



Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae)

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Insects with facultative social behaviour permit direct examination of factors associated with the expression of division of labour: why do some females remain in their natal nest as nonreproductive foragers, while others disperse? The facultatively social halictid bee *Megalopta genalis* shows strong reproductive division of labour, associated with body size (foragers tend to be smaller than queens and dispersers). We used *M. genalis* to test two hypotheses for the expression of worker behaviour: (1) queens suppress reproduction by subordinates, which then forage, and (2) small-bodied females are handicapped as reproductives, and therefore take on a foraging role to assist a more fertile relative (the 'subfertility' hypothesis). We removed queens from 19 nests and found that the remaining foragers enlarged their ovaries and reproduced at the same rate as solitary reproductives from unmanipulated (nonremoval) nests. This observation suggests that queen dominance limited reproduction by subordinates, and that foragers were not handicapped reproductives. To investigate the effect of body size variation on reproductive rate in the absence of social interactions, we placed single, newly eclosed females into 31 observation nests. Body size was not correlated with reproductive output or with the females' tenure in the observation nests. Nor was there any correlation between body size and number of brood cells in 21 solitary-female nonremoval nests. Taken together these data show that small females were not inherently poor reproductives. We also found that ovaries of reproductive females from social groups were larger than those of solitary reproductives, suggesting that social structure shapes ovary development.

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Facultatively eusocial species are those in which females can reproduce solitarily or live within social groups as a reproductive or a worker. Such taxa are critical for studying the origins and evolution of reproductive division of labour because they permit direct examination of the factors that promote a switch from solitary to social life (Wcislo 1997, 2000; Field et al. 2000; Smith et al. 2003, 2007, 2008; Schwarz et al. 2007). The facultatively social bee *Megalopta genalis* (Hymenoptera: Halictidae) shows strong reproductive division of labour associated with body size: foragers tend to be smaller than queens, dispersers and solitary reproductives (Smith et al. 2008). An association between body size and social

role (caste) could result from multiple mechanisms, two of which we test here. First, if smaller individuals are less fecund, they may be more likely to act as workers because they can realize higher fitness by helping than by nesting alone (the subfertility hypothesis; West-Eberhard 1975, 1978, 1981; Craig 1983). It is often assumed that insect body size generally correlates with reproductive ability (e.g. Lin & Michener 1972; Crespi & Ragsdale 2000), although the evidence across bee species is mixed (Bosch & Vicens 2006) and there is presently no evidence supporting the subfertility hypothesis in a facultatively social bee or wasp (Field & Foster 1999).

Second, caste differentiation may result from social competition. A reproductive queen may suppress the reproduction of some nestmates, even if those nestmates are fully capable of reproducing (Michener & Brothers 1974; West-Eberhard 1987, 1996). Previous studies in other sweat bee species have correlated observed aggression with body size and the expression of foraging behaviour, suggesting that maternal aggression towards younger or smaller females may induce them to become subordinate workers

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(Michener & Brothers 1974; Kukuk & May 1991; Richards & Packer 1994; Richards et al. 1995). These two hypotheses, subfertility and queen dominance, are not mutually exclusive, and in both cases the queen of a social insect colony could manipulate factors such as body size, thus influencing whether a given daughter stays as a nonreproductive helper (Alexander 1974; Michener & Brothers 1974; West-Eberhard 1975).

Approximately one-half of *M. genalis* nests are social, and these typically contain a queen, primary forager, and sometimes young female(s) possibly waiting to disperse (Smith et al. 2003, 2007, 2008; Wcislo et al. 2004; Wcislo & Gonzalez 2006). Even in two-bee groups, division of labour is strong (Wcislo & Gonzalez 2006; Smith et al. 2008), and foragers can gain indirect fitness through helping (Smith et al. 2003, 2007). At the species level, behaviour varies from solitary to eusocial, but the behavioural and reproductive flexibility of individual females has not been directly assessed.

We removed the queen and any other bees from the nest to study the reproductive behaviour of the primary foragers when social competitors are removed. This manipulation allowed us to test whether nonreproductive foragers become replacement reproductives (Michener 1990; Chandrashekar & Gadagkar 1992; Mueller 1996), and, if so, whether they enlarge their ovaries and reproduce at the same rate as females in naturally solitary nests (hereafter 'nonremoval solitary nests').

We next compared the ovaries of reproductive females in social nests, including replacement reproductives, to those of solitary reproductives. Ovary size may reflect increased endocrine activity associated with aggression (Röseler et al. 1984, 1985; Bloch et al. 2000, 2002; Geva et al. 2005). If generally true, then reproductive females within socially competitive environments should have larger ovaries than those of solitary reproductives.

Finally, to test whether body size is correlated with reproductive ability in the absence of maternal social interactions, we placed newly emerged females singly into observation nests in the field. If larger females are better reproductives, then body size should be correlated with reproductive output.

METHODS

Study Site

All studies were conducted on Barro Colorado Island (BCI; 9°09'N, 79°51'W), Republic of Panama. BCI is a lowland tropical semideciduous moist forest (Rau 1933; Leigh 1999).

Study Species

Megalopta genalis females excavate tunnel nests in dry, rotting sticks (dead branches or lianas) that are usually suspended in vegetation or otherwise raised above the ground, and nests are singly founded (Smith et al. 2003; Wcislo et al. 2004; Tierney et al. 2008a). On BCI, emerging *M. genalis* adult females can disperse to reproduce solitarily or they can remain in their natal nest and act as workers. Solitary nests may later become social colonies. Workers may be mated or unmated (A.R.S., K.M.K. & W.T.W., unpublished data). Mean body size of workers is about 90% that of social queens and solitary reproductives (Smith et al. 2008). Nests need not have helpers: some nests remain solitary despite rearing many female offspring (Wcislo & Gonzalez 2006; Smith et al. 2007). Based on observation nests (Wcislo & Gonzalez 2006), newly eclosed females are presumably daughters of the nest foundress, but this has not been confirmed with genetic analyses. About one-third–one-half of all nests are multifemale during the tropical dry and early wet seasons (December–July), when the bees are reproductively most

active. Foraging and cell provisioning continues through the wet season at diminishing levels until approximately October, when it largely ceases because of a scarcity of floral resources. Bees initiate new nests throughout this time, and there is little synchrony in offspring production among nests (Wcislo et al. 2004). Both males and females are produced throughout the year, although male offspring are most common in late February and early March (Wcislo et al. 2004).

