of protein was therefore about five times higher in the 5 maggots than in the 1 maggot/week treatment. The experimental nests were monitored every seven days, and the pupae of each sex and caste counted. Sexual pupae were removed after each census. To estimate the weekly production of worker pupae, we divided by two the number of worker pupae counted weekly because the mean lifetime of worker pupae is about 14 days in laboratory conditions (Benois 1973).

To study the effect of protein availability on the size of individuals produced, we determined the fresh and dry weights of pupae (worker, unsupplemented nests: number of colonies n=14, number of individuals N=64; worker, medium protein treatment: n=15, N=57; worker, high protein treatment: n=14, N=62); male, unsupplemented nests: n=9, N=17; male, medium protein treatment: n=12, N=50; male, high protein treatment: n=10, N=28; queen, unsupplemented nests: n=11, N=43; queen, medium protein treatment: n=15, N=133; queen high protein treatment: n=13, N=145). Dry weights were determined to the nearest 0.01 mg after the individuals had been dried at 70°C for 24 hours. Because the number and/or mass of the pupae are not independent when they come from the same nest, allocations ratios were estimated from the biomass of each caste produced per nest. Hence, sex allocation ratio (proportional investment in males among sexual pupae), caste allocation ratio (relative investment in new female sexuals among diploids), and reproductive allocation ratio (proportional investment in sexual pupae among all castes) were estimated on the basis of the biomass reared by each nest in each food treatment. Estimations of energetic costs were not corrected for differential metabolic rates (Boomsma 1989; Boomsma, Keller & Nielsen 1995), because only pupae were considered in our experiments. We therefore used the biomass of pupae produced (dry weight) to estimate sex, caste and reproductive allocation ratios.

For statistical comparisons, the appropriate parametric or non-parametric tests were employed. Because our data were obtained from nests of identical composition and reared in standardized laboratory conditions, all nests were assigned an equal weight. Numerical and investment ratios were calculated as arithmetic means of experimental nests ratios. All significance levels refer to two-tailed tests.

Results

Forty-two of the 45 nests produced both worker and sexual pupae. The first sexual pupae were produced eight weeks after the experimental nests were set up and the last pupae on week 13. We first tested whether the two protein-supplemented treatments (1 versus 5 maggots/week) had different influences on the number of worker, male and female pupae produced, the dry and fresh weight of these pupae, and the relative investment (numerical and mass ratios) between the three castes. No significant difference was found (Mann-Whitney test: $p > 0.05$ for all comparisons), suggesting that, in our experiments, differences in number of maggots provided did not affect reproductive decisions nor the weight of the pupae produced. We therefore pooled data from the medium and high protein treatments for all subsequent comparisons.

The addition of protein significantly influenced the number of pupae produced (Table 1). Protein-supplemented nests reared significantly more pupae. The number of male and queen pupae was about twice higher in protein-supplemented nests than in nests that did not receive protein (Mann-Whitney test, both $p < 0.004$). However, there was no significant effect of protein availability on the mean number of worker pupae produced. The positive effect of protein addition on the number of male and queen but not worker pupae produced resulted in a significantly higher proportion of queen pupae among the female pupae, as well as higher proportion of males among all individuals produced (Table 1). The proportion of males among sexuals was similar (64% and 67%) and not significantly different between both treatments. Within each treatment group, there was no association between the number of sexual (queen and

male) and worker pupae produced (Spearman rank correlation: unsupplemented nests: r_s=- 0.47, p> 0.08; protein-supplemented nests: r_s=- 0.19, p> 0.3)

Pupae of the three castes reared on protein-supplemented nests were heavier than those from unsupplemented nests (Fig. 1). The effect of treatment was significant for each caste and for both fresh and dry weights (t-tests, fresh weight: queens: t_{37}=6.38, males: t_{29}=3.17, workers: t_{41}=6.58, all p< 0.01; dry weight: t_{37}=2.79, males: t_{29}=-2.30, workers: t_{41}=4.86, all p< 0.05). Two-way ANOVAs showed that there was a significant interaction between treatment and caste for fresh weights (treatment: $F_{1,107}$=67.68, p< 0.001; caste: $F_{2,107}$=1556.87, p< 0.001; treatment x caste: $F_{2,107}$=16.64 p< 0.001), indicating that the addition of protein differently affected the weight of each caste. The difference in fresh weight between protein-supplemented and unsupplemented nests was higher for workers (25%) and queens (23%) than males (16%). By contrast, although both treatment and caste have a significant effect on the dry weights (treatment: $F_{1,107}$=18.17, p< 0.001; caste: $F_{2,107}$=1303.72, p< 0.001), there was no significant interaction between both parameters, suggesting that food supplementation did not differently affect the dry weight of each caste ($F_{2,107}$=2.75, p=0.11).

