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Foundress polyphenism and the origins of eusociality in a facultatively eusocial sweat bee, *Megalopta genalis* (Halictidae)

Karen M. Kapheim · Adam R. Smith · Peter Nonacs · William T. Wcislo · Robert K. Wayne

Abstract The reproductive (queen) and nonreproductive (worker) castes of eusocial insect colonies are a classic example of insect polyphenism. A complementary polyphenism may also exist entirely among females in the reproductive caste. Although less studied, reproductive females may vary in behavior based on size-associated attributes leading to the production of daughter workers. We studied a bee with flexible social behavior, *Megalopta genalis,* to better understand the potential of this polyphenism to shape the social organization of bee colonies and, by extension, its role in the evolution of eusociality. Our experimental design reduced variation among nest foundresses in life history variables that could influence reproductive decisions, such as nesting quality and early adulthood experience. Within our study population, approximately one third of *M. genalis* nests were eusocial and the remaining nests never produced workers. Though they do not differ in survival, nest-founding females who do not attempt to produce workers (which we refer to as the solitary phenotype) are significantly smaller and become reproductive later than females who attempt to recruit workers (the social phenotype). Females with the social phenotype are more likely to produce additional broods but at a cost of having some of their first offspring become nonreproductive workers. The likelihood of eusocial organization varies with body size across females of the social phenotype. Thus, fitness consequences associated with size-based plasticity in foundress behavior has colony level effects on eusociality. The potential for size-based polyphenisms among reproductive females may be an important factor to consider in the evolutionary origins of eusociality.

Keywords Alternative reproductive behavior · Facultative eusociality · Maternal manipulation · Social evolution · Polyphenism

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Introduction

Polyphenism, where two or more phenotypes are produced without implicit genetic variation (Mayr 1963; Simpson et

al. 2011), is a fundamental component of eusociality and likely played a key role in its evolutionary origins (Michener 1961; West-Eberhard 1986, 2003). In eusocial Hymenoptera colonies, the reproductive caste (queens) are typically larger in body size, more physiologically primed for reproduction, and behave as dominant egg layers within a colony, relative to nonreproductives (workers) that are typically smaller, with smaller ovaries, lower levels of nutrient stores, and specialize on nonreproductive tasks such as nest construction, foraging, and brood care. Workers and future queens produced in the same colony thus represent separate morphological, physiological, and behavioral phenotypes.

A second polyphenism may also exist in some social insects, entirely within the reproductive caste. In facultatively eusocial species, colonies with workers exhibit reproductive and behavioral division of labor typical of most eusocial insects, but some nests remain solitary (without workers) for their duration (Michener 1974, 1990; Wcislo 1997a; Schwarz et al. 2007). The suites of environmental and genetic factors that shape this variation are not well understood (Packer et al. 1989; Packer 1990; Eickwort et al. 1996; Yanega 1997; Plateaux-Quenu et al. 2000; Wcislo 2000; Soucy 2002; Soucy and Danforth 2002; Field et al. 2010, 2012). We hypothesized that colony level variation in social organization could result from polyphenism among nest founding, reproductive females. The development of eusociality could, therefore, depend on the degree to which nest foundresses influence their daughters to become nonreproductive workers (Alexander 1974; Michener and Brothers 1974). An investigation of this polyphenism highlights a focus on the individual-based nature of this process, independent of a more common focus on 'facultative sociality' at the colony level. Preliminary evidence for this hypothesis was described for a marginal population of a temperate sweat bee, *Augochlorella aurata* (=*striata*), where half the foundresses produced females as their first offspring and half of all the nests developed into social colonies, though whether these were the same nests was not clear (Packer et al. 1989; Packer 1990). This pattern could suggest that colony level social organization was a function of foundress reproductive behavior, but the mechanism by which this variation arose is unknown. We hypothesized that such a behavioral polyphenism could be associated with variation in body size and reproductive physiology.

We assessed the significance of variation in reproductive behavior among foundresses in a neotropical halictid bee, *Megalopta genalis*, for which social organization varies among nests. Within one population of this species, some nest-founding females become the queens of social nests, while the rest reproduce solitarily, without workers (Smith et al. 2003, 2007, 2008, 2009; Wcislo et al. 2004; Kapheim et al. 2011, 2012). Social nests are characterized by a strong division of labor in which workers perform most of the

foraging effort and feed the queen and other nestmates through trophallaxis (Wcislo and Gonzalez 2006; Smith et al. 2008). Facultative eusociality represents discrete alternatives among nest foundresses that are not ontogenetic stages of a single life history (Wcislo and Gonzalez 2006; Smith et al. 2007).