Queen-removal Nests

We modified 20 naturally occurring multifemale nests in the field for censusing and marking, by cutting away part of the side of the stick that formed the tunnel wall. We covered the exposed tunnel with flexible, transparent acetate secured with rubber bands, creating a window into the nest. The nest was then hung inside an opaque PVC pipe. One end of the pipe was sealed and the other end was covered with opaque plastic through which the first ~2 cm of the nest (including the nest entrance) protruded. Thus, the portion of the nest exposed for observation was protected from light and water inside the pipe, but the bees could enter and exit through the natural entrance. Bees were marked on the thorax with unique combinations of white dots from a Decocolor paint pen.

Foraging Observations

We recorded foraging trips using a Sony MiniDV infrared camcorder mounted on a tripod at the nest entrance during the two main periods of *Megalopta* foraging activity: approximately 90 min before sunrise and 60 min after sunset (Wcislo et al. 2004; Kelber et al. 2006). After at least five observed foraging trips, the female with the most trips was designated the primary forager. The queen performs less than 10% of all foraging trips in multifemale *Megalopta* nests (Wcislo & Gonzalez 2006), so extensive observations of foraging were not necessary to identify behavioural roles. In cases where a second female emerged into a single-female nest, we waited until the second female began foraging before removing the queen, as solitary queens forage for nectar and feed newly emerged offspring for approximately 1 week before the offspring either disperse or begin foraging (Wcislo & Gonzalez 2006).

Queen Removals

After the primary forager was identified, all other bees were removed from the nest ($N = 20$ nests; 33 individuals removed). We placed the heads and thoraces of the removed bees in 100% ethanol and their abdomens (metasoma) in Kahle's fixative (18:1:1 solution of 70% ethanol, formalin and glacial acetic acid) for subsequent dissections. The nest, now containing just the primary forager and brood, was left in the field for 4 weeks and checked once weekly for numbers of adult females and males, and the number and status of brood cells. These inspections were the only instances in which the nests were removed from the PVC pipes. After 4 weeks, the nests, the original primary foragers, and any newly emerged bees (presumably offspring from the original queen) were collected. Queen removals took place between 31 January and 11 March 2004, and nests were collected between 28 February and 8 April 2004.

Nonremoval Nests

We used solitary and social nests from which no females were removed for comparison with the experimental nests. Fifteen nests were marked during 14–24 February 2004 and collected after 4 weeks (3–13 March 2004; 9 were solitary, 6 were social). We collected eight more natural nests (4 solitary, 4 social) during 16–22

March 2004. Eight nests that we modified for observation were solitary, and a ninth developed into a social colony but too late to be included in the removal experiment. These nine nests were collected during 6–24 March 2004. Together, these constituted 21 solitary and 11 social nonremoval nests for comparisons with the experimental nests.

Dissections and Measurements

The metasoma ('abdomens') of all bees were dissected for ovarian measurement. We used a stereomicroscope fitted with a digital camera to photograph ovaries dorsally at 20× magnification, and calculated area using Adobe Photoshop 6.0 calibrated with photos of a similarly photographed millimetre standard slide (Reichert CAT 1400). We report the mean of the left and right ovary areas. We had no a priori knowledge of the minimum size of reproductively active ovaries, and therefore we used the smallest value for mean (left and right) ovary area seen in the 21 solitary, reproductively active nonremoval nests (1.56 mm^2), as the cutoff for 'enlarged ovaries' (following Smith et al. 2008).

As a metric of body size, we measured head width (the distance across the eyes and head, measured through the antennal sockets and perpendicular to the frontal midline) from digital photographs as described above. This is a reliable measure of body size in augochlorine bees (Tierney et al. 2008b).

Queen Confirmation

We used ovary area measurements to confirm our behavioural definition of which female was the queen (Smith et al. 2008). All of the removed queens had enlarged ovaries that were characteristic of reproductive females, and mean ovary area did not differ from the presumed reproductives (i.e. females with largest ovaries) of multifemale nonremoval nests (mean \pm SD: removed queens: 2.76 ± 0.49 , nonremoval presumed queens: 3.01 ± 0.71 ; $t_{15} = 1.04$, $P = 0.30$). If an experimental nest had three or more females, all other bees were removed in addition to the queen to isolate the primary forager. These were probably future reproductives waiting to disperse (Wcislo & Gonzalez 2006). None of the predispersal females had enlarged ovaries (see Smith et al. 2008 for details).

Categories of Brood Development

We classified all brood cells as being in one of the following developmental categories: 'empty' refers to apparently intact cells with no brood or pollen (broken cells were excluded); 'open with pollen' refers to cells being provisioned with pollen, but not yet containing an egg; 'egg' is a sealed cell with an egg laid on a pollen mass; 'small,' 'medium,' and 'large' larvae all refer to feeding larvae with pollen in the cell; 'prepupa' is a larva that has consumed its pollen and has defecated but has not yet pupated; 'light-eyed pupa' is a pupa with white or pink eyes; 'dark-eyed pupa' is a pupa with dark red or black eyes; 'pigmented pupa' is a pupa with the entire cuticle pigmented; and 'callow adult' is a fully formed adult with extended wings that has not emerged from its cell. To compare the distribution of brood stages between nonremoval and experimental groups, we pooled 'open with pollen' and 'egg' cells into one category, all larval cells (including prepupae) into the category 'larvae', and all pupal cells (including callow adults) into the category 'pupae'.

To distinguish between the offspring of replacement reproductives and those of the original removed queens, we assigned to the replacement reproductive all offspring that were at the stage 'medium larvae' or younger. This is a conservative measure that is unlikely to err and assign immatures left behind from the removed queens to their replacements, because egg-to-adult development

time is about 36 days (Wcislo et al. 2004; Wcislo & Gonzalez 2006) and our experimental period was 28 days. We could not record cell contents at the time of queen removal because the cells were closed.

