

Frequency of social nesting in the sweat bee *Megalopta genalis* (Halictidae) does not vary across a rainfall gradient, despite disparity in brood production and body size

S. M. Tierney · C. N. Fischer · S. M. Rehan ·
K. M. Kapheim · W. T. Weislo

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Abstract Local environmental conditions can facilitate or preclude the development of eusocial colonies in insects that facultatively express behavioural-caste polyphenism. To explore how environmental variability relates to the expression of social behaviour, we collected 120 nests of the facultatively social sweat bee, *Megalopta genalis* (Halictidae: Augochlorini), along a nearly twofold rainfall gradient in central Panama. Brood rearing activity of bees in seasonal neotropical forests should track flowering phenologies, which are typically set by rainfall and phylogenetic patterns.

Nests were collected at roughly similar times of year from three sites comprising wet, moist and dry lowland tropical forests. There were significant differences in ovarian development, brood production and body size across sites for some comparisons, but no effect on the proportion of social colonies collected at each site. Results show that phenotypes of *M. genalis* relevant to social behaviour (ovarian development, brood production, body size) may be responsive to variation in local environment over distances of <20 km.

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Keywords Social evolution · Seasonality · Rainfall gradient · Eusocial behaviour · Augochlorini

S. M. Tierney (✉) · C. N. Fischer · S. M. Rehan ·
K. M. Kapheim · W. T. Weislo (✉)
Smithsonian Tropical Research Institute, Apartado Postal,
0843-03092 Panama, Republic of Panama
e-mail: simon.tierney@flinders.edu.au

W. T. Weislo
e-mail: wcislow@si.edu

S. M. Tierney
School of Biological Sciences, Flinders University, GPO Box
2100, Adelaide, SA 5001, Australia

C. N. Fischer
Department of Molecular, Cellular and Developmental Biology,
Yale University, 219 Prospect Street, 904 Kline Biology Tower,
New Haven, CT 06511, USA

S. M. Rehan
Department of Biological Sciences, University of Pennsylvania,
433 S University Avenue, Philadelphia, PA 19104, USA

K. M. Kapheim
Department of Entomology and Institute for Genomic Biology,
University of Illinois at Urbana-Champaign, 1206 W Gregory,
Urbana, IL 61801, USA

Introduction

Social arthropods that facultatively switch between solitary and eusocial-group living provide opportunities to explore the factors thought to be influential in the development and maintenance of societies (reviewed by Michener, 1974; West-Eberhard, 1996; Weislo, 1997; Schwarz et al., 2007; Purcell, 2011). Comparisons of social polyphenism (sensu Michener, 1961) within and among natural populations permit an understanding of how local environmental conditions shape social phenotypes (e.g. Weislo, 1996; Richards, 2004). In addition to shaping seasonal activity, environmental factors can also influence body size, which often has fitness repercussions relating to social hierarchies, reproductive asymmetries, and other traits (Roulston and Cane, 2000; Weislo, 2000; Richards, 2004; Cronin et al., 2011; Kapheim et al., 2011; Smith et al., 2012).

The physical environment can preclude or promote the expression of social behaviour in populations of facultatively eusocial bees. Eusocial societies are characterized by an overlap of generations among nest-mates (Michener,

1974), which require a growing season sufficiently long to permit the development of at least two broods (Wcislo and Danforth, 1997). For example, in temperate montane sites, brood rearing seasons may be short relative to egg-to-adult developmental rates, and an overlap of generations would be precluded due to insufficient time to rear a second brood. Thus in some bee species, populations that live at low elevations express eusocial behaviour, while those living at high elevations, are solitary (Sakagami and Munukata, 1972; Eickwort et al., 1996; Wcislo, 1996; Soucy, 2002). Comparable patterns are known for latitudinal gradients (e.g. Richards, 2004; reviewed by Wcislo, 1997; Purcell, 2011) and have been demonstrated experimentally using reciprocal transplants of nests from southern and northern populations of a sweat bee, *Halictus rubicundus* (Halictidae: Halictini) (Field et al., 2010, 2012).