Previous research indicates that physical traits are associated with this social polyphenism. *M. genalis* queens are significantly larger, with larger ovaries, and higher juvenile hormone (a gonadotropin) levels than age-matched reproductive females who remain solitary, without workers (Kapheim et al. 2012; Smith et al. 2012). Of these factors, body size and ovary development are highly predictive of this social phenotype (Smith et al. 2007; Kapheim et al. 2012), but causality is not known. It is unclear whether these differences arise as a consequence of social interactions or influence the probability that a foundress will recruit workers through maternal behavior. Unlike ovary size, insect body size is determined during larval development and does not change in the adult phase of the life cycle. Thus, physiological and behavioral correlates of body size may influence whether a nest foundress attempts to generate a social colony or remains solitary.

We tested the hypothesis that variation in female reproductive behavior influences colony level social organization and that this variation is the behavioral output of physiological and size-based differences among reproductive females stemming from larval development. To test this hypothesis, we placed newly emerged females into standardized observation nests and monitored the social development of each nest. We then assessed factors contributing to differences in reproductive success among these nest foundresses. We discuss our results with respect to how size-based behavioral variation among reproductive females can lead to eusociality at the nest level.

Methods

Behavioral and reproductive observations

We studied *M. genalis* during the reproductive season from January to May in both 2008 and 2009, on Barro Colorado Island, Republic of Panama. BCI is a 1,500-ha island characterized by semi-deciduous tropical moist forest (see Leigh 1999 for details of the site). *M. genalis* is a nocturnal bee that commonly nests in dead sticks or branches suspended off the ground on BCI (Wcislo et al. 2004; Wcislo and Tierney 2009). Reproductive activity is highest in this population during the dry season and early wet season (mid-December–September) (Wcislo et al. 2004). During this period, nesting is asynchronous on a population level, but egg laying patterns within nests are partitioned into semi-discrete broods (see Supplementary Material for additional

details). We constructed standardized balsa wood observation nests that mimicked natural nests and allowed resident bees to live freely in their natural environment (see Kapheim et al. 2012 for details). Each nest was seeded with a single adult female within 1–2-day post-eclosion, which we obtained by collecting developing larvae and pupae from natural nests and rearing them in tissue culture trays under ambient conditions. Observation nests were distributed among eight clusters; nests within a cluster were spaced from 1–100-m apart and were subject to similar environmental conditions. These methods reduced variation in factors that could influence direct fitness outcomes, including early social experience, nest quality, resource availability, and seasonal effects.

We tracked survivorship, cell building, cell closing and opening, new emergences, and disappearances through censuses every 4 days. These observations spanned the production of one to three offspring broods for each foundress. Newly emerged males were collected and stored at −20 °C in 95 % EtOH. New females were measured with calipers (head width) and marked with a white Decocolor® paint pen in the field and returned to their nest. If all adults disappeared from a nest, we reared the developing brood to emergence. Nests and their developing brood do not usually survive if left unprotected (Smith et al. 2003, 2007; Wcislo et al. 2004). These offspring were included in the analyses of egg-laying patterns, but were not counted as successfully reared offspring in calculations of foundress reproductive success. Additional details relating to nest censuses, brood cycles, and collection methods are given in the Supplementary Material.

Observed nests were collected toward the end of the study within a 5-week time period (26 April 2008 to 31 May 2008 and 23 April 2009 to 24 May 2009) at the same time of day, when all bees were present in the nest. The contents of each nest and its brood cells were recorded and stored in 95 % ETOH at −20 °C. To investigate the sociogenetic structure of M. genalis nests, we genotyped adults and developing brood collected from a subset of 30 social and eight solitary observation nests at eight polymorphic microsatellite loci, including some nests sampled in 2007 (Kapheim et al. 2009). See the Supplementary Material for additional details. Voucher specimens are in the Museo de los Invertebrados "Graham Fairchild," Universidad de Panamá.

Phenotypic assignment

We assigned each nest foundress to a social phenotype based on her behavior. A female was considered to be a nest foundress if she began building an entrance collar at the open end of the tunnel, even if she later disappeared, because this is one of the first architectural features

constructed when a female establishes a new nest, and all nests have this feature (Smith et al. 2003; Wcislo et al. 2004). Females that disappeared without showing any signs of nesting were excluded from analyses. Foundresses that successfully raised offspring through emergence were further classified based on whether they attempted to rear workers. Workers were daughters of nest foundresses who remained in their natal nests for at least 10 days and were observed leaving and returning to the nest during foraging hours. Foundresses were dissected to measure ovarian development, and their spermathecae were examined for spermatazoa.