Plexiglas Observation Nests

To test whether body size influences reproductive output in the absence of social interactions, we constructed 31 observation nests made of balsa wood between two panes of Plexiglas, and hung these in the field under plastic roofs (Wcislo & Gonzalez 2006). We reared immature females collected from natural nests until 1–2 days before eclosion, at which time they were placed inside a nest (one female per nest). We checked the nests every 4 days to record any newly constructed cells and emerging offspring. Nests were placed in the field between 28 January and 25 March 2007, and monitored until 13 June 2007.

Statistics

We calculated all statistics in SPSS 17.0 (SPSS Inc., Chicago IL, U.S.A.). All means are reported \pm SD, and all P values are two tailed. The analysis of queen tenure and head width uses Spearman rank correlation because queen tenure was not normally distributed. All other variables did not significantly differ from a normal distribution (Shapiro–Wilk test: $P > 0.05$), so parametric tests were used.

RESULTS

Forager Behavioural Responses to Queen Removal

In 19 of the 20 queen-removal nests (95%), the primary foragers remained at the nest until offspring emerged. These offspring were presumably those of the original queen. One nest was abandoned, and ants consumed the cell contents. Two of the 19 primary foragers that remained at their nest until brood emergence disappeared before collection.

Foragers Enlarged their Ovaries following Queen Removal

Of the 17 primary foragers collected after 4 weeks, all but one (94%) had enlarged ovaries (Fig. 1). Ovary size did not differ between the postremoval primary foragers (hereafter referred to as 'replacement reproductives') and the queens that they replaced (paired t test: $t_{14} = 1.17$, $P = 0.26$; mean of replacement reproductives = $2.46 \pm 0.85 \text{ mm}^2$, mean of queens removed from the same nests = $2.71 \pm 0.56 \text{ mm}^2$; Fig. 1). The visibly enlarged ovaries of one replacement reproductive were sufficiently damaged during dissection to preclude measurement.

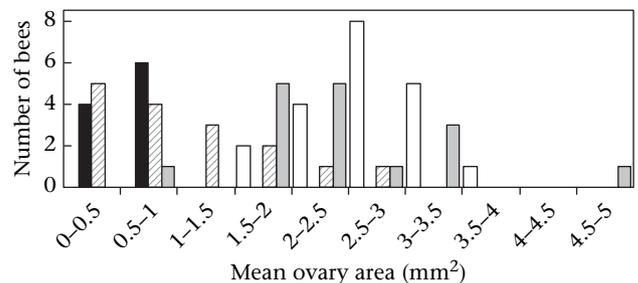


Figure 1. Ovary area of all females from experimental queen-removal nests. Removed queens (□) and predispersal females (those bees present at removal besides the queens and primary foragers; ▨) were removed at the beginning of the study. Primary foragers left alone after queen removal (■) and females that emerged after queen removal (▩) were removed at the conclusion of the study. Data on removed queens and predispersal females are reported in Smith et al. (2008).

It is impossible to know the size of the primary foragers' ovaries before queen removal. However, analysis of the 11 multifemale nonremoval nests suggests that the primary foragers had small ovaries before removal. Only one (9%) of the multifemale nonremoval nests contained more than one bee with an ovary area above the 1.56 mm² threshold for 'enlarged' (see *Methods*), and it was only slightly above this threshold (1.57 mm²). A second female from another nest had an ovary area just under the threshold (1.55 mm²). With the exception of these two bees, there was no overlap in ovary area between the presumed workers of multifemale nests and the reproductives of either the solitary or multifemale nonremoval nests (Fig. 2). Likewise, only 1 of 11 (9%) multifemale nests from a separate collection at the same site in 2003 contained two females with developed ovaries (Smith et al. 2008). Thus, while some of the primary foragers may have had enlarged ovaries prior to queen removal, the observed values in our experiment (16 of 17 primary foragers with enlarged ovaries) differed significantly from those expected based on the frequency of nonqueens with enlarged ovaries from the nonremoval nests (Pearson chi-square test: $\chi^2_1 = 66.73$, $P < 0.001$; this analysis conservatively included the 1.55 mm² value as 'enlarged'). These findings suggest that the primary foragers enlarged their ovaries following queen removal.

Foragers Reproduced after Queen Removal

Fifteen of 16 primary foragers with enlarged ovaries also laid eggs. We conservatively assigned only medium-sized and smaller (younger) larvae, but not large larvae or pupae, to the replacement reproductives, while large larvae and pupae were not attributed to her to avoid any possibility of misassigning the removed queen's offspring to her replacement (see *Methods*). Cells provisioned with fresh pollen, but not yet containing an egg, were also assigned to the replacement reproductives. Replacement reproductives provisioned 2.19 ± 0.98 brood cells during the study. This output is similar to the mean number of cells with pollen and/or medium-sized or smaller larvae in the solitary nonremoval nests during the same time (2.00 ± 1.25; two-sample *t* test: $t_{37} = -0.58$, $P = 0.56$).

In each of the three nests in which the primary forager did not develop into a replacement reproductive (two disappearances and one without enlarged ovaries), one of the newly emerged females from the removed queen's brood had enlarged ovaries. All three nests also had early stage larvae or cells provisioned with pollen.

