

# Control of body size of *Lasius niger* ant sexuals — worker interests, genes and environment

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## Abstract

In most animals, the survival and reproductive success of males and females is linked to their size. The ability of individuals to control environmental influences on size will therefore have consequences for their fitness. In eusocial insects, individual males and reproductive females do not have to forage for themselves or control their local environment. Instead, they are reared by nonreproductive siblings (workers) inside colonies. Workers should benefit from controlling the size of sexuals because these sexuals are usually the only means for workers to transmit their genes to future generations. Nevertheless, considerable intraspecific variation exists around mean sexual size in social hymenopterans, even in species with monomorphic sexuals. This variation could result from genetic influences on sexual size, for instance sexuals may be selected to not agree to worker interests, or be due to strong, unpredictable environmental conditions constraining the efforts of workers to control sexual size. In a study that is the first of its kind I investigated genetic and environmental components of sexual body size variation in the ant *Lasius niger*, examining sexuals from wild colonies with one or several fathers (paternity levels established through microsatellite DNA offspring analysis). Evidence was found for a genetic component of size (broad-sense heritability of up to 42%) but strong common-colony effects (among-colony variation in food availability or in worker capacities to restrain sexual selfishness) also increased the size differences among colonies. Workers thus seem to only have partial control over sexual size, but may be doing the best of a bad job.

*Keywords:* conflict, inclusive fitness, phenotype, resource allocation, social insects

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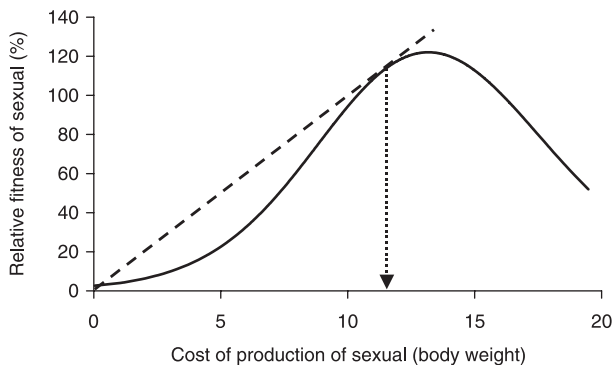
## Introduction

The size and condition of organisms greatly affect their survival and reproductive success (e.g. Endler & May 1986; Blanckenhorn 2000; Jennions *et al.* 2001; Simmons 2001; Bennett & Owens 2002) and are usually strongly related to nutrition. The ability of individuals to exploit their environment when conditions are favourable and to counter the impact of a poor environment will therefore have consequences for their fitness. In eusocial Hymenoptera (ants and many bees and wasps), individual males and reproductive females (queens) cannot directly modulate their foraging

rates to ensure a good body size, in contrast to the situation in most other animals. Developing sexuals do not forage for themselves, being instead reared to maturity inside colonies by their nonreproductive sisters, the workers, who forage and distribute the food to the brood (Hölldobler & Wilson 1990; Bourke & Franks 1995).

Workers, however, should have an interest in regulating sexual size because the sexuals are generally the workers' only means of contributing genes to future generations (Hamilton 1964; Bourke & Franks 1995; Crozier & Pamilo 1996). The inclusive fitness of workers (Hamilton 1964; Bourke & Franks 1995) is a function of the number of sexuals produced, the relatedness of workers to these sexuals and the fitness of each sexual. If a certain sexual size is linked to a higher sexual fitness per amount of resources invested by workers in its production, such as is often the case in species with monomorphic sexuals (cf. e.g. Davidson 1982; Abell *et al.* 1999; Wiernasz *et al.* 2001; Wiernasz &

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**Fig. 1** Worker interests in controlling the size of sexuals, assuming that the fitness of individual sexuals as a function of the resources invested in them follows a decreasing-returns curve. From the workers' point of view, optimal sexual size should maximize the fitness of a sexual per resource unit invested. This size is represented by the steepest possible tangent to the curve while constraining it to pass through the origin, and hence does not coincide with the size conferring maximal personal fitness on a sexual. Sexual sizes below or above the theoretically optimal sexual size for workers would lower the inclusive fitness gains of workers per resource unit invested (lower tangent slopes). Note that the figure does not take into account the possibility that optimal sexual size may change along a season. Such seasonal changes are, however, unlikely in most European ants, e.g. *Lasius niger*, because mating flights occur during a short time period.

Cole 2003; Fjerdingstad & Keller 2004; see also Smith & Fretwell 1974; Rosenheim *et al.* 1996), workers should benefit from making all sexuals that size (all other things equal) (Fig. 1). Hence we would expect that variation in resource availability should lead workers to vary the number of sexuals produced, not their size (Fig. 1; cf. e.g. Rosenheim *et al.* 1996; Wiernasz *et al.* 2001; Parker *et al.* 2002), if queens are able to supply sufficient numbers of eggs.

Nevertheless, considerable size variation is found in sexuals of monomorphic ants, both among (e.g. Ward 1983; Herbers 1990; Backus 1993; Fjerdingstad & Boomsma 1997; Wiernasz *et al.* 2001) and within colonies of a given species (Fjerdingstad & Boomsma 1997; Fjerdingstad 2004) (see also Davidson 1982; Vander-Meer *et al.* 1992; Keller & Ross 1993, 1995; Tschinkel 1993, 1996; Sundström 1995; Wiernasz *et al.* 1995, 2001; Bernasconi & Keller 1996, 1999; Abell *et al.* 1999; Wagner & Gordon 1999; DeHeer 2002). For most of the species studied there is no obvious reason to assume that this variation is due to colonies being egg limited and therefore only capable of increasing body size, not the number of sexuals, in response to resource variation. This is because most of these species (except the *Rhytidoponera* studied by Ward 1983) possess large queens with a physiology specialized for high egg production rates (very enlarged gaster and very large numbers of ovarioles; see, e.g. Tschinkel 1987).

Size variation in ant sexuals could be mainly due to environmental factors that workers cannot control completely. For instance, it may be difficult for workers to distribute the same amount of food to each larva, especially in ants where brood is reared in loose brood piles and fed many times by different workers. Also, variation among colony-sites in the amount of resources available for feeding the developing sexuals may be great and unpredictable along a season, or may occur at times when resources cannot be used optimally. For example, an abundance of resources may occur late in the season when colonies cannot start to rear more sexuals (sexuals maturing in spring often develop from eggs laid the previous autumn, see, e.g. Brian 1983; Kipyatkov 1993; Jemielity & Keller 2003). In such cases, extra resources can only be used for making existing sexuals larger (Rosenheim *et al.* 1996). Conversely, a resource shortage may occur so late in the season that it will no longer be favourable to cull some sexuals and feed them to the others (cf. Chapuisat *et al.* 1997), resulting in colonies that produce small sexuals. Finally, offspring conflict over resources may make it difficult for workers to control the allocation of resources to individual larvae. For instance, sexual larvae arising from early laid eggs could be stronger competitors for food (better at begging) (cf. Creemers *et al.* 2003) than their (slightly) younger siblings, leading to size differences between early-hatching and late-hatching larvae.