Statistical analysis

Due to departures from normality in the distribution of several metrics, we used nonparametric tests for several comparisons as indicated. For dependent variables that were in the form of counts (e.g., number of eggs laid, number of offspring successfully reared), we used negative binomial regressions to test the significance of independent variables. In these regressions, number of days observed prior to death, disappearance, or collection (tenure) was the exposure variable. The exposure variable accounts for differences in the number of times an event could have happened. In the case of multiple pairwise tests, we used a Bonferroni correction. All tests were two-tailed, and means are presented ± one standard deviation. All statistical tests were done in Stata v. 9.2.

Results

Social phenotypes

Nest foundresses exhibited variable patterns of offspring sex allocation. Of the foundresses that had completed their first brood at the time they disappeared, died, or were collected, 39 (34 %) laid only male eggs in their first brood, despite having mated. Both males and females were produced in the first brood of 74 (64 %) nests, and no nests produced only females (Table 1). Though some of the nests producing only males in the first brood did produce female eggs in subsequent broods, none became social (i.e., had a worker) prior to the end of the study period. Among the nests in which both males and females were produced in the first brood, the first offspring was female in 67 (91 %) of these nests. Most (76 %) of the females emerging in the first brood remained in their nests for at least 10 days [approximately 3–5 days past the age when workers begin foraging (Wcislo and Gonzalez 2006)], excluding nests that were collected earlier. Video evidence showed that daughter(s) still present in the natal nest at the time of nest collection were foraging worker(s).

The remaining 24 % of females emerging in the first brood disappeared within 10 days of emerging. These females may have dispersed to found nests of their own or died. Our data cannot distinguish between these alternatives.

We categorized foundresses producing at least one female in their first brood as the social phenotype (hereafter SOC) and those producing exclusively males in the first brood as the solitary phenotype (hereafter SOL) (Table 1). With these labels, we do not intend to imply that all SOC foundresses ended up with social nests, but rather they had the *potential* to end up with a social nest. In contrast, SOL foundresses did not have this potential, because by laying only male eggs, they did not produce any potential workers in their first brood. Indeed, SOC foundresses faced variable outcomes. Of 74 such foundresses, 53 (72 %) had at least one worker at the time of collection, and these were classified as social nests (Table 1). The daughters disappeared (either died or dispersed) shortly (<10 days) after emergence before we obtained evidence of them working in seven (9 %) of SOC nests, and these were characterized as 'failed social' nests (Table 1). The nest foundress disappeared or died before the daughter in the remaining 14 (19 %) SOC nests (Table 1), and these were categorized as superseded nests (Table 1). Male offspring typically disappear within 4 days of emerging, presumably to mate, though nothing is known of *M. genalis* mating behavior. Three post-emergence foundresses were problematic to categorize and were excluded from analyses. In these nests, several male eggs were laid at short intervals (0–5 days), and then a female egg was laid (10–17 days later), all during the first brood (Table 1). Nests were collected prior to female offspring emergence, so the phenotype was unknown.

Environmental factors

Variation in foundress reproductive behavior in this study could not be attributed to seasonal or local resource conditions. Social and solitary nests developed within close proximity of one another, without significant differences in the proportion of solitary or social outcome across nest clusters (chi-squared test: $\chi^2=21.47$, $p=0.43$, $n=113$). Foundresses transferred to observation nests eclosed between 27 January to 6 March 2008 and 22 January to 24 February 2009. There were no significant differences in the date SOC and SOL foundresses eclosed and were transferred to observation nests (Wilcoxon Mann–Whitney test: $Z=0.05$, $p=0.96$, $n=113$).

Sociogenetic structure

The number of eggs laid by females other than the nest foundress was low in both social and solitary nests. Genotypes in seven of the eight solitary nests used in genetic analysis were consistent with a single matriline. Patrilines could not be detected because most solitary nests did not produce females. Genotypes in 28 of 30 social nests were consistent with a single matriline and a single patriline. In these 28 nests, there was no evidence of worker reproduction, and relatedness between daughters was 0.75. Two social nests had evidence of worker reproduction and/or social parasitism. Overall, one of 24 (4 %) genotyped offspring from solitary nests and 11 of 190 (6 %) genotyped offspring from social nests were excluded as foundress offspring. In social nests, six offspring (four males, two females) were likely worker produced. The probability of detecting worker-laid males and females was 0.97 and 0.93, respectively, based on calculations derived from allele frequencies (see Supplementary Material and Table SI 1). This suggests estimates of worker-laid males (3 %) and females (2 %) based on our genetic parentage sampling accurately reflect the population average. The remaining six unrelated offspring were likely the result of intraspecific parasitism because they could not be assigned to any female from the nest, but this rate was not significantly different among