Although replacement reproductives were able to match the solitary nonremoval reproductives' output of young larvae, analysis

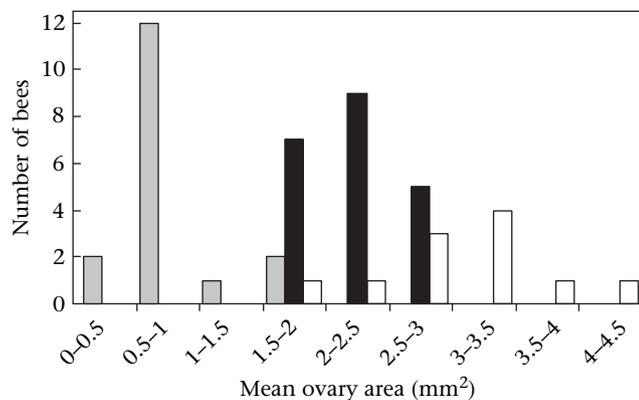


Figure 2. Ovary area of females from nonremoval nests. ■: solitary reproductives; □: the female with the largest ovaries (presumed queen) from multifemale nests; ◻: other females from the multifemale nests.

of the cell contents of both groups of nests suggests that there was a lag time before the replacements began to reproduce (Fig. 3). While both groups contained similar numbers of early stage brood, the nonremoval nests had more pupae. This pattern suggests that oviposition declined immediately after queen removal, as the eggs laid at the beginning of the experiment would probably be the pupae collected 4 weeks later. The experimental nests also had more empty cells, consistent with a lag before empty cells were refurbished and provisioned. The distribution of brood stages differed significantly between the two groups (Pearson chi-square test: $\chi^2_3 = 23.45$, $N = 121$ queen removal cells, 231 nonremoval cells, $P < 0.001$; Fig. 3). There was little difference between nonremoval and experimental nests in the percentage of cells containing large larvae or prepupae, suggesting that the prepupal stage may be a more accurate, if less conservative, cutoff for assigning brood to the replacement reproductives (3.1% versus 2.6% large larvae, and 15.5% versus 13.3% prepupae in nonremoval versus experimental nests, respectively). We could not compare offspring sex ratio between the two groups because sex cannot be determined until the pupal stage.

Age

We found no correlation between age of replacement reproductives and ovary size at collection (Pearson correlation: $r_9 = 0.01$, $P = 0.85$). However, this analysis was limited only to those nests in which the primary forager emerged during the study ($N = 11$; 58%). Eight (42%) of the nests already had primary foragers present when the nest was discovered, so we did not know their age. If they were generally older than the bees that emerged during the study, this would bias our analysis. There was no significant difference, however, between the mean ovary size after removal of known-age replacement reproductives (2.61 ± 0.82 mm²) and those present when their nest was discovered (2.01 ± 0.92 mm²; two-sample *t* test: $t_{14} = 1.41$, $P = 0.18$). In at least four of these nests, the primary forager was not the first adult female to emerge into the nest. The other eight nests may have had previous emergences before we discovered them.

Emerged Bees

At the conclusion of the experiment, 10 nests (53%) contained adult females that emerged after queen removal ($N = 16$ bees).

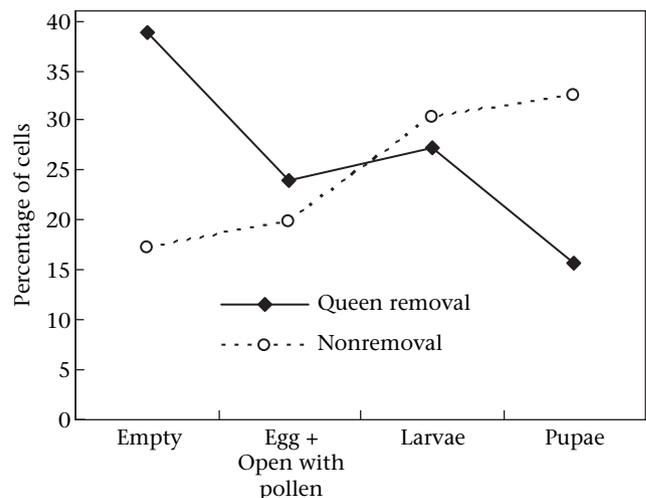


Figure 3. Cell contents of experimental and nonremoval nests, expressed as the percentage of all the cells in each treatment group. Empty cells contained no brood. Other stages are listed in order of increasing maturity.

These bees were presumably offspring of the original queen. In one nest, the emerged bee had enlarged ovaries, while the original primary forager did not, suggesting that the original forager had been usurped as replacement reproductive. The two cases in which the primary forager disappeared before collection and newly emerged bees became replacement queens may also represent cases of usurpation. Most other newly emerged bees did not have enlarged ovaries (Fig. 1), but because they were generally young, they may not have had time to fully develop their ovaries. However, one experimental nest had two females with enlarged ovaries: the former primary forager (4.56 mm^2 ; the largest value in the study) and a female that had emerged into the nest 12–18 days before collection (2.53 mm^2 ; the largest value of any female that did not have the largest ovaries in her nest). In two other nests, bees that emerged into the nest had intermediately developed ovaries (1.29 mm^2 , 16–22 days old, and 1.33 mm^2 , 1–6 days old, respectively). The younger of these bees had one ovary that was 2.0 mm^2 , showing that *M. genalis* is capable of rapid ovarian development. The queen of the nest with the 1.33 mm^2 emerged bee had the second-largest ovaries of all replacement queens (3.46 mm^2), while the queen of the 1.29 mm^2 emerged bee had average-sized ovaries (2.47 mm^2).

Multifemale Queens have Larger Ovaries than Solitary Reproductives

The presumed queens from multifemale nonremoval nests (those females with the largest ovaries in their nest) had significantly larger ovaries than the reproductives from solitary nonremoval nests (multifemale nonremoval mean = $3.01 \pm 0.71 \text{ mm}^2$, single-female nonremoval mean = $2.20 \pm 0.38 \text{ mm}^2$; two-sample *t* test: $t_{29} = 4.18$, $P < 0.001$). This could be due to increased productivity in multifemale nests (Smith et al. 2007), as ovary size and number of brood cells were positively correlated (Pearson correlation: $r_{29} = 0.55$, $P = 0.001$; Fig. 4a). However, analysis of the residuals of a linear regression of cell number on ovary size showed that queens in multifemale nests had larger ovaries than expected even after accounting for differences in productivity. Ovaries of seven of the 11 multifemale queens were larger than expected based on cell number, whereas ovaries of 16 of 20 solitary reproductives were smaller than expected (Pearson chi-square test: $\chi^2_1 = 5.90$, $P < 0.05$). Finally, the average residual value for social queens (0.24 ± 0.70) was significantly greater than that for solitary reproductives (-0.16 ± 0.38 ; two-sample *t* test: $t_{29} = 2.09$, $P < 0.05$; Fig. 4a).