In contrast to temperate zone bees, the relationships between seasonality of resource availability, brood production and sociality are less well understood in the tropics. Colonies of a Costa Rican sweat bee, *Lasioglossum (Dialictus) umbripenne* (Halictidae: Halictini), for example, showed markedly different patterns in seasonal activity, social structure, and caste-related morphology, when comparing a highly seasonal Pacific coast population (Wille and Orozco, 1970), with a less seasonal Caribbean slope population (Eickwort and Eickwort, 1971), although bees were studied at different times of the year. Understanding such patterns is complicated by the fact that flowering phenology can vary dramatically even over relatively short geographic distances (Janzen, 1967; Roubik, 1989; Wright and Calderon, 1995; Frankie et al., 2004), and the species composition of forest communities can vary as well (Pyke et al., 2001; Condit et al., 2005).

Here, we examine whether expression of eusociality varies with respect to variation in local environmental conditions, using three populations of the sweat bee *Megalopta genalis* Meade-Waldo (Halictidae: Augochlorini), along a rainfall gradient parallel to the Panama Canal in central Panama. We used a 50-km north–south transect as a proxy for variation in resource availability. We used nest collections, census data, and dissections to provide snap-shots of social colony structure, assessed as both the proportion of social colonies and colony size within a population. We also used morphometric data to compare body size with ovarian development.

Methods

Précis of natural history

Megalopta is a genus of nocturnally foraging bees that nest within dead tree branches, lianas or vines (Wcislo et al.,

2004; Tierney et al., 2008a; Wcislo and Tierney, 2009; Santos et al., 2010). In central Panama the main brood rearing period occurs during the dry season (December to May). The dry season begins slightly earlier (2–3 weeks) on the Pacific coast but ends at roughly the same time at all three sites in mid- to late-April (Condit et al., 2000). Brood rearing continues through the early- to mid-wet season (until August), tapering off as the rains intensify, with little or no foraging activity at the end of the wet season (Wcislo et al., 2004). The egg-to-adult developmental rate of *M. genalis* in central Panama is approximately 35 days (Wcislo et al., 2004; Wcislo and Gonzalez, 2006). On Barro Colorado Island, bees collect pollen from at least 64 species of angiosperms, but rely most heavily on about five species (Smith et al., 2012).

Field sites

We examined *M. genalis* nesting biology at three lowland (<200 m elevation) sites along a transect in central Panama, roughly in parallel with the transoceanic Canal (see Table S1 for details on site descriptions and meteorological data):

(1) *Caribbean site* Santa Rita Arriba (SRA) [9°19'48"N, 79°46'48"W] is near the Atlantic coast and has mean annual precipitation of 3,054 mm. Some rain falls in all months of the year, and at the Atlantic entrance to the Canal the dry season lasts approximately 102 days (Condit et al., 2000). The forest is diverse with 162 species of trees and shrubs in a 1-ha plot having a diameter at breast height (dbh) \geq 10 cm (Condit et al., 2005).

(2) *Mid-isthmus site* Barro Colorado Island (BCI) [9°9'0"N, 79°51'36"W] has mean annual precipitation of 2,581 mm. The forest is intermediate in diversity with 90 species in a 1-ha plot having a dbh \geq 10 cm (Condit et al., 2005).

(3) *Pacific site* Parque Natural Metropolitano (PNM) [8°59'34"N, 79°32'24"W] is near the Pacific terminus of the Canal, where there is a distinct dry season from December through late April to early May (approximately 129 days), and on average the area receives <1,875 mm of rain per year, nearly all of this during the wet season (see Table S1). Surrounding Pacific forests are generally less diverse with a mean of 57 species in a 1-ha plot having a dbh \geq 10 cm (Condit et al., 2005), around 80 species of trees and lianas have been recorded from PNM (Kalacska et al., 2007).

In central Panama there are 1,142 tree and shrub morphospecies. Of these, 492 are restricted to wet forest only, such as the SRA site, 87 are restricted to dry sites, such as PNM, and 197 species are widespread and occur across the isthmus (Pyke et al., 2001; Condit et al., 2005).