Table 1 Categories of social organization for *M. genalis* observation nests

Category	Nests	Description
Social	53	At least 1 female worker in first brood that remained in the nest at least 10 days before disappearing or video evidence showed a worker foraging; first offspring was usually female
Superseded	14	Nest foundress disappeared or died before worker disappeared or died
Failed social	7	First offspring was female but potential worker either died or disappeared <10 days after emergence
Solitary	39	Entire first brood is male; finished building cells for first brood when collected or disappeared
Other	3	First offspring are males, laid at regular intervals (0–5 days) but female egg is last in first brood (10–17 days); equivocal whether these would have been social or solitary

The social, superseded, and failed social categories result from foundresses with the SOC phenotype (i.e., foundresses laying female eggs in their first brood of offspring); the solitary nests result from foundresses with the SOL phenotype (i.e., foundresses laying exclusively male eggs in their first brood of offspring). Nests is the number of nests in each category for 2008 and 2009 combined

social and solitary nests (Fisher's exact, $p=0.38$). Complete details of microsatellite performance and socio-genetic structure within observation nests are available in the Supplementary Material.

Foundress survival

SOC and SOL foundresses did not have significantly different survival probabilities. Twelve (31 %) solitary females and 21 (28 %) queens disappeared or died before they could be collected (chi-squared test: $\chi^2=0.071$, $p=0.79$, $n=113$; Table 2). The date of collection followed a similar distribution for SOC and SOL foundresses (chi-squared test: $\chi^2=7.29$, $p=0.30$, $n=77$), and age at time of collection was not significantly different (average age at collection: SOL$=81.6\pm15.2$ days, SOC$=83.5\pm16.9$ days; Wilcoxon Mann–Whitney test: $Z=-1.24$, $p=0.22$, $n=77$). Thus, similarity in foundress disappearance rates likely reflects a biological, rather than methodological, phenomenon.

The above results were obtained from foundresses that had successfully reared at least one offspring to emergence. To assess survival probability of each phenotype *prior* to offspring emergence, we used the sex of the first egg laid as a predictor of future phenotype (e.g., SOC or SOL) among foundresses that disappeared, died, or were collected prior to emergence of any offspring. There were no significant differences in number of disappearances or deaths depending on the sex of first offspring (chi-squared test: $\chi^2=0.17$, $p=0.68$, $n=36$). To ensure we were not biasing the results by not accounting for the 9 % of SOC females that produce males first, we repeated the analysis, but with two of the

male-first foundresses that disappeared early being counted as female-first nests that disappeared early (chi-squared test: $\chi^2=1.08$, $p=0.3$). Female-first and male-first foundresses were collected across a similar distribution of dates (chi-squared test: $\chi^2=1.81$, $p=0.94$, $n=15$) and ages (Wilcoxon Mann–Whitney test: $Z=-0.49$, $p=0.62$, $n=9$). Similarities in disappearance probabilities likely reflect a biological, rather than methodological, phenomenon.

Sex ratio and offspring investment

The population sex ratio, based on the offspring produced in social and solitary nests, was male biased (27 % female offspring, 72 % male offspring, and the sex of 1 % of offspring could not be determined). This translates to a sex investment ratio of 35 % females and 65 % males, based on the ratio of the dry weight of average larval provisions (F/M$=1.42$) (Kapheim et al. 2011). The sex ratio was even more male-biased when nonreproductive workers were excluded (16 % female, 83 % male, 1 % unknown sex). The numerical primary sex ratio within social and solitary nests was not significantly different between years (Wilcoxon Mann–Whitney test: $Z=-1.65$, $p=0.10$, $n=113$ nests; Fig. SI 1). SOC foundresses had an average sex ratio (proportion males) of 0.54 in the first brood, 0.65 in the second brood, and 0.50 in the third brood. SOL foundresses, by definition, produced first broods composed entirely of males and had an average sex ratio of 0.90 and 0.50 in each subsequent brood, respectively (Table 2).

SOC and SOL foundresses provisioned cells and laid eggs at similar rates overall, but this rate was significantly

Table 2 Reproductive factors associated with foundress strategies

Values represent combined mean ± SD for 39 SOL (i.e., foundresses laying exclusively male eggs in their first brood of offspring) foundresses and 74 SOC (i.e. foundresses laying female eggs in their first brood of offspring) foundresses in 2008 and 2009. Patterns were not significantly different between years. See main text for statistical analysis of each metric for each corresponding p.