The replacement reproductives, including both those that were originally primary foragers and those that emerged into the nest after queen removal, showed a similar difference between multifemale and solitary reproductive ovary size (multifemale replacement mean = $2.85 \pm 0.90 \text{ mm}^2$, single-female replacement mean = $2.02 \pm 0.34 \text{ mm}^2$; $t_{17} = 2.69$, $P = 0.02$). There was no significant difference in reproductive output between the replacement reproductives collected alone and those collected with newly emerged nestmates, and the correlation between ovary size and productivity was not significant (Pearson correlation: $r_{17} = 0.37$, $P = 0.11$; Fig. 4b). Analysis of the residuals of a linear regression of cell number on ovary size showed that multifemale replacement reproductives had significantly larger residuals than solitary replacement reproductives (0.36 ± 0.44 and -0.33 ± 0.81 , respectively; two-sample *t* test: $t_{17} = 2.34$, $P < 0.05$), although the distribution of positive and negative residuals did not differ significantly (Pearson chi-square test: $\chi^2_1 = 1.35$, $P = 0.25$; Fig. 4b).

Body Size was not Correlated with Reproductive Output

Because nest survival and foundress tenure in our constructed Plexiglas observation nests varied from 24 to 128 days

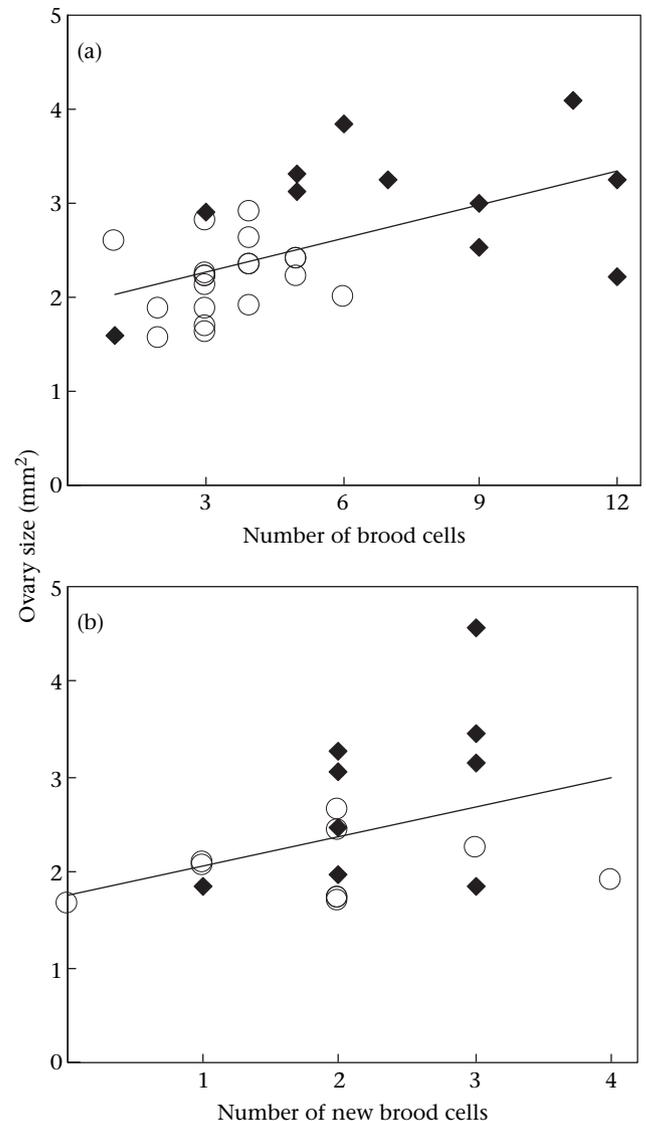


Figure 4. Relation between ovary size of reproductives and (a) number of brood cells produced by natural reproductives collected from nonremoval nests and (b) number of new brood cells produced by replacement reproductives from the experimental queen-removal nests. \blacklozenge : reproductives from multifemale nests; \circ : solitary reproductives. In both (a) and (b), the linear regression line is for all individuals.

(mean = 90.74 ± 27.49), we measured reproductive output as the rate of offspring produced per day of tenure. Reproductive rate ranged from 0 to 0.11 offspring per day (mean = 0.06 ± 0.03). Reproductive rate was not correlated with head width, our measure of body size for these bees (Pearson correlation: $r_{29} = 0.04$, $P = 0.84$), and foundress tenure was not correlated with head width (Spearman rank correlation: $r = 0.01$, $N = 31$, $P = 0.99$). There was no correlation between body size and number of brood cells at collection in the natural single-female nonremoval nests (Pearson correlation: $r_{29} = -0.19$, $P = 0.65$).

DISCUSSION

Most primary foragers were able to enlarge their ovaries and reproduce after their queens were removed. Thus, *M. genalis* foragers are capable of reproduction, but apparently are precluded from doing so by the presence of another, presumably dominant, female. This conclusion is consistent with most previous studies of sweat bees

(Michener 1990; Mueller et al. 1994), although foragers in some species do not retain reproductive ability (Michener 1990; Cronin & Hirata 2003), and replacement queens of temperate species are not always capable of entering diapause and subsequently establishing new nests (Yanega 1989). Some replacements may have already had enlarged ovaries at the beginning of our study, but the rarity of nests containing more than one female with developed ovaries among the nonremoval collections and among an independent sample of nests from the same population in 2003 (Smith et al. 2007, 2008) suggests that most replacements began the study with small ovaries, and enlarged them following release from social competition.

Not only could *M. genalis* replacements enlarge their ovaries, but they could reproduce as well as queens in naturally occurring nonremoval nests. Our study only followed the replacement reproductives for 4 weeks, so it is not clear whether these females would be able to sustain a similar reproductive output over a longer duration. Furthermore, we do not know whether the replacements in our study would have been able to find and initiate new nests as well as natural dispersers. Nevertheless, the similar short-term reproductive output of replacements relative to naturally occurring queens does not support the hypothesis that foragers are inherently subfertile or otherwise poor reproductives.