Nest collections

Between 1 April and 17 June 2009, 40 nests were collected at each site on the following dates: SRA, 1–13 April ($n = 12$) and 1–16 June ($n = 28$); BCI, 8–22 May ($n = 20$) and 10–11 June ($n = 20$); PNM, 4–25 May ($n = 33$) and 9–17 June ($n = 7$). Nests were collected between 0800 and 1600 hours, when bees were not foraging and all residents were assumed to be present. Nests were transported back to the laboratory, split longitudinally and then all contents were recorded as a snap-shot of nesting biology and social structure for each nest. Nest architecture was noted and brood were categorized as follows: egg, small larva (first and second instars), medium larva (third and early fourth instar), large larva (late fourth instar), pre-pupa (post-defecating fourth instar), pupa, or callow adult. We recorded the number of sealed and open brood cells; open brood cells that only contained pollen were presumed to represent provisions for future egg-laying.

Dissections and morphometrics

Adult females were measured and dissected under a stereomicroscope to record the following morphometrics on body size and reproductive condition. Intertegular width (Cane, 1987; Tierney et al., 2008b) and forewing length (axillary sclerites to the base of the stigma) provided measures of body size. Wing wear was scored by the number of nicks in the distal wing margin, which provides an estimate of foraging effort or age (Mueller and Wolf-Mueller, 1993; Tierney and Schwarz, 2009). Spermathecae were examined for presence of sperm as evidence of mating. The lengths of the three largest terminal oocytes were summed, as a measure of ovarian development. We used an ANCOVA on solitary nesting females to assess potentially confounding body size scaling effects on ovary size, prior to conducting other analyses. Females were ranked within each nest according to summed oocyte length, as a proxy for their reproductive caste; ranks lower than fourth were excluded from analyses due to small sample sizes. We subsequently compared means of this metric within and between sites, and used ovarian rank to assess relationships between body size and wing wear.

Re-sampling to account for asynchronous nest initiation

Ovarian development is associated with social status within a nest (e.g., Smith et al., 2008), and our analyses are potentially confounded by the fact that nest initiation is not necessarily synchronous among females within a population, or among populations. To address this problem, we used a re-sampling procedure (1,000 replicates) to calculate

the mean difference in ovary size between randomly selected pairs of individuals, drawn from the pool of recorded individual values (Rehan et al., 2009; Tierney and Schwarz, 2009). We then calculated the observed mean difference and identified its percentile position within a simulated stochastic distribution. This result gives an indication of the deviation of observed data from a random assortment of pairs. We ran analyses for all multi-female colonies pooled, then independently for each site, and repeated these for two-female colonies only. We also used two resampling procedures, whereby the sampled pairs were either (1) excluded from or (2) returned to the pool.

Statistics

Re-sampling analyses were performed using R (R Development Core Team, 2011); code is available from the first author. All other statistical analyses were performed with SPSS© v11. Data were tested for normality (Kolmogorov–Smirnov or Shapiro–Wilk tests) prior to analyses, and Levene’s homogeneity of variance tests were undertaken prior to all parametric analyses. When assumptions were violated we used non-parametric tests, as indicated in the text, and alpha values for multivariate tests were corrected using the sequential Bonferroni technique.

Results

Nest censuses

Colonies contained up to five adult females and nine brood (Table 1). The modal group size was one female per nest, with 25–33 % of colonies containing two or more females. The number of adult females per nest did not differ among collection sites (Table 1; Fig. S1). There were significant differences among sites in total brood numbers (see Table 1; Fig. 1). Colonies from BCI contained significantly more total brood than both SRA ($U = 520.5$, $P = 0.006$) and PNM ($U = 494$, $P < 0.002$); these patterns were maintained when classes of brood were analyzed separately as eggs or larvae, but were not significant for pupae. When total brood numbers were considered separately for solitary nests and pooled multi-female colonies, then the effect of site on brood variables was upheld for solitary nests, but not for multi-female colonies from different sites (Table 1). An analysis of open brood cells that were actively being provisioned also showed an effect of collection site (Table 1), with greater numbers of such cells in solitary nests from BCI than at SRA ($U = 256.5$, $P = 0.014$), but not between BCI and PNM ($U = 313$, $P = 0.06$).

Table 1 Colony census data, giving the minimum, maximum and mean values for numbers of adults, immature brood and open brood cells that were actively being provisioned (active cell provisions)

	%	<i>n</i>	Mean					
			Adult females	