The effect of protein availability on the biomass of pupae produced is reported in Table 2. As expected from the data reported above, the addition of protein significantly increased the total biomass of pupae produced. The increase was significant for both male and queen pupae, but not worker pupae. The proportional investment in males among all castes was not different between both treatments; by contrast, the proportion of investment allocated to queens among females was higher in the protein-supplemented treatments. Protein-supplemented nests also invested proportionally more into sexual production than unsupplemented nests. There was, however, no significant association between the relative amount of resources invested in sexuals and total biomass production per nest (unsupplemented nests: r_s=0.33, n=14; supplemented nests, r_s=0.22, n=28, both p> 0.2).

Sex allocation ratio (proportion of sexual investment allocated to males) was not significantly different between supplemented nests (49%) and unsupplemented nests (56%) (Table 2). Moreover, there was no significant correlation between the proportional investment in males and total nest sexual productivity (Spearman rank correlation; unsupplemented nests: r_s= - 0.12, n=14; supplemented nests, r_s= - 0.09, n=28, both p> 0.6).

Discussion

The addition of protein in the diet provided to experimental nests of *L. humile* had a significant effect on the number of pupae produced. Protein-supplemented nests (either with 1 or 5 maggots/week) raised significantly more queen and male pupae than non-supplemented nests. However, increased levels of protein did not affect the number of workers produced. These results indicate that the amount of resources available do not merely influence the process of caste determination and thus the proportion of females developing into queens versus workers. The hypothesis that the main effect of food availability is to change the proportion of females developing in queens had been proposed to account for the positive association between colony productivity and more female biased sex allocation in ants (Nonacs 1986a,b). However, if increased levels of protein would only have an effect on caste determination, the production of workers should be lower in protein-supplemented nests because more female brood would have been directed to the queen developmental pathway. This clearly was not the case. In fact, our results show that more females (workers and queens) were raised in the protein-supplemented nests. Similarly, protein-supplemented nests produced about twice as many males as unsupplemented ones. These results indicate that a significant proportion of both the male and female brood most likely was cannibalized in unsupplemented nests.

Food shortage has been shown to trigger cannibalism of siblings in various animals, including ants, bees, amphibians and birds (Hölldobler & Wilson 1990; Crespi 1992; Kukuk 1992; Choe & Crespi 1997; Mock & Parker 1997). In the Argentine ant, it has been shown that workers

discriminate between sex, caste and age of the brood and selectively eliminate larvae according to social environment (Aron *et al*. 1994; Passera & Aron 1996). Cannibalism in unsupplemented nests may have been particularly important in our experimental design because nests were initially given *ad libitum* amount of food, which probably translated into high egg production (Nonacs 1991). When the treatment begun and protein was withdrawn, unsupplemented nests may have produced eggs in line with an amount of food they were no longer receiving. Hence, workers may have been forced to cut back because of the immediate food stress. Overproduction of brood and culling of offspring because of limited resources is common in many animal species (Mock and Forbes 1995). Interestingly, unsupplemented nests were able to produce workers and some sexuals. Workers most likely used stored energy, for example fat. In several ant species (e.g., *Formica rufa*-pratensis Gösswald, *Myrmica rubra* Linné) workers partly use lipids and other stored reserves to raise sexuals (Bier 1954; Elmes *et al*. 1999).

The addition of protein significantly increased both the fresh and dry weights of queen, male and worker pupae. Workers therefore react to a higher protein diet not only by rearing more sexuals, but also by producing larger individuals. Protein supplementation had a significantly greater effect on the increase of fresh weight of females than males. By contrast, no differential response between males and females was found for dry weight, suggesting that protein supplementation might also have resulted in females retaining proportionally more water. The reason for such a difference between sexes remains unknown. The increase of weight for all three castes that occurs in the Argentine ant contrasts with data on food availability reported for other ant species. Indeed, dry weight of workers, males and new queens did not vary with variation of food treatment in the ant *Myrmica punctiventris* (Herbers & Banschbach 1999). In *Leptothorax longispinosus*, Herbers & Banschbach (1998) found that food supplementation translated in a higher dry weight for new queens only.