*$p<0.05$

[a] Number of successfully reared offspring with reproductive potential. Discounts nonreproductive workers, abandoned brood, and parasitized brood

	Foundress phenotype		
	SOL	SOC	p
Head width (mm)	3.62 ± 0.19	3.80 ± 0.31	0.01*
Disappeared before all brood emerged	15 % (6/39)	26 % (19/74)	0.21
Nest tenure (days)	75.90 ± 14.32	79.89 ± 17.08	0.30
Age at first reproduction (days)	25.33 ± 7.58	22.62 ± 6.23	0.02*
Age when brood 2 began (days)	74.15 ± 14.41	73.93 ± 13.54	0.80
Produced more than 1 brood	33 % (13/39)	57 % (42/74)	0.02*
Number of broods produced	1.36 ± 0.54	1.61 ± 0.57	0.02*
Sex ratio of brood 1 (proportion males)	1 ± 0.00	0.54 ± 0.27	<0.0001*
Sex ratio of brood 2 (proportion males)	0.90 ± 0.28	0.65 ± 0.40	0.04*
Sex ratio of brood 3 (proportion males)	0.50	0.5 ± 0.71	>0.99
Number of eggs laid	4.38 ± 2.32	5.27 ± 2.59	0.02*
Eggs laid in brood 1	3.64 ± 1.60	3.80 ± 1.36	0.42
Eggs laid in brood 1, discounting workers	3.64 ± 1.60	2.58 ± 1.55	0.003*
Eggs laid in broods 2 and 3	0.74 ± 1.35	1.47 ± 1.83	0.01*
Reproductive success[a]	3.95 ± 2.45	3.85 ± 2.68	0.76

higher for nests with social queens during the period after workers emerged. The average number of days between laying eggs within each brood did not differ significantly between SOC and SOL nests (Wilcoxon Mann–Whitney test: $Z=-1.55$, $p=0.12$, $n=109$; Table SI 2). SOL foundresses laid eggs in shorter intervals than SOC foundresses in the first brood, though this result is not quite statistically significant (Wilcoxon Mann–Whitney test: $Z=-1.85$, $p=0.06$, $n=310$). Within SOC nests, there was a significant decrease in the intervals between egg laying in the second brood compared to the first (Wilcoxon Mann–Whitney test: $Z=2.05$, $p=0.04$, $n=261$), but no such pattern was seen in SOL nests (Wilcoxon Mann–Whitney test: $Z=-0.55$, $p=0.58$, $n=117$).

Body size and productivity

SOC foundresses were significantly larger than SOL foundresses (Wilcoxon Mann–Whitney test: $Z=-2.42$, $p=0.02$, $n=108$; Table 2). However, a negative binomial regression model of number of eggs laid by each foundress that included body size, social phenotype, and their interaction as independent variables, with number of days resident in the nest (tenure) as the exposure variable, was not significant overall (negative binomial regression/likelihood ratio $\chi^2=5.00$, $r^2=0.01$, $p=0.08$, $n=108$). SOC foundresses began laying eggs at a younger age than SOL foundresses (Wilcoxon Mann–Whitney test: $Z=2.42$, $p=0.02$, $n=113$; Table 2), and laid significantly more eggs overall (Wilcoxon Mann–Whitney test: $Z=-2.41$, $p=0.02$, $n=113$; Table 2). SOC foundresses were more likely to produce more than one brood (number of broods produced: SOC, 1.61 ± 0.57; SOL, 1.36 ± 0.54; chi-squared test: $\chi^2=5.61$, $p=0.02$, $n=113$; Table 2) and laid more eggs in later broods than SOL foundresses (eggs laid in later broods: SOC=1.47 ± 0.53; SOL=0.74 ± 1.35; Wilcoxon Mann–Whitney test: $Z_{eggs}=-2.44$, $p=0.01$, $n=113$; Table 2). This was not the result of getting an earlier start on producing a second brood, as the age at which females laid the first egg of the second brood was not significantly different across phenotypes (Wilcoxon Mann–Whitney test: $Z=0.25$, $p=0.80$, $n=55$; Table 2). The number of days each foundress spent in the observation nest did not significantly differ between SOC and SOL foundresses (Wilcoxon Mann–Whitney test: $Z=-1.03$, $p=0.89$, $n=113$), but it is unknown how long they would have remained alive and reproductive in their nests if they were not collected at the end of the study period.