Also, genetic variation for size could constrain the ability of workers to control the size of sexuals. For instance, genetic variation may exist for size at larval hatching or pupation, or for larval adeptness at begging food from workers. Parent-offspring resemblance (e.g. Buschinger 1975, 1978; Heinze & Buschinger 1989; McInnes & Tschinkel 1995; Heinze 1998; Ruppell *et al.* 1998, 2001) and sib-resemblance [Keller & Ross 1993, 1995, 1999; Goodisman *et al.* 1999; Fraser *et al.* 2000 (on workers)] have been observed in a number of ants, consistent with the hypothesis of genetic influence on size. Genetic variation for size of sexuals is particularly interesting in the context of potential conflict between workers and developing sexuals over the amount of resources that each sexual should receive (Fig. 1; Herbers 1990; Backus 1993; Rosenheim *et al.* 1996; Kaptein *et al.* 2005) (conceptually similar to standard parent-offspring, Trivers 1972). Conflict is expected because each developing sexual is more closely related to itself than to its siblings, especially in species with multiple queens per colony or multiple mates per queen. Such co-evolutionary conflict between workers and sexuals could have as consequence that workers of different genetic backgrounds differ in their capacity to restrain sexual selfishness. This could inflate among-colony differences in the size of sexuals (sexual size being kept at the worker optimum in some colonies, and closer to the individual sexual optimum in other colonies).

One step towards understanding how well social insect workers are able to control sexual size, and hence control an important component of their own inclusive fitness, is to study genetic and environmental effects on sexual size in natural populations. Such studies can provide insight into the likely importance of different factors for sexual size variation in nature (e.g. environmental variation, worker–sexual conflicts over resource allocation) and thus give stimulus for targeted experimental studies.

I here carry out the first quantitative study on genetic and environmental influences on sexual size in ants. I examine sexuals from natural colonies of the ant *Lasius niger* which is a monogynous (one queen per colony) and polyandrous (multiple mates per queen) species (Van der Have *et al.* 1988; Fjerdingstad *et al.* 2002, 2003) with large, long-lived colonies that produce many hundreds to thousands of monomorphic sexuals in brood piles each spring (Fjerdingstad *et al.* 2002). These sexuals are subjected to natural and sexual selection during their mating flight and (for queens) colony-founding stages, and sexual fitness covaries with body size (Fjerdingstad & Keller 2004; E. J. Fjerdingstad, unpublished). I estimate genetic and environmental components of sexual size in *L. niger* by examining size variation between colonies, between patriline within colonies and between individuals within patrilines inside colonies. I examine two components of size: head width that depends on prepupal feeding (i.e. early-season events) and body weight that depends also on post-pupal events (late-season events) and I discuss the ability of workers to control the colony environment and sexual size.

## Materials and methods

### Collection and measurement of samples and analyses of colony structure

In July 1997 and 1998, more than 50 000 young unmated but mature *Lasius niger* queens and males were collected in tent traps as they left their natal colonies for mating flights on the campus of Lausanne University in Switzerland

(Fjerdingstad *et al.* 2002). Following capture, body weights and head widths of a sample of young queens (mean  $\pm$  SD number of young queens per colony, 1997:  $19.7 \pm 0.7$  for 23 colonies; 1998:  $19.5 \pm 1.2$  for 34 colonies) and males (1997:  $4.96 \pm 0.20$  for 22 colonies; 1998:  $5.0 \pm 0.0$  for 26 colonies) were measured. For males, dry weight was used to estimate body weight (methods in Fjerdingstad *et al.* 2002) but for young queens fresh weights had to be used because subsequent DNA analysis to assign queens to patriline could not be performed on dried material. Fresh and dry weights are, however, strongly correlated in young *L. niger* queens ( $r = 0.93$ ,  $n = 137$ ,  $P < 0.0001$ , E. Fjerdingstad, University of Lausanne, Lausanne, unpublished data). Weighing was performed using a Toledo-Mettler balance (accurate to the nearest  $\mu\text{g}$ ) and head widths were measured with the use of a Nikon projector-screen micrometer. All measurements were highly repeatable ( $r > 0.97$ ,  $P < 0.0005$  for all traits, based on 39 replicate measurements for each trait).

Genetic analyses using two highly variable microsatellite markers were performed on the young queens from the sampled colonies to establish the number of patrilines per colony (Fjerdingstad *et al.* 2002, 2003). Twelve colonies sampled in 1997 and 19 sampled in 1998 had multiple fathers, and young queens could be unambiguously assigned to patriline. I used these queens (Table 1) to test for a genetic component of size by examining whether queen size differed significantly between patrilines that had shared the same colony environment. At the same time, I quantified the environmental components of size variation (details below).

For the study on size variation in males, I used samples from all colonies that had produced males (22 and 26 colonies in 1997 and 1998, respectively, Table 1) regardless of whether the mother queens in these colonies had mated with one or more males. Males arise from unfertilized eggs in the Hymenoptera (Cook 1993) and hence all male offspring of a single queen are full-brothers even if the queen had several mates. Additionally, worker production of males is extremely rare in *L. niger* (Fjerdingstad *et al.* 2002) and so can be ignored. Hence, all males inside a given colony can be considered full-brothers.