Our second study, in which we placed reared females alone into observation nests, suggests that body size is not correlated with reproductive ability in the absence of social competition. This is consistent with the data from the naturally occurring single-female nests in this study and naturally occurring social nests from Smith et al. (2008), neither of which showed a correlation between the body size of reproductive females and their reproductive output. Taken together, the results of the queen removal and the artificial nest experiments suggest that body size influences caste expression through dominance interactions rather than through variation in reproductive capabilities among small females. Although body size is determined during larval development, its correlation with caste appears to result from adult interactions.

The lack of late-stage brood in queen-removal nests relative to the nonremoval nests suggests that the replacements did not begin laying eggs immediately after queen removal. These replacements were left alone in nests, so this lag in reproductive activity was not due to conflict over assuming the reproductive role, as assumed by some theoretical models (e.g. Reeve & Ratnieks 1993). Presumably, it reflects the time required for the replacement queen to enlarge her ovaries, as seen in other bees and wasps (Mueller et al. 1994; Mueller 1996; Strassmann et al. 2004). The preponderance of empty cells in experimental nests relative to nonremoval nests suggests that as the original queens' offspring emerged, the replacements did not keep pace by reprovisioning them. As mentioned above, this is probably due to the lag time required for the replacements to enlarge their ovaries, and possibly also because the original queen had a forager to help provision cells, while the replacement was alone. Some queen-removal nests were collected with pupae. While some of these may have been left from the original queen, the presence of early stage (light-eyed) pupae in four nests suggests that at least some of the replacements were able to lay eggs soon after removal, perhaps because they already possessed somewhat enlarged ovaries.

Our results are consistent with the hypotheses that all *M. genalis* females are reproductively totipotent, and that foragers are fully competent reproductives, even if they rarely reproduce in nature. We found no correlation between age of replacement queens and ovary size, suggesting that females that worked as foragers for several weeks could still enlarge their ovaries. This raises the question of why, if foragers can become replacement reproductives, do they not leave to initiate their own nests? Also, if body size does not affect reproductive ability, why are the daughters that stay to

work smaller than those that disperse? Unlike temperate-zone halictids, *Megalopta* in the low-elevation wet tropics are not strongly seasonally limited; for most of the year at least some dispersing individuals successfully initiate new nests (Wcislo et al. 2004). One answer may be that body size is correlated with some aspect of reproduction that we did not measure, such as the ability to resist usurpation or find and initiate a new nest (Bosch & Vicens 2006; Zobel & Paxton 2007). If so, the indirect fitness benefits of helping would be relatively greater for smaller females.

To the extent that queens control the amount of provisions in a cell (see Mueller et al. 1994), the smaller body size of foragers may be a result of parental manipulation to create more easily dominated subordinates (Alexander 1974; Michener & Brothers 1974). Small-bodied offspring may result from factors other than variation in parental provisioning, such as variation in pollen protein levels (Roulston & Cane 2002), resource scarcity (Bosch 2008), or cleptoparasitism of provisions (Smith et al. 2008). However, foragers are significantly smaller than sisters that emerge just a few days earlier or later (Smith et al. 2008), and forager emergence is not temporally coordinated across nests (A.R.S., W.T.W. & K.M.K., personal observation), suggesting that resource availability and type do not play a strong role. Also, small-bodied foragers are produced in nests with and without cleptoparasitism (Smith et al. 2008). Such considerations suggest that body size differences result from differential maternal provisioning. Unlike most social halictine bees (Michener 1990), adult *Megalopta* share liquid food via trophallaxis within the nest (Wcislo & Gonzalez 2006), so mothers may bias postnatal nutrition as well by withholding food from some daughters but not others. We hypothesize that *Megalopta* queens may be manipulating their offspring's body size or nutritional status to create smaller and more easily dominated daughters (Michener & Brothers 1974; Kukuk & May 1991). This hypothesis predicts that those daughters that stay as foragers do so after receiving more aggression from the queen as young bees than do those that leave to disperse (Brothers & Michener 1974; Smith & Weller 1989; Pabalan et al. 2000), and that this aggression suppresses ovarian development to the extent that staying as a worker outweighs the benefit of dispersing (Michener & Brothers 1974).

In addition to indirect benefits of helping (Smith et al. 2003, 2007), this study demonstrates that foragers may also be 'hopeful reproductives' (West-Eberhard 1978), remaining at the nest as helpers with the potential to inherit the nest when the queen dies, with subsequent fitness gains from direct reproduction. We do not have data on queen mortality rates for natural *M. genalis* nests, but in nests of the sweat bee *Augochlorella striata*, 43.2% of queens died within 4 weeks and were superseded by a daughter (Mueller et al. 1994). Although foraging is risky (O'Donnell & Jeanne 1995; Kukuk et al. 1998), foragers born to relatively old mothers may outlive them to inherit their nest.

The comparison of reproductives from multi- and single-female nests suggests that dominant *M. genalis* enlarge their ovaries in response to social cues beyond what is needed for egg production. Even after accounting for differences in nest productivity, social reproductives had larger ovaries than solitary reproductives. The latter may have less energy to invest in reproductive physiology, due to energetic costs incurred by foraging. However, we found this difference in replacement reproductives as well. *Megalopta genalis* helpers only begin foraging after about 1 week of age (W. T. Wcislo & V. H. Gonzalez, unpublished data), and therefore most newly emerged bees presumably had little opportunity to forage for the replacement reproductive, if they did so at all (we did not conduct foraging observations in the postremoval nests). It is therefore unlikely that differences in mean ovary size between these groups resulted merely from differences in foraging effort. Also, ovary size was not correlated with body size, and body sizes did not significantly differ between solitary and social reproductives, suggesting

that ovary size differences do not result from body size differences (Smith et al. 2008). Sharing a nest with another bee may lead the queen to enlarge her ovaries even more. This may be a direct response to social competition, as suggested by two of the three primary forager-emerged bee comparisons in which the emerged bee had relatively large ovaries. In two of these pairs, the larger female had particularly large ovaries (in the third she did not). In paper wasps (*Polistes*) and bumblebees (*Bombus*), ovaries produce ecdysteroids that, along with juvenile hormone from the corpora allata, lead to increased aggression and dominance (Röseler et al. 1984, 1985; Bloch et al. 2000, 2002; Geva et al. 2005). Increased ovary size may maintain high ecdysteroid titres and aggressive behaviour, thereby suppressing ovarian development and hormone expression in subordinates. Future studies measuring hormone titres and aggression will test this hypothesis.