The number of eggs laid in the first brood was similar in both phenotypes (Wilcoxon Mann–Whitney test: $Z=-0.81$, $p=0.42$, $n=113$; Table 2), but SOL foundresses achieved significantly higher reproductive success (i.e., number of successfully reared offspring with reproductive potential) from their first brood due to differences in the reproductive potential of their offspring (Wilcoxon Mann–Whitney test: $Z=3.03$, $p=0.002$, $n=113$; Table 2). All SOL offspring were

potentially reproductive, but 1.22 ± 0.73 (mean±SD) SOC daughters per nest remained as nonreproductive workers. SOC and SOL foundresses did not, however, have significantly different reproductive success (i.e., number of successfully reared offspring with reproductive potential) within the study period overall (Wilcoxon Mann–Whitney test: $Z=0.31$, $p=0.76$, $n=113$; Table 2). A negative binomial regression model of number of successfully reared offspring with reproductive potential that included foundress phenotype, year, and their interaction term as independent variables, with tenure time as the exposure variable, was not statistically significant (negative binomial regression/likelihood ratio $\chi^2=5.09$, $r^2=0.02$, $p=0.17$, $n=113$).

Body size and social outcomes

Not all SOC foundresses ended up with a successful eusocial nest. There were significant differences in body size (i.e., head width) among foundresses that ended up with solitary, social, failed social, or superseded nests (Kruskal–Wallis test: $\chi^2=16.02$, $p=0.01$, $n=108$). Solitary foundresses (SOL) were significantly smaller than SOC that became social queens (Wilcoxon Mann–Whitney test: $Z=3.47$, $p=0.0005$, $n=88$). SOC foundresses of superseded nests were also significantly smaller than social queens (Wilcoxon Mann–Whitney test: $Z=3.10$, $p=0.002$, $n=65$; Fig. 1a). Superseded foundresses were also significantly closer in size to, or smaller than, their first brood daughters than were queens of social nests (Wilcoxon Mann–Whitney test: $Z=2.78$, $p=0.01$, $n=64$ foundresses; Fig. 1b). The proportional size difference between queens (q) and workers (w) was $(q-w)/q=0.067$ and 0.009 in social and superseded nests, respectively. The egg-laying rate of superseding daughters was similar to that of their mothers' (queens, 0.07 ± 0.02 eggs per day; replacements, 0.06 ± 0.06 eggs per day; Wilcoxon matched pairs signed rank test: $Z=0.41$, $p=0.68$, $n=14$). SOC females laid similar numbers of eggs per day, regardless of social outcome (Kruskal–Wallis test: $\chi^2=0.79$, $p=0.67$, $n=74$; Fig. 2a). SOC females that failed to produce workers, despite laying female eggs in the first brood, had a significantly lower rate of reproductive success than those who successfully produced workers and were superseded (Kruskal–Wallis test: $\chi^2=8.02$, $p=0.02$, $n=74$; Fig. 2b).

Discussion

Foundress polyphenism and the evolution of eusociality

Castes of eusocial insects are one of the best studied examples of polyphenisms (Michener 1961; West-Eberhard 2003; Simpson et al. 2011). Our results suggest that the reproductive caste also exhibits a polyphenism, in that not all
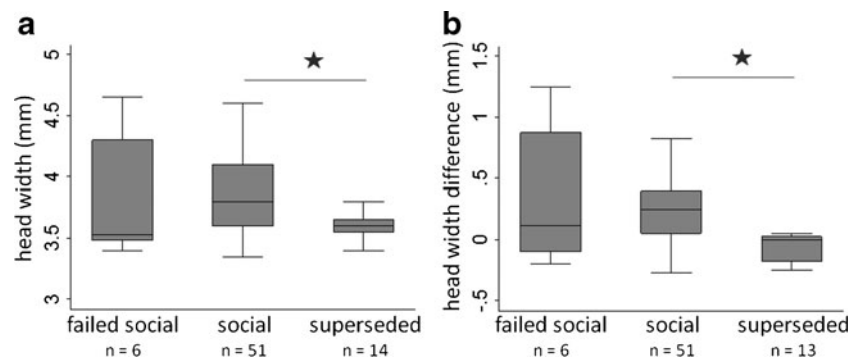
foundresses attempt to produce workers. Additionally, some females were intraspecific nest parasites and a few nests did not appear to follow a typical solitary or social pattern. These observations are consistent with the exceptional plasticity found in reproductive strategies of halictid bees (Michener 1990; Wcislo 1997b; Schwarz et al. 2007).