**Table 1** Sample sizes

| Trait | Young queens          |  |                |               |                 |                       | Males   |                 |
|-------|-----------------------|--|----------------|---------------|-----------------|-----------------------|---|-----------------|
|       | $N_{\text{colonies}}$ | $n_{\text{sexuals}}/\text{patriline (mean } \pm \text{ SD)}$ |                |               |                 | $N_{\text{colonies}}$ | $n_{\text{sexuals}}/\text{colony (Mean } \pm \text{ SD)}$ |                 |
|       |                       | 1  | 2              | 3             | 4               |                       |   |                 |
| 1997  | Body weight           | 12   | $13.8 \pm 2.7$ | $5.8 \pm 2.6$ | $0.17 \pm 0.39$ | —                     | 22  | $4.95 \pm 0.21$ |
|       | Head width            | 12   | $12.5 \pm 4.3$ | $5.3 \pm 2.8$ | $0.08 \pm 0.28$ | $0.08 \pm 0.28$       | 21  | $5.00 \pm 0.0$  |
| 1998  | Body weight           | 19   | $14.2 \pm 2.3$ | $5.3 \pm 2.3$ | $0.26 \pm 0.56$ | —                     | 26  | $4.96 \pm 0.20$ |
|       | Head width            | 19   | $14.2 \pm 2.2$ | $5.6 \pm 2.6$ | $0.37 \pm 1.01$ | —                     | 26  | $4.96 \pm 0.20$ |

(a)

| Source                                 | d.f.        | Mean squares | Components of MS  |
|--|-------------|--------------|---|
| Between colonies                       | $q - 1$     | $MS_Q$       | $\sigma_{\text{offspring}}^2 + k'_0 \sigma_{\text{father}}^2 + (mk)_0 \sigma_{\text{colony}}^2$ |
| Between patriline<br>(within colonies) | $q(m - 1)$  | $MS_F$       | $\sigma_{\text{offspring}}^2 + k_0 \sigma_{\text{father}}^2$                                    |
| Offspring within patrilines            | $qm(k - 1)$ | $MS_W$       | $\sigma_{\text{offspring}}^2$   |

(b)

| Observational pure components of variance | Covariance components                          | Causal components estimated              |
|---|--|--|
| Colonies $\sigma_{\text{colony}}^2$       | cov(half-sib)                                  | $1/4 V_A + V_{\text{Ecommon}}$           |
| Patriline $\sigma_{\text{father}}^2$      | cov(full-sib) - cov(half-sib)                  | $1/2 V_A + 1/2 V_D$                      |
| Offspring $\sigma_{\text{offspring}}^2$   | $V_{\text{Pop}} - \text{cov}(\text{full-sib})$ | $1/4 V_A + 1/2 V_D + V_{\text{Ewithin}}$ |

### Analyses of variance of sexual size between and within colonies

Through nested analyses of variance (ANOVA) I tested whether patriline (nested within colony) and colony explained significant parts of the variance in young queen size. In case they did, the variance component attributable to each level (Table 2) was isolated using the statistics program *SPSS* 11.0.1 (SPSS Inc.), according to standard procedures for unbalanced designs (see Sokal & Rohlf 1995, pp. 296–297).

These variance components were broken down into their causal genetic components based on the male-haploid, female-diploid genetic system of Hymenoptera, following Liu & Smith (2000) (see also Falconer & Mackay 1996, p. 167 for a classic diploid case), but taking into the multiple-paternity set-up (corresponding to a sire-within-dam set-up) and the colonial lifestyle of *L. niger* (Table 2). Of the genetic variance components, only additive and dominance components were considered because epistatic variance components are difficult to estimate in breeding analyses (Falconer & Mackay 1996). The genetic variance partitioning assumes large, outbred and random mating populations, and earlier microsatellite DNA marker studies have shown that these requirements are fulfilled for *L. niger* (Fjerdingstad *et al.* 2002, 2003).

I took into account the fact that individuals from a given colony could resemble each other because they had shared the same environment, i.e. that differences among colonies would be increased by a common-colony environmental component ( $V_{\text{Ecommon}}$ ) (Table 2). Maternal effects, and genetic differences among workers of different colonies in their capacity to control the allocation of resources to sexuals, would also inflate among-colony differences in sexual body size. Such effects could not be separated from the common-colony environment ( $V_{\text{Ecommon}}$ ) variance

**Table 2** (a) ANOVA design used for young queens.  $q$  is the number of colonies,  $m$  the mean number of mates per mother-queen, and  $k$  the mean number of offspring examined per patriline.  $k'_0$  and  $k_0$  are different modified mean numbers of offspring per father.  $(mk)_0$  is a modified product of the mean numbers of patrilines and offspring per patriline (see text). (b) Interpretation of the pure variance components isolated from the ANOVAS

component. All patrilines within a common colony were assumed to experience the same local environment. Offspring within patrilines might, however, have had different environmental experiences (increasing size differences among offspring within patrilines by a component  $V_{\text{Ewithin}}$  Table 2), for instance depending on whether they by chance eclosed early or late.

Estimates of broad-sense heritability  $[(V_A + V_D)/V_{\text{pop}}]$  (Falconer & Mackay 1996) of body weight and head width of young queens were obtained as twice the pure 'patriline' variance divided by the total variance  $V_{\text{pop}}$  (Table 2). Lower 90% confidence limits for the broad-sense heritability estimates were calculated according to Becker (1984, pp. 53–63) based on among-patriline mean squares, but substituting the coefficient '4' (cf. Becker 1984, p. 58) by '2' as appropriate because the patriline variance component in my sire-within-dam set-up estimates  $1/2$  of the heritability, not  $1/4$  as in dam-within-sire set-ups for diploids. Note that the causal content of the among-patriline component given in this study (Table 2) differs from that of Bargum *et al.* (2004) who incorrectly assumed that the patriline variance component for a sire-within-dam set-up (such as mine and theirs) was the same as that for the dam-within-sire set-up described in Liu & Smith (2000).

For males, the analyses applied were one-way ANOVAS with 'colony' as the sole factor because all males inside a colony have the same mother and no father. When the colony level explained a significant part of the variance, the variance component attributable to that level was isolated using *SPSS* (see above) and was decomposed into its constituent genetic and environmental components (Table 3), taking into account male haploidy following Liu & Smith (2000). Because the colony-level variance and the offspring-within-colonies variance both contained an environmental component (Table 3) no estimator of the genetic variance of size could be obtained for males.