Our study shows that despite the strong behavioural and reproductive division of labour expressed in social *M. genalis* nests (Wcislo & Gonzalez 2006; Smith et al. 2008) most helpers are capable of laying eggs, even if most do not. The reason that most do not appears to be social competition: in the absence of the queen, nonreproductives readily become replacement queens and reproduce at the same level as natural reproductives. Body size does not predict fecundity: among both field-collected solitary reproductives and those experimentally assigned to observation nests, there was no correlation between body size and reproductive output. Additionally, comparisons of ovary size between solitary-female and multifemale reproductives suggest that *M. genalis* enlarge their ovaries coincident with social dominance. Thus, reproductive division of labour and caste expression, including effective sterility, can be both expressed and reversed by facultatively reproductive individuals in response to their social environment, although the social environment may be manipulated by queens that control the body sizes of their daughters.

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References

Alexander, R. D. 1974. The evolution of social behavior. *Annual Review of Ecology and Systematics*, **9**, 449–474.
 Bloch, G., Hefetz, H. & Hartfelder, K. 2000. Ecdysteroid titer, ovary status, and dominance in adult worker and queen bumble bees (*Bombus terrestris*). *Journal of Insect Physiology*, **46**, 1033–1040.

Bloch, G., Wheeler, D. E. & Robinson, G. E. 2002. Endocrine influences on the organization of insect societies. In: *Hormones, Brain and Behaviour*. Vol. III (Ed. by D. W. Pfaff), pp. 195–235. San Diego: Academic Press.
 Bosch, J. 2008. Production of undersized offspring in a solitary bee. *Animal Behaviour*, **75**, 809–816.
 Bosch, J. & Vicens, N. 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology and Sociobiology*, **60**, 26–33.
 Brothers, D. J. & Michener, C. D. 1974. Interactions in colonies of primitively social bees. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **90**, 129–168.
 Chandrashekara, K. & Gadagkar, R. 1992. Queen succession in the primitively eusocial tropical wasp *Ropalidia marginata* (Lep.) (Hymenoptera: Vespidae). *Journal of Insect Behavior*, **5**, 193–209.
 Craig, R. 1983. Subfertility and the evolution of eusociality by kin selection. *Journal of Theoretical Biology*, **100**, 379–397.
 Crespi, B. J. & Ragsdale, J. E. 2000. A skew model for the evolution of sociality via manipulation: why it is better to be feared than loved. *Proceedings of the Royal Society B*, **267**, 821–828.
 Cronin, A. L. & Hirata, M. 2003. Social polymorphism in the sweat bee *Lasioglossum (Evylaeus) baleicum* (Cockerell) (Hymenoptera, Halictidae) in Hokkaido, northern Japan. *Insectes Sociaux*, **50**, 379–386.
 Field, J. F. & Foster, W. 1999. Helping behaviour in facultatively eusocial hover wasps: an experimental test of the subfertility hypothesis. *Animal Behaviour*, **57**, 633–636.
 Field, J., Shreeves, G., Sumner, S. & Casiraghi, M. 2000. Insurance-based advantage to helpers in a tropical hover wasp. *Nature*, **404**, 869–870.
 Geva, S., Hartfelder, K. & Bloch, G. 2005. Reproductive division of labor, dominance, and ecdysteroid levels in hemolymph and ovary of the bumble bee *Bombus terrestris*. *Journal of Insect Physiology*, **51**, 811–823.
 Kelber, A., Warrant, E. J., Pfaff, M., Wallé, R., Theobald, J. C., Wcislo, W. T. & Raguso, R. A. 2006. Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behavioral Ecology*, **17**, 63–72.
 Kukuk, P. F. & May, B. 1991. Colony dynamics in a primitively eusocial halictine bee *Lasioglossum (Dialictus) zephyrum* (Hymenoptera: Halictidae). *Insectes Sociaux*, **38**, 171–189.
 Kukuk, P. F., Ward, S. A. & Jozwiak, A. 1998. Mutualistic benefits generate an unequal distribution of risky activities among unrelated group members. *Naturwissenschaften*, **85**, 445–449.
 Leigh, E. G., Jr. 1999. *Tropical Forest Ecology: a View from Barro Colorado Island*. Oxford: Oxford University Press.
 Lin, N. & Michener, C. D. 1972. Evolution of sociality in insects. *Quarterly Review of Biology*, **47**, 131–159.
 Michener, C. D. 1990. Reproduction and castes in social halictine bees. In: *Social Insects: an Evolutionary Approach to Castes and Reproduction* (Ed. by W. Engels), pp. 77–121. Berlin: Springer-Verlag.
 Michener, C. D. & Brothers, D. J. 1974. Were workers of eusocial Hymenoptera initially altruistic or oppressed? *Proceedings of the National Academy of Sciences, U.S.A.*, **71**, 671–674.
 Mueller, U. G. 1996. Life history and evolution of the primitively eusocial bee *Augochlorella striata* (Hymenoptera: Halictidae). *Journal of the Kansas Entomological Society, Supplement*, **69**, 116–138.
 Mueller, U. G., Eickwort, G. C. & Aquadro, C. F. 1994. DNA fingerprinting analysis of parent-offspring conflict in a bee. *Proceedings of the National Academy of Sciences, U.S.A.*, **91**, 5143–5147.
 O'Donnell, S. & Jeanne, R. L. 1995. Implications of senescence patterns for the evolution of age polyethism in eusocial insects. *Behavioral Ecology*, **6**, 269–273.
 Pabalan, N., Davey, K. G. & Packer, L. 2000. Escalation of aggressive interactions during staged encounters in *Halictus ligatus* Say (Hymenoptera: Halictidae), with a comparison of circle tube behaviors with other Halictine species. *Journal of Insect Behavior*, **13**, 627–650.
 Rau, P. 1933. *Jungle Bees and Wasps of Barro Colorado Island*. St Louis, Missouri: Von Hoffmann Press.
 Reeve, H. K. & Ratnieks, F. L. W. 1993. Queen–queen conflicts in polygynous societies: mutual tolerance and reproductive skew. In: *Queen Number and Sociality in Insects* (Ed. by L. Keller), pp. 45–85. Oxford: Oxford University Press.
 Richards, M. H. & Packer, L. 1994. Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee. *Behavioral Ecology and Sociobiology*, **34**, 385–391.
 Richards, M. H., Packer, L. & Seger, J. 1995. Unexpected patterns of parentage and relatedness in a primitively eusocial bee. *Nature*, **373**, 239–241.
 Röseler, P. F., Röseler, I., Strambi, A. & Augier, R. 1984. Influence of insect hormones on the establishment of dominance hierarchies among foundresses of the paper wasp, *Polistes gallicus*. *Behavioral Ecology and Sociobiology*, **15**, 133–142.
 Röseler, P. F., Röseler, I. & Strambi, A. 1985. Role of ovaries and ecdysteroids in dominance hierarchy establishment among foundresses of the primitively social wasp, *Polistes gallicus*. *Behavioral Ecology and Sociobiology*, **18**, 9–13.
 Roulston, T. H. & Cane, J. H. 2002. The effect of pollen protein concentration on body size in the sweat bee *Lasioglossum zephyrum* (Hymenoptera: Apiformes). *Evolutionary Ecology*, **16**, 49–65.
 Schwarz, M. P., Richards, M. H. & Danforth, B. N. 2007. Changing paradigms in insect social evolution: insights from halictine and allodapine bees. *Annual Review of Entomology*, **52**, 127–150.
 Smith, A. R., Wcislo, W. T. & O'Donnell, S. 2003. Assured fitness returns favor sociality in a mass-provisioning sweat bee, *Megalopta genalis* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **54**, 22–29.