Our study demonstrates that these individual reproductive behaviors have consequences that affect the extended phenotype of the colony, shaping subsequent social selection (Wcislo 2000). By measuring these consequences as foundress reproductive success, without considering indirect fitness accrued by workers, we revealed an important influence on social selection without making unwarranted assumptions concerning the relative contribution of each worker toward nest level reproduction (Wolf and Wade 2001). Foundress polyphenism and its effects on eusociality has also been suggested for a marginal population of a widespread temperate halictine bee, *A. aurata* (Packer et al. 1989; Packer 1990), suggesting that this aspect of social flexibility may be more common than has been previously recognized and may play an important role in the evolutionary origins of eusociality. Foundresses on a solitary trajectory have more immediate, but potentially limited, reproductive success with less investment. Foundresses on

a social trajectory delay reproductive success in favor of long-term investment in workers but may have higher reproductive rates in subsequent broods in the long run (Smith et al. 2007) or in populations with favorable ecological or demographic conditions (Eickwort et al. 1996; Tierney et al., in revision). Indeed, our results suggest that if left undisturbed, foundresses of social nests would be more likely to produce additional broods and thus achieve a higher reproductive success than foundresses of solitary nests. Over evolutionary time, fitness differentials could cause eusociality to spread and become fixed in some populations or completely lost in other populations (Wcislo and Danforth 1997).

Physical underpinnings of foundress polyphenism

Queens of *M. genalis* social nests are larger, have larger ovaries, and higher juvenile hormone levels than age-matched solitary reproductive females (Smith et al. 2008; Kapheim et al. 2012; Smith et al. 2012). Ovarian development and hormone levels may vary across an individual's lifetime as a function of reproductive status, nutritional status, and social environment. Body size, however, is fixed largely as a function of larval diet and could potentially influence reproductive behavioral decisions among females.
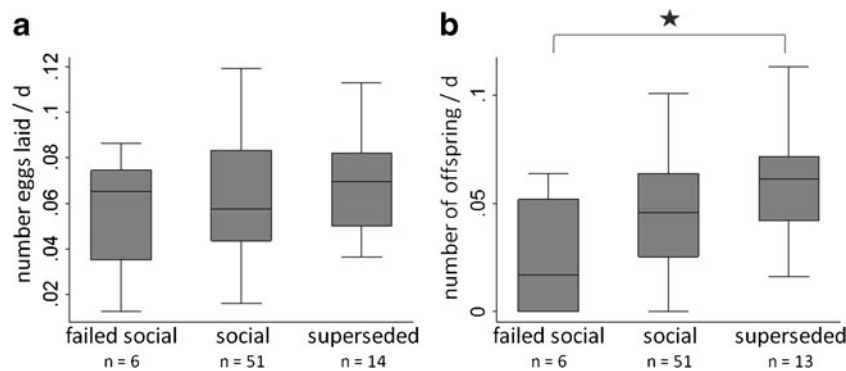
Fig. 2 Reproductive outcomes of social nests with varying social outcomes. (**a**) Comparisons of number of eggs laid and (**b**) reproductive success rate. Reproductive success is number of successfully reared offspring with reproductive potential divided by nest tenure. This metric discounts offspring that would not have survived (parasitized or abandoned) or were nonreproductive workers. Failed social foundresses have significantly lower reproductive success rate than superseded foundresses. A *star* represents statistically significant differences in a Wilcoxon Mann–Whitney test, after a Bonferroni correction for multiple testing

Our experiment isolated the effects of developmentally related physical variation on reproductive decisions, independent of early social interactions and variation in nest quality, resource availability, and other environmental factors.

Maternal size is an important factor in the sex allocation strategies of other bees (Willmer and Stone 2004; Rehan and Richards 2010; Field et al. 2012). Our results further suggest that foundress reproductive phenotype may be linked to physical condition at the time of emergence. There are two ways in which physical conditions could influence the social reproductive phenotype of a nest foundress. First, small individuals may be poor provisioners or otherwise handicapped (West Eberhard 1978). Consistent with this hypothesis, solitary foundresses laid their first egg at a later age and were less likely to lay more than one brood of offspring. In contrast, *M. genalis* replacement queens (i.e., workers that become queens following the disappearance of the foundress) are equally fecund and show no signs of reproductive handicaps, at least under short-term experimental conditions, though provisioning rate was not measured (Smith et al. 2009). In temperate halictid bees (e.g., *Lasioglossum malachurum*), over-wintering foundresses require substantial lipid reserves to successfully establish nests, and the amount of lipids left over after nest founding may influence whether a queen produces one or two worker broods (Strohm and Bordon-Hauser 2003; Mitesser et al. 2007; Weissel et al. 2012). In *M. genalis*, female offspring receive more maternal investment through larger provisions, with higher protein and sugar content, than males (Kapheim et al. 2011). In this case, investing in less costly sons would minimize reproductive delays for poor provisioners.