**Table 3** (a) ANOVA design used for males.  $q$  is the number of colonies,  $o$  the number of offspring examined per colony,  $o_0$  a modified mean number of offspring examined per colony (modification applied due to the slightly unequal numbers of males per colony, see text). (b) Interpretation of the pure variance components isolated from the ANOVAs. Note that no dominance variance is involved because males are haploid (a)

| Source                    | d.f.       | MS     | Components of MS  |
|---------------------------|------------|--------|---|
| Between colonies          | $q - 1$    | $MS_Q$ | $\sigma_{\text{offspring}}^2 + o_0\sigma_{\text{colony}}^2$ |
| Offspring within colonies | $q(o - 1)$ | $MS_W$ | $\sigma_{\text{offspring}}^2$                               |

| Observational pure components of variance | Covariance components                          | Causal components estimated    |
|---|--|--------------------------------|
| Colonies $\sigma_{\text{colony}}^2$       | $\text{cov}(\text{full-sib})$                  | $1/4 V_A + V_{\text{Ecommon}}$ |
| Offspring $\sigma_{\text{offspring}}^2$   | $V_{\text{Pop}} - \text{cov}(\text{full-sib})$ | $3/4 V_A + V_{\text{Ewithin}}$ |

## Results

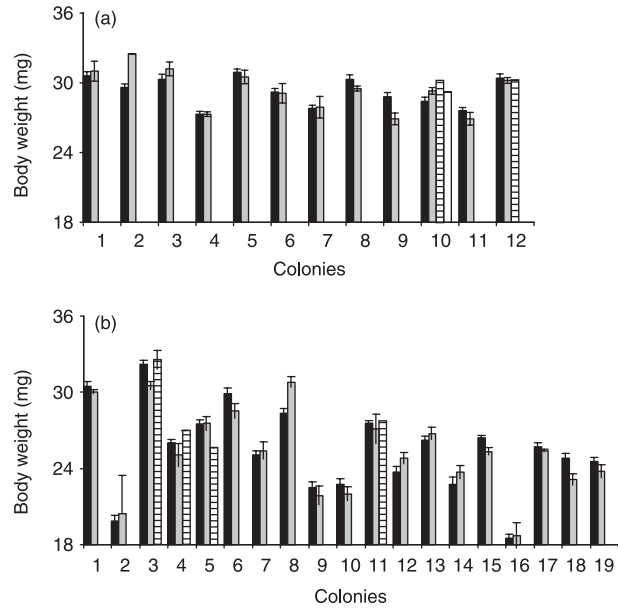
### Overall

At the population level, body weights of young queens and males showed considerable variation (coefficient of variation corrected for sample size bias cf. Sokal & Braumann 1980; CV\* in percentage: 6.0–14.5 for queens and 13.8–18.6 for males, in 1997 and 1998, respectively) while head width was less variable (CV\* in percentage: 2.2–2.0 for queens and 3.3–3.6 for males, in 1997 and 1998). This difference in degree of variability (CV\* body weight > CV\* head width) was significant both for young queens ( $t_{1997} = 13.0$ , d.f. = 469;  $t_{1998} = 22.91$ , d.f. = 747) and males ( $t_{1997} = 10.6$ , d.f. = 211;  $t_{1998} = 12.2$ , d.f. = 256) ( $P < 0.0001$  for all tests; tests cf. Sokal & Braumann 1980).

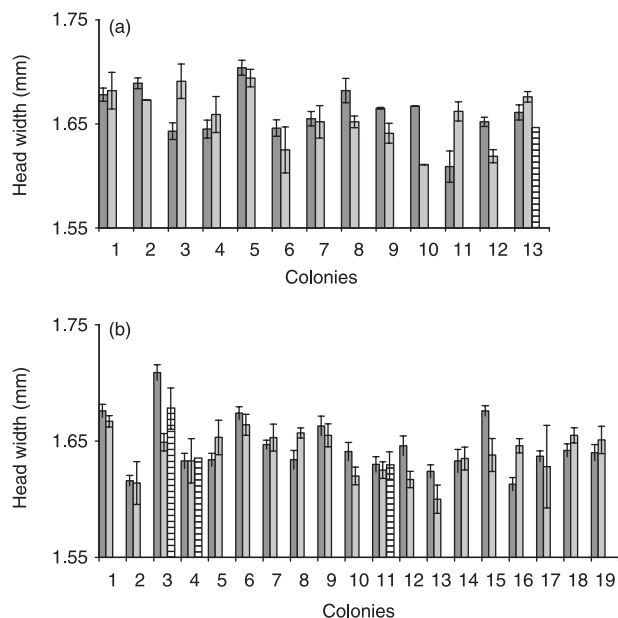
### Young queens

Body weight and head width varied significantly between colonies, meaning that the daughters of queens living in different colonies varied significantly in body size (also after controlling for patriline effects) (Figs 2a, b and 3a, b). Additionally, the daughters of different patrilines (within colonies) varied marginally significantly in body weight and highly significantly in head width (Figs 2a, b and 3a, b). This strongly suggests genetic variation for size of young queens.

Evidence was also found that environmental effects increased body size differences between colonies, thus increasing the relative size resemblance of individuals within colonies. This was established in the following way:



**Fig. 2** Mean ( $\pm$  SE) body weight of young queens in different colonies and patrilines in 1997 (a) and 1998 (b). For each colony, patrilines are shown side by side in different colours. Differences among patrilines within colonies were marginally significant in 1997 ( $F = 1.7$ , d.f. = 15, 212,  $P = 0.05$ ) and significant in 1998 ( $F = 1.6$ , d.f. = 21, 335,  $P < 0.05$ ), and among-colony differences were highly significant in both years (1997:  $F = 12.6$ , d.f. = 11, 15,  $P < 0.0005$ ; 1998:  $F = 73.0$ , d.f. = 18, 21,  $P < 0.0005$ ).



**Fig. 3** Mean ( $\pm$  SE) head width of young queens in different colonies and patrilines in 1997 (a) and 1998 (b). For each colony, patrilines are shown side by side in different colours. Differences among patrilines within colonies were highly significant in both years (1997:  $F = 3.0$ , d.f. = 15, 205,  $P < 0.0005$ ; 1998:  $F = 2.7$ , d.f. = 21, 334,  $P < 0.0005$ ), while among-colony differences were significant for 1997 ( $F = 3.1$ , d.f. = 11, 15,  $P < 0.05$ ) and highly significant for 1998 ( $F = 5.6$ , d.f. = 18, 21,  $P < 0.0005$ ).