- Smith, A. R., Wcislo, W. T. & O'Donnell, S.** 2007. Survival and productivity benefits to social nesting in the sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **61**, 1111–1120.
- Smith, A. R., Wcislo, W. T. & O'Donnell, S.** 2008. Body size shapes caste expression, and cleptoparasitism reduces body size in the facultatively eusocial bees *Megalopta* (Hymenoptera: Halictidae). *Journal of Insect Behavior*, **21**, 394–406.
- Smith, B. H. & Weller, C.** 1989. Social competition among gynes in halictine bees: the influence of bee size and pheromones on behavior. *Journal of Insect Behavior*, **2**, 397–411.
- Strassmann, J. E., Fortunato, A., Cervo, R., Turillazzi, S., Damon, J. M. & Queller, D. C.** 2004. The cost of queen loss in the social wasp *Polistes dominulus* (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society*, **77**, 343–355.
- Tierney, S. M., Gonzalez-Ojeda, T. & Wcislo, W. T.** 2008a. Biology of a nocturnal bee, *Megalopta atra* (Hymenoptera: Halictidae; Augochlorini), from the Panamanian highlands. *Journal of Natural History*, **43**, 1841–1847.
- Tierney, S. M., Gonzalez-Ojeda, T. & Wcislo, W. T.** 2008b. Nesting biology and social behavior of *Xenochlora* bees (Hymenoptera: Halictidae: Augochlorini) from Perú. *Journal of the Kansas Entomological Society*, **81**, 61–72.
- Wcislo, W. T.** 1997. Behavioral environments of sweat bees (Halictinae) in relation to variability in social organization. In: *Social Behavior in Insects and Arachnids* (Ed. by J. C. Choe & B. J. Crespi), pp. 316–332. Cambridge: Cambridge University Press.
- Wcislo, W. T.** 2000. Environmental hierarchy, behavioral contexts, and social evolution in insects. In: *Ecologia e Comportamento de Insetos. Supplement. Vol. 8* (Ed. by R. P. Martins, T. M. Lewinsohn & M. S. Barbeitos), pp. 49–84. Rio de Janeiro: Oecologia Brasiliensis.
- Wcislo, W. T. & Gonzalez, V. H.** 2006. Social and ecological contexts of trophallaxis in facultatively social sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera, Halictidae). *Insectes Sociaux*, **53**, 220–225.
- Wcislo, W. T., Arneson, L., Roesch, K., Gonzalez, V. H., Smith, A. R. & Fernandez, H.** 2004. The evolution of nocturnal behaviour in sweat bees, *Megalopta genalis* and *M. ecuadoria* (Hymenoptera: Halictidae): an escape from competitors and enemies? *Biological Journal of the Linnean Society*, **83**, 377–387.
- West-Eberhard, M. J.** 1975. The evolution of social behavior by kin selection. *Quarterly Review of Biology*, **50**, 1–33.
- West-Eberhard, M. J.** 1978. Polygyny and the evolution of social behavior in wasps. *Journal of the Kansas Entomological Society*, **51**, 832–856.
- West-Eberhard, M. J.** 1981. Intragroup selection and the evolution of insect societies. In: *Natural Selection and Social Behavior* (Ed. by R. D. Alexander & D. W. Tinkle), pp. 3–17. New York: Chiron Press.
- West-Eberhard, M. J.** 1987. Flexible strategy and social evolution. In: *Animal Societies: Theories and Facts* (Ed. by Y. Itô, J. L. Brown & J. Kikkawa), pp. 35–51. Tokyo: Scientific Societies Press.
- West-Eberhard, M. J.** 1996. Wasp societies as microcosms for the study of development and evolution. In: *Natural History and Evolution of Paper Wasps* (Ed. by S. Turillazzi & M. J. West-Eberhard), pp. 290–317. Oxford: Oxford University Press.
- Yanega, D.** 1989. Caste determination and differential diapause within the first brood of *Halictus rubicundus* in New York (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, **24**, 97–107.
- Zobel, M. U. & Paxton, R. J.** 2007. Is big the best? queen size, usurpation and nest closure in a primitively eusocial sweat bee (*Lasios glossum malachurum*). *Behavioral Ecology and Sociobiology*, **61**, 435–447.