Second, small females may be less likely to force larger daughters into nonreproductive worker roles. Among temperate sweat bees, worker behavior is often attributed to the ability of larger (and older) queens to aggressively dominate their smaller daughters (Michener and Brothers 1974; Michener 1990; but see Schwarz et al. 2007 for additional factors). *M. genalis* workers are typically smaller than their queens (Smith et al. 2008; Kapheim et al. 2012), and this relationship may be enforced through aggressive dominance interactions (Arneson and Wcislo 2003). If the value of female offspring depends on their size, then producing male offspring may be favored over producing tiny daughters that can be dominated among small foundresses. The 12 % of foundresses that were superseded tended to be small, suggesting they could have achieved similar reproductive success if they had followed a solitary trajectory of producing all males.

Direct fitness outcomes of foundress polyphenism

Solitary females produce significantly more offspring with reproductive potential in the first brood with less investment because males are less costly to provision (Kapheim et al. 2011), This highlights the risk incurred by delayed reproductive success for the social phenotype. In *M. genalis*, nearly 10 % of the foundresses that followed a social trajectory failed to retain any workers. Similarly, nearly 43 % of foundresses in an *A. aurata* population died and were superseded by a daughter (Mueller et al. 1994). The potential payoff from having daughter workers may outweigh this risk if it results in larger later broods and if the queen dies. With respect to the latter, assured fitness benefits ensure that daughters continue to protect and raise the queen's offspring (Queller 1989; Gadagkar 1990; Nonacs 1991). These benefits have been demonstrated for *M. genalis* (Smith et al. 2003, 2007) and other social insects (Field et al. 1999; Bull and Schwarz 2001; Landi et al. 2003). Even superseded females are thus likely to insure the protection of their other developing offspring without reducing the potential for future grand-offspring, though this benefit is greatest for older queens with decreasing fecundity. Another advantage of social nesting is it saves surviving daughters from the putatively risky task of initiating nests. If daughters can reproduce using their natal nest after their mother's death, this greatly facilitates the evolution of worker behavior (Nonacs 2011). *M. genalis* workers may gain this benefit during the dry and early wet seasons, but nesting substrate decays throughout the rainy season, reducing the value of nest inheritance (Wcislo et al. 2004).

The fitness payoffs of male and female offspring also depend on the population sex ratio (Fisher 1958; Crozier and Pamilo 1996). The sex investment pattern in our study showed an overabundance of males, despite the fact that all foundresses in our study were mated and suggests that both solitary foundresses and queens were overinvesting in males, which would potentially lower their overall fitness if mating competition was high. A similar male bias sex ratio was observed early in the nesting cycle of a socially polyphenic population of *A. aurata* (Packer 1990). A biased investment in sons among solitary nests is expected by split sex ratio theory, if it is balanced by a female biased investment among social nests (Grafen 1986; Godfray and Grafen 1988; Boomsma and Eickwort 1993; Meunier et al. 2008). Our results indicate that social nests were more female-biased than male nests, suggesting that split sex ratios may play a role in the social dynamics of this speces. It is unknown, however, how well the sex ratio measured over the course of our study reflects the population sex ratio. *M. genalis* have a long and asynchronous reproductive period, and sex ratio may be more female biased during the early part of the season, which is not captured in the current study (A.R. Smith, personal observation). Furthermore, nothing is known of the mating biology of *M. genalis*. It is also unknown whether all-male broods are common among eusocial halictid bees because sex ratio data are often pooled

among nests. Understanding the role of sex ratio on the social dynamics of this species requires further study.

Conclusions

Intraspecific polyphenisms may be important for some major evolutionary transitions in life history (West-Eberhard 1986; Maynard Smith and Szathmáry 1995). The facultative expression of both the putative ancestral and derived states within a single population of *M. genalis* supports this hypothesis. Facultative expression of a social behavioral phenotype is mostly discrete but is associated with a physical phenotype (size) that is continuous and overlapping. Most foundresses that produce females in their first brood become queens of a social colony. By producing nonreproductive offspring in their first brood; however, these females delay reproductive success. This cost is balanced by increases in subsequent broods and assuring reproductive continuity on the nest in case of queen death. Most smaller foundresses choose to lay male eggs in their first brood, possibly because they are less capable of provisioning female offspring or physically dominating them into working. They accrue earlier reproductive success because all of their offspring were reproductively capable, but they are less likely to produce additional broods. Overall, variation in foundress reproductive behaviors appears to be an important factor in the evolution of eusociality that requires future study.

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