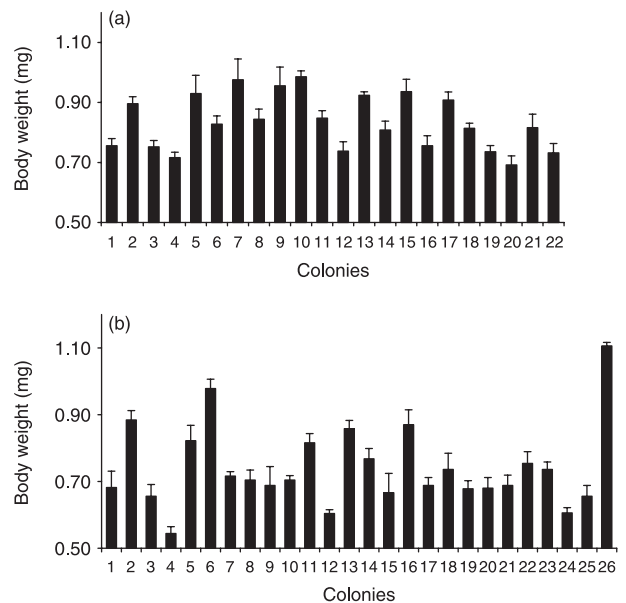
**Table 4** Proportion of variance in body size and head width of sexuals explained at different levels, and estimates of broad-sense heritability ( $h^2$ ) in young queens

| Trait       | Sexual type | Level                       | % variance explained |      | Broad sense $h^2$ (lower 90% CI) |               |
|-------------|-------------|-----------------------------|----------------------|------|----------------------------------|---------------|
|             |             |                             | 1997                 | 1998 | 1997                             | 1998          |
| Body weight | Young queen | Colonies                    | 46.0                 | 82.4 |                                  |               |
|             |             | Patrilines within colonies  | 5.3                  | 1.4  | 0.11 (–0.032)                    | 0.03 (–0.026) |
|             |             | Offspring within patrilines | 48.2                 | 16.3 |                                  |               |
|             | Male        | Colonies                    | 53.7                 | 71.8 |                                  |               |
|             |             | Offspring within colonies   | 45.6                 | 28.2 |                                  |               |
| Head width  | Young queen | Colonies                    | 15.2                 | 31.2 |                                  |               |
|             |             | Patrilines within colonies  | 20.5                 | 13.8 | 0.42 (0.167)                     | 0.28 (0.146)  |
|             |             | Offspring within patrilines | 64.3                 | 55.0 |                                  |               |
|             | Male        | Colonies                    | 33.9                 | 50.8 |                                  |               |
|             |             | Offspring within colonies   | 66.1                 | 49.2 |                                  |               |

if environmental effects had been negligible, the pure 'colony' variance component (representing differences among colonies) ( $1/4V_A + V_{\text{Ecommon}}$ , Table 2) would have been less than one-half of the 'patriline' component (differences among patrilines within colonies) ( $1/2V_A + 1/2V_D$ ) (Table 2). Instead the former was usually greater than the latter (Table 4), and this was significant for body weight ( $F_{1997} = 8.7$ , d.f. = 11, 15,  $P < 0.0005$ ;  $F_{1998} = 5.1$ , d.f. = 18, 21,  $P < 0.001$ ) but not for head width ( $F_{1997} = 0.74$ , d.f. = 11, 15,  $P > 0.50$ , NS;  $F_{1998} = 2.3$ , d.f. = 18, 21,  $P < 0.10$ , NS).

In general, the amounts of variance explained at different levels were not the same for body weight and head width, and this was the case in both years. For body weight, the greatest part of the variance was explained by differences among colonies (most of it being environmental variance, see above), while only little of the variance was due to genetic effects (differences among patrilines within colonies) and the amount of variance at the within-patriline level was modest (Table 4). For head width, by contrast, the proportion of the variance due to colony differences was lower, the genetic component much higher, and the proportion of the variance found at the within-patriline level higher (Table 4). For both body weight and head width, heritability did not vary significantly between years, because the lowest estimates (from 1998) lay within the lower 90% confidence limit of the biggest estimates (1997) (Table 4).

A considerable part of the variation in size of individual young queens within patrilines may have been constituted by environmentally caused variance ( $V_{\text{Ewithin}}$ ). This could be seen from the fact that the pure 'offspring' variance component (variance between offspring within patrilines) ( $1/4V_A + 1/2V_D + V_{\text{Ewithin}}$ , Table 2) was considerably larger than the pure 'patriline' (differences among patrilines) variance component ( $1/2V_A + 1/2V_D$ ) (Tables 2 and 4). This was significant for both body weight and head width (body-weight:  $F_{1997} = 9.0$ , d.f. = 212, 15,  $P < 0.0001$ ;  $F_{1998} = 11.6$ ,



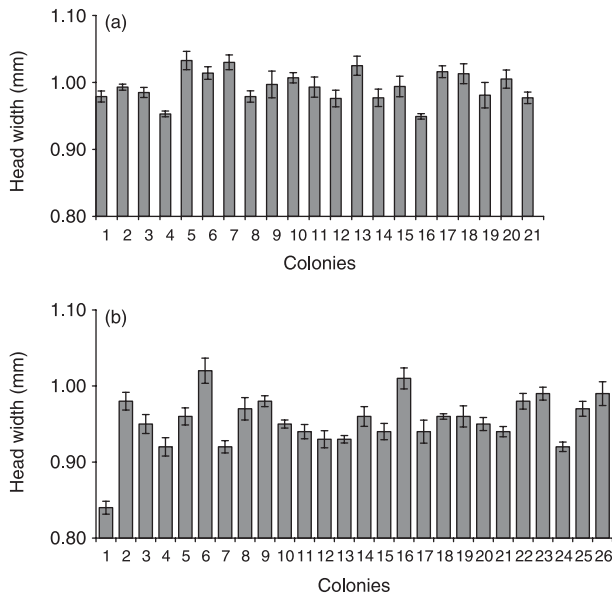
**Fig. 4** Mean ( $\pm$  SE) body weight of males in different colonies in 1997 (a) and 1998 (b). Differences among colonies were highly significant in both years (1997:  $F = 6.9$ , d.f. = 21, 87,  $P < 0.0005$ ; 1998:  $F = 16.3$ , d.f. = 25, 103,  $P < 0.0005$ ).

d.f. = 335, 21,  $P < 0.0001$ ; head width:  $F_{1997} = 3.1$ , d.f. = 205, 15,  $P < 0.02$ ;  $F_{1998} = 4.0$ , d.f. = 334, 21,  $P < 0.001$ ).

### Males

As in young queens, male body weight and head width showed significant variation among colonies, that is variation among the sons of different mother-queens living in different colonies (Figs 4a, b and 5a, b). Like in young queens, the percentage of the total variation explained by differences





**Fig. 5** Mean ( $\pm$  SE) head width of males in different colonies in 1997 (a) and 1998 (b). Differences among colonies were highly significant in both years (1997:  $F = 3.5$ , d.f. = 20, 83,  $P < 0.0005$ ; 1998:  $F = 6.2$ , d.f. = 25, 104,  $P < 0.0005$ ).

between colonies was greater for body weight than for head width (Table 4), and so the proportion of the variance explained at the within-colony level was smaller for body weight than for head width also in males (Table 4). The percentages of the total variation in body weight due to differences between colonies were similar in males and young queens in both years (Table 4).