The higher number and greater weight of the queen and male pupae in protein-supplemented nests resulted in a significant higher investment in sexual production in these nests. Increased

production of sexuals with increased resources has been reported in several ant species (Boomsma *et al*. 1982; Deslippe & Savolainen 1995; Morales & Heithaus 1998; Herbers & Banschbach 1998, 1999). However, in most of these studies, increased sexual production in food supplemented nests was associated with a change in sex ratio investment, generally a shift toward more females, or exceptionally towards more males (Herbers & Banschbach 1999). By contrast, our data show that increased level of protein resulted in no difference in sex ratio investment in *L. humile*. A possible explanation for these discrepancies is that in some species, or under some ecological conditions, colonies are limited in the quantity of male or female brood so that increased levels of resources result in higher investment in the sex less limited at the egg or larval stage. Unfortunately, the available data do not show whether increased investment in one sex is generally associated with brood limitation of the other sex. Similarly, field studies generally do not allow one to determine whether increased investments in queens in food-supplemented colonies occurs at the expense of worker production. Additional studies in the laboratory such as the one performed here might be useful to determine why food supplementation has different effect on weight, sex and reproductive allocation depending on the species, year, and perhaps timing of the developmental cycle of the brood where additional food is provided.

No significant difference between the two protein-supplemented treatments (1 versus 5 maggots/week) was detected in our experiments. Both types of nests produced similar numbers of queen, male and worker pupae. Moreover, the dry and fresh weights of these pupae were similar. As a result, the relative investment (numerical and mass ratios) in each of the three caste was independent of the amount of protein provided. The absence of difference between the two protein treatments could be due to a lack of quantitative difference between 1 and 5 maggots per week. However, this explanation is unlikely because blowfly maggots are quite large (see methods) and represent sizeable amount of food. Moreover, the addition of a single maggot significantly influenced the number and size of the pupae produced. The possibility that one

maggot left for 5 days could be functionally equivalent to a new maggot being added every day can also be ruled out; Argentine ant workers are able to feed on maggots only during 1–2 hours, before the hemolymph coagulates. Alternatively, there could be a threshold over which additional amounts of protein have no more effect on reproductive allocation or the size of the individuals produced. This hypothesis could explain why experimental addition of food in the field has sometimes provided inconsistent results, for example because untreated colonies might have been differently limited in the amount of protein available. In a similar experiment Nonacs (1991) also found that colony productivity in the ant *Camponotus floridanus* Buckley does not always increase with increased protein availability. In fact, colony productivity was maximal with intermediate protein availability. Nonacs suggested that decreased productivity with more protein might stem from a shift in reproductive strategy, but it is unclear what could be the benefits of reducing productivity when resources are more abundant.

Interestingly, the numerical proportion of males among all castes in protein-supplemented nests (0.29) was similar to that reported from field experiments (0.30-0.38; Aron *et al*. 1994). However, the proportion of males among sexuals was lower in our experimental nests (0.64) than in the field (0.80–0.98; Markin 1970; Passera & Keller 1992; Aron *et al*. 1994). There are at least two possible explanations for this difference. First, resource availability and/or worker to brood ratio may differ between the field and our laboratory colonies and influence the ratio of females developing into queens versus workers. In the Argentine ant, a higher worker to brood ratio generally increases the number of sexuals produced (Passera *et al*. 1988b). In the present study, workers were well fed and the amount of brood was relatively small because it comprised only the brood produced by the queens over a 3-weeks period. The worker to brood ratio is probably much lower under natural conditions because in spring workers rear not only the brood produced by the queens after hibernation but also the overwintering brood. Thus, it is possible that fewer females develop into queens in the field, which may account for the higher proportion of males among sexuals, compared to our laboratory colonies. Alternatively, the lower

proportion of males among sexuals in the field might stem from some sexualization of female larvae being inhibited by queens. Mated queens of *L. humile* exert a strong pheromonal inhibition on the development of new female sexuals, and this inhibition is much higher for queen than male production (Passera *et al*. 1988a; Vargo & Passera 1991). Although 90% of queens are executed at the beginning of the reproductive season in the field (Keller *et al*. 1989), the remaining queens may still limit sexualization of new female larvae. Because no queen was left in our experimental nests, this may explain why they produced a higher ratio of queens to workers thus accounting for the less male-biased sex ratio among sexuals than in the field.