## Discussion

My study gave evidence that both genetic and environmental factors affect size variation of sexuals in *Lasius niger* ants and that these may constrain worker control over sexual size. The genetic component of size variation was strongly suggested by the fact that daughters of different fathers differed significantly in body weight and head width despite having the same mother and having been reared in the same colony, at the same time and by the same worker force. Evidence for genetic components of sexual size has been found previously for polygynous ants with dimorphic queens (e.g. McInnes & Tschinkel 1995; R uppell *et al.* 2001), although these studies could not completely reject that maternal effects might underlie the size similarities between mothers and daughters.

Evidence for genetic components of sexual size unaffected by maternal effects had been established for only two other ants previously. The first is the imported fire ant, *Solenopsis invicta* in which body weight depends mainly on a single gene (Keller & Ross 1993, 1995; DeHeer *et al.* 1999; Goodisman

*et al.* 1999; DeHeer 2002). The fire ant system is, however, not likely to be representative for other ants because it includes several peculiarities (such as selective execution of some queen types, Keller & Ross 1993; Ross & Keller 1998) resulting perhaps from the bottleneck to which the population was subjected when introduced to North America (but see Krieger & Ross 2002). The second ant for which a genetic component of body size (head width) had previously been indicated is the polyandrous wood ant *Formica truncorum*. For this ant, the broad-sense estimate of heritability ( $h^2 = 0.51$ , Bargum *et al.* 2004) was similar to the ones for *L. niger*, for the 1 year that heritability was significant for *F. truncorum*. Thus, my heritability estimates for *L. niger* lay within the lower 90% confidence limit for the *F. truncorum* estimate (0.26) (Bargum *et al.* 2004). By contrast, heritability of body size did not vary significantly between years in *L. niger* while it did so for *F. truncorum* (Bargum *et al.* 2004), likely because my study included far more colonies. Some year-to-year variation in heritability estimates (30% in this study) for natural populations is, however, common (e.g. Falconer & Mackay 1996), because among-year environmental differences influences the total phenotypic variance, and hence the ratio of genetic variance to total variance that estimates heritability.

The causes behind a genetic component of sexual body size in *Formica truncorum* and other ants with dimorphic queens (e.g. *Leptothorax rugatulus* ants, R uppell *et al.* 1998, 2001; black fire ants *Solenopsis geminata*, McInnes & Tschinkel 1995) may however not be the same as in *Lasius niger*. This is because queens of different sizes in *F. truncorum* and queen-dimorphic species tend to follow different reproductive strategies, larger queens preferentially dispersing far and founding nests independently while smaller queens mate near their natal nests and seek re-adoption (McInnes & Tschinkel 1995; Sundstr m 1995; R uppell *et al.* 1998, 2001). Hence in such ants a genetic component of body size variation could be adaptive from the point of view of colonies and hence workers, allowing them to exploit divergent colony-founding strategies (see also Bargum *et al.* 2004).

In species such as *L. niger* where queens are monomorphic and all follow the same reproductive strategy (dispersal, mating on nuptial flights, independent colony founding), workers are not likely to benefit from queens of different patriline reaching different sizes and hence probably receiving unequal amounts of food. Differential investment in queens should only be in the fitness interests of workers in such species if they could recognize and nepotistically favour their own full-sisters. While it cannot be ruled out that such nepotism occurs in *L. niger*, no evidence of kin recognition has ever been found for polyandrous ants (Keller *et al.* 1997), although it seems to occur in some polygynous ants (Hannonen & Sundstr m 2003) where maternal effects may contribute to differentiate the brood of different lineages. The size differences among young

*L. niger* queens might be due to among-patriline variation in eclosion times. An earlier study on young colonies, however, found no evidence for genetic variation in the eclosion dates of female larvae in this ant (mean and variance of time to eclosion of first workers the same in 45 single- and 82 multiple-paternity colonies,  $P = 0.71$ , Fjerdingstad & Keller 2004). That daughters of some patrilines are larger seems more likely to result from effects arising after eclosion. For instance, daughters of different patrilines may vary in the size at which they are able to undergo pupation or they may carry different alleles that affect their success at begging food from workers. Such selfish strategies could increase the fitness of young queens (larger queens have a higher reproductive output, Fjerdingstad & Keller 2004), while lowering overall colony fitness. The opposing selective pressures (direct benefits for individuals achieving a large size; workers attempting to limit sexual size by limiting food and so likely decreasing the fitness of lines carrying genes for large size) might explain why genetic variation for size exists in *L. niger*.

The mechanism for genetically based size similarities between related individuals (e.g. through similar minimum sizes at pupation or through similar begging levels) has not been addressed in social insects. However, for the particular case of the fire ant, *Solenopsis invicta*, it has been shown that the sizes achieved by young queens depend not only on their own genotypes but also on the genotypic composition of the workers that rear them (Keller & Ross 1995). This suggests that *S. invicta* workers of different genotypes feed developing larvae of varying genotypes differently, but the mechanistic factors underlying this (for instance, variation in begging levels and in responsiveness to begging) are not known. Genetic variation in begging levels of sexuals and responsiveness of workers to begging, however, fit a scenario of conflict between workers and developing sexuals over resource allocation.

Variation among workers from different colonies in their responsiveness to begging, or more generally, in their capacity to restrain selfishness by developing sexuals, may have contributed to inflating among-colony differences in sexual body weight in *L. niger*. The pattern of significant common-colony effects for body weight (strongly dependent on post-eclosion feeding) but not head width (determined by feeding during the larval period) might be consistent with this. Controlling food allocation to imagos (eclosed sexuals) who can interact aggressively and move very actively about, may be more difficult for workers than controlling feeding of larvae who can only beg. If so, differences between workers of different colonies in the capacity to restrain sexual selfishness would precisely be reflected most strongly in post-eclosion traits of sexuals. Cross-fostering studies could allow a decisive test of whether workers of different colonies vary in their capacity to control resource allocation to individual sexuals.

Other explanations for the common-colony effects inflating among-colony differences in sexual size in *L. niger* are possible. Colonies producing large sexuals may have been egg-limited and so may not have had the option to use extra resources for producing more sexuals. *Lasius* queens are, however, extremely well endowed for egg production, having large gasters and a very large number of ovarioles (Tschinkel 1987; personal observation). It seems more likely that colonies investing more in individual sexual body weight had experienced an unpredicted resource abundance late in the season, when it was too late to start rearing more new sexuals (Rosenheim *et al.* 1996; see also Ode & Rissing 2002). That is, *L. niger* workers may not be able to control the flow of resources during the entire reproductive season but they may be trying to make the 'best of a bad job' when investing resources into each sexual at any given time. That ant workers will feed individual sexuals more when more resources are available late in the season has been demonstrated in experimental studies (e.g. Aron *et al.* 2001). Two lines of evidence are consistent with the hypothesis that between-colony size variation in *L. niger* sexuals is inflated by variation in late-season resource availability. First, as I noted above, common-colony environmental effects significantly inflated among-colony differences in body weight (determined by pre- and post-pupal feeding) but not head width (determined only by prepupal feeding). Second, the population-level coefficient of variation in sexuals was greater for body weight than head width. Thus, *L. niger* workers seem to start out investing similar sex-specific amounts in each male and young queen larva but some colonies (those experiencing resource shortages) end up investing relatively little in late season and others (those encountering resource abundance) more. Experimental studies varying resource availability along a season will, however, be needed, because the greater among-colony variation in sexual body weight than head width also fits the worker–sexual conflict scenario (see above).

My study suggested that a considerable part of sexual size variance within colonies may result from workers by error not feeding exactly the same amount of food to every developing larva (*L. niger* brood is kept in loose brood piles), or from selfish competition for food among individual queen larvae. A substantial proportion of the total variance in body weight and head width of young queens was due to variation between offspring within patrilines and was mainly explained by environmental effects. Behavioural studies of queen larvae and workers could distinguish between the hypotheses of worker errors and brood conflict over resource allocation (if more actively begging larvae are fed more and become larger than other larvae, this would confirm the conflict scenario) (see also Backus 1993; Rosenheim *et al.* 1996; Wiernasz *et al.* 2001). Such studies could also establish whether the size differences among



young queens of different patriline were due to differences in begging rates, as hypothesized under the conflict scenario.

In conclusion, my analyses on sexual size variation in *L. niger* demonstrated genetic effects but also strong common-colony environment effects. These findings suggest that ant workers are far from completely able to control the nest environment in the face of variation in food availability or selfishness by sexuals, and therefore seem to have only partial control over an important component of colony fitness, the size of sexuals.

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## References

- Abell AJ, Cole BJ, Reyes R, Wiernasz DC (1999) Sexual selection on body size and shape in the Western harvester ant, *Pogonomyrmex occidentalis* Cresson. *Evolution*, **53**, 535–545.
- Aron S, Keller L, Passera L (2001) Role of resource availability on sex, caste and reproductive allocation ratios in the Argentine ant *Linepithema humile*. *Journal of Animal Ecology*, **70**, 831–839.
- Backus VL (1993) Packaging of offspring by nests of the ant *Leptothorax longispinosus*: parent–offspring conflict and queen–worker conflict. *Oecologia*, **95**, 283–289.
- Bargum K, Boomsma JJ, Sundström L (2004) A genetic component to size in queens of the ant, *Formica truncorum*. *Behavioral Ecology and Sociobiology*, **57**, 9–16.
- Becker WA (1984) *Manual of Quantitative Genetics*, 4th edn. Academic Enterprises, Pullman Washington.
- Bennett P, Owens I (2002) *Evolutionary Ecology of Birds: Life Histories, Mating Systems, and Extinction*. Oxford University Press, Oxford.
- Bernasconi G, Keller L (1996) Reproductive conflicts in cooperative associations of fire ant queens (*Solenopsis invicta*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **263**, 509–513.
- Bernasconi G, Keller L (1999) The effect of queen phenotype and social environment on early queen mortality in incipient colonies of the fire ant, *Solenopsis invicta*. *Animal Behaviour*, **57**, 371–377.
- Blanckenhorn W (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, **75**, 385–407.
- Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.
- Brian MV (1983) *Social Insects: Ecology and Behavioural Biology*. Chapman and Hall, London.
- Buschinger A (1975) Eine genetische komponente im polymorphismus der dulotischen ameise *Harpagoxenus sublaevis*. *Naturwissenschaften*, **62**, 239.
- Buschinger A (1978) Genetisch bedingte entstehung geflügelter weibchen bei der sklavenhaltenden ameise *Harpagoxenus sublaevis* (Nyl.) (Hym.: Form.). *Insectes Sociaux*, **25**, 163–172.
- Chapuisat M, Sundström L, Keller L (1997) Sex-ratio regulation: the economics of fratricide in ants. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **264**, 1255–1260.
- Cook JM (1993) Sex determination in Hymenoptera: a review of models and evidence. *Heredity*, **71**, 421–435.
- Creemers B, Billen J, Gobin B (2003) Larval begging behaviour in the ant *Myrmica rubra*. *Ethology Ecology and Evolution*, **15**, 261–272.
- Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.
- Davidson DW (1982) Sexual selection in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). *Behavioral Ecology and Sociobiology*, **10**, 245–250.
- DeHeer CJ (2002) A comparison of the colony-founding potential of queens from single- and multiple-queen colonies of the fire ant *Solenopsis invicta*. *Animal Behaviour*, **64**, 655–661.
- DeHeer CJ, Goodisman MAD, Ross KG (1999) Queen dispersal strategies in the multiple-queen form of the fire ant *Solenopsis invicta*. *American Naturalist*, **153**, 660–675.
- Endler JA, May RM (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton, New Jersey.
- Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics*, 4th edn. Longman, Essex.
- Fjerdingstad EJ (2004) Multiple-paternity and colony homeostasis in *Lasius niger* ants. *Behavioral Ecology and Sociobiology*, **56**, 50–58.
- Fjerdingstad EJ, Boomsma JJ (1997) Variation in size and sperm content of sexuals in the leafcutter ant *Atta colombica*. *Insectes Sociaux*, **44**, 209–218.
- Fjerdingstad EJ, Keller L (2004) Relationships between phenotype, mating behaviour, and fitness of queens in the ant *Lasius niger*. *Evolution*, **58**, 1056–1063.
- Fjerdingstad EJ, Gertsch PJ, Keller L (2002) Why do some social insect queens mate with several males? Testing the sex ratio manipulation hypothesis in *Lasius niger*. *Evolution*, **56**, 553–562.
- Fjerdingstad EJ, Gertsch PJ, Keller L (2003) The relationship between multiple mating by queens, within-colony genetic variability and fitness in the ant *Lasius niger*. *Journal of Evolutionary Biology*, **16**, 844–853.
- Fraser VS, Kaufmann B, Oldroyd BP, Crozier RH (2000) Genetic influence on caste in the ant *Camponotus consobrinus*. *Behavioral Ecology and Sociobiology*, **47**, 188–194.
- Goodisman MAD, Mack PD, Pearse DE, Ross KG (1999) Effects of a single gene on worker and male body mass in the fire ant *Solenopsis invicta*. *Annals of the Entomological Society of America*, **92**, 563–570.
- Hamilton WD (1964) The genetical evolution of social behaviour. I, II. *Journal of Theoretical Biology*, **7**, 1–52.
- Hannonen M, Sundström L (2003) Worker nepotism among polygynous ants. *Nature*, **421**, 910.
- Heinze J (1998) Intercastes, intermorphs, and ergatoid queens: who is who in ant reproduction? *Insectes Sociaux*, **45**, 113–124.
- Heinze J, Buschinger A (1989) Queen polymorphism in *Leptothorax* spec. A: its genetic and ecological background (Hymenoptera: Formicidae). *Insectes Sociaux*, **36**, 139–155.
- Herbers JM (1990) Reproductive investment and allocation ratios for the ant *Leptothorax longispinosus*: sorting out the variation. *American Naturalist*, **136**, 178–208.
- Hölldobler B, Wilson EO (1990) *The Ants*. Springer-Verlag, Berlin.
- Jemielity S, Keller L (2003) Queen control over reproductive decisions — no sexual deception in the ant *Lasius niger*. *Molecular Ecology*, **12**, 1589–1597.
- Jennions MD, Moller AP, Petrie M (2001) Sexually selected traits and adult survival: a meta-analysis. *Quarterly Review of Biology*, **76**, 3–36.