In conclusion, this study demonstrates that the main effect of protein supplementation was to increase the number of individuals raised, and that *only* additional sexuals (queens and males) were produced. Because the initial amount of brood was similar in unsupplemented and protein-supplemented nests, this indicates that an important effect of the amount of protein available is to alter the proportion of brood that is culled. Although numerous studies have shown that a high proportion of the brood is culled in colonies of social insects (see Bourke & Franks, 1995), the fact that workers may have to eliminate part of the brood is rarely considered in theoretical investigations of the outcome of queen-worker conflict over reproductive decisions. In *L. humile*, workers discriminate between male and female brood and selectively eliminate males after egg eclosion, i.e. at the first larval stage (Passera & Aron 1996). In *Formica exsecta*, Chapuisat *et al*. (1997) showed that workers eliminated male larvae later, when they were close to pupation. In fact, workers had already invested so much in these males that they could not increase their inclusive fitness by replacing them with new queens. Chapuisat *et al*. (1997) thus proposed that male destruction was probably triggered by resource limitation and that the evolution of fratricides in ants is best explained by a combination of ecological, demographic and genetic parameters. The increasing amount of experimental data showing that brood culling is an important part of the biology of ants and other social insects emphasis the need to combine

kin selection and life-history approaches to study the outcome of queen-worker conflicts on the dynamics of colony growth and sex allocation.

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Figure 1. Effects of protein availability on the fresh and dry weights (mg) (means ± s.d.) of queen, male and worker pupae produced in experimental nests of *L. humile*. For protein-supplemented nests, data from medium (1 maggot/week) and high (5 maggots/week) protein treatments were pooled. Sample sizes (number of nests) are indicated by numbers above error bars. Student *t*-test: *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$.

Table 1. Effects of protein availability on the total number (means ± s.d) of worker, queen and male pupae produced, and numercial ratios in experimental nests of *L. humile*. n =number of nests.

Caste	Unsupplemented nests (n=14)	Protein-supplemented nests (n=28)	Mann-Whitney *U*-test *p* values
Workers	202 ± 93.5	201.3 ± 85.3	*U*=191; *p*=0.9
Queens	25.1 ± 23.5	54.3 ± 30.1	*U*=78.5; *p*< 0.002
Males	56.6 ± 34.2	104.6 ± 58.5	*U*=86.5; *p*< 0.004
Total N. pupae	283.8 ± 92.5	360.2 ± 116.6	*U*=102; *p*< 0.013
Prop. of queens among females	0.15 ± 0.17	0.23 ± 0.12	*U*=104.5; *p*< 0.02
Prop. of males among all castes	0.20 ± 0.11	0.29 ± 0.10	*U*=107.5; *p*< 0.02
Prop. of males among sexuals	0.67 ± 0.23	0.64 ± 0.17	*U*=174.5; *p*> 0.5

Table 2. Effects of protein availability on the biomass (means ± s.d.) of worker, queen and male pupae produced, and on sex allocation ratio (proportion of sexual investment allocated to males), on caste allocation (proportion of investment allocated to queens among diploids), and on reproductive allocation ratio (proportion of overall investment allocated to sexuals) in experimental nests of *L. humile*. Data are derived from the results reported on Table 1 and Figure 1. n: number of nests.

Caste	Unsupplemented nests (n=14)	Protein-supplemented nests (n=28)	Mann-Whitney *U*-test *p* values
Worker biomass	38.39 ± 19.96	44.61 ± 22.70	*U*=157; *p*> 0.3
Queen biomass	24.67 ± 26.01	54.61 ± 30.57	*U*=78; *p*< 0.002
Male biomass	27.14 ± 17.09	53.36 ± 31.19	*U*=80; *p*< 0.002
Total biomass	90.20 ± 31.55	152.59 ± 46.93	*U*=50; *p*< 0.001
Prop. of investment allocated to queens among females	0.36 ± 0.29	0.53 ± 0.18	*U*=114.5; *p*< 0.03
Prop. of investment allocated to males among all castes	0.28 ± 0.14	0.34 ± 0.12	*U*=152; *p*> 0.2
Prop. of investment allocated to males among sexuals	0.56 ± 0.26	0.49 ± 0.19	*U*=169; *p*> 0.4
Prop. of overall investment allocated to sexuals	0.54 ± 0.23	0.71 ± 0.10	*U*=122; *p*< 0.05