- Kaptein N, Billen J, Gobin B (2005) Larval begging for food enhances reproductive options in the ponerine ant *Gnamptogenys striatula*. *Animal Behaviour*, **69**, 293–299.
- Keller L, Ross KG (1993) Phenotypic basis of reproductive success in a social insect: genetic and social determinants. *Science*, **260**, 1107–1110.
- Keller L, Ross KG (1995) Gene by environment interaction: effects of a single gene and social environment on reproductive phenotypes of fire ant queens. *Functional Ecology*, **9**, 667–676.
- Keller L, Ross KG (1999) Major gene effects on phenotype and fitness: the relative costs of Pgm-3 and Gp-9 in introduced populations of the fire ant *Solenopsis invicta*. *Journal of Evolutionary Biology*, **12**, 672–680.
- Keller L, Sundström L, Chapuisat M (1997) Male reproductive success: paternity contribution to queens and workers in *Formica* ants. *Behavioral Ecology and Sociobiology*, **41**, 11–15.
- Kipyatkov VE (1993) Annual cycles of development in ants: diversity, evolution, regulation. *Proceedings of the Colloquia on Social Insects*, **2**, 25–48.
- Krieger MJB, Ross KG (2002) Identification of a major gene regulating complex social behavior. *Science*, **295**, 328–332.
- Liu F-H, Smith SM (2000) Estimating quantitative genetic parameters in haplodiploid organisms. *Heredity*, **85**, 373–382.
- McInnes DA, Tschinkel WR (1995) Queen dimorphism and reproductive strategies in the fire ant *Solenopsis geminata* (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology*, **36**, 367–375.
- Ode PJ, Rissing SW (2002) Resource abundance and sex allocation by queen and workers in the harvester ant, *Messor pergandei*. *Behavioral Ecology and Sociobiology*, **51**, 548–556.
- Parker GA, Royle NJ, Hartley IR (2002) Intrafamilial conflict and parental investment: a synthesis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **357**, 295–307.
- Rosenheim JA, Nonacs P, Mangel P (1996) Sex ratios and multifaceted parental investment. *American Naturalist*, **148**, 501–535.
- Ross KG, Keller L (1998) Genetic control of social organization in an ant. *Proceedings of the National Academy of Sciences, USA*, **95**, 14232–14237.
- Rüppell O, Heinze J, Hölldobler B (1998) Size-dimorphism in the queens of the North American ant *Leptothorax rugatulus* (Emery). *Insectes Sociaux*, **45**, 67–77.
- Rüppell O, Heinze J, Hölldobler B (2001) Complex determination of queen body size in the queen dimorphic ant *Leptothorax rugatulus* (Formicidae: Hymenoptera). *Heredity*, **87**, 33–40.
- Simmons LW (2001) *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton University Press, Princeton, New Jersey.
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *American Naturalist*, **108**, 499–506.
- Sokal RR, Braumann CA (1980) Significance tests for coefficients of variation and variability profiles. *Systematic Zoology*, **29**, 50–66.
- Sokal RR, Rohlf FJ (1995) *Biometry*. W.H. Freeman and Co., New York.
- Sundström L (1995) Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. *Behavioral Ecology*, **6**, 132–139.
- Trivers RL (1972) Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (ed. Campbell B), pp. 136–179. Heineman, London.
- Tschinkel WR (1987) Relationship between ovariole number and spermathecal sperm count in ant queens: a new allometry. *Annals of the Entomological Society of America*, **80**, 208–211.
- Tschinkel WR (1993) Resource allocation, brood production and cannibalism during colony founding in the fire ant, *Solenopsis invicta*. *Behavioral Ecology and Sociobiology*, **33**, 209–223.
- Tschinkel WR (1996) A newly-discovered mode of colony founding among fire ants. *Insectes Sociaux*, **43**, 267–276.
- Van der Have TM, Boomsma JJ, Menken SBJ (1988) Sex investment ratios and relatedness in the monogynous ant *Lasius niger* (L.). *Evolution*, **42**, 160–172.
- Vander-Meer RK, Morel L, Lofgren CS (1992) A comparison of oviposition rates from monogyne and polygyne fire ants, *Solenopsis invicta*. *Physiological Entomology*, **17**, 384–390.
- Wagner D, Gordon DM (1999) Colony age, neighborhood density and reproductive potential in harvester ants. *Oecologia*, **119**, 175–182.
- Ward PA (1983) Genetic relatedness and colony organization in a species complex of ponerine ants. II. Patterns of sex ratio investment. *Behavioral Ecology and Sociobiology*, **12**, 301–307.
- Wiernasz DC, Cole BJ (2003) Queen size mediates queen survival and colony fitness in harvester ants. *Evolution*, **57**, 2179–2183.
- Wiernasz DC, Yenchis J, Cole BJ (1995) Size and mating success in males of the Western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *Journal of Insect Behaviour*, **8**, 523–531.
- Wiernasz DC, Slater AK, Abell AJ, Cole BJ (2001) Male size, sperm transfer, and colony fitness in the Western harvester ant, *Pogonomyrmex occidentalis*. *Evolution*, **55**, 324–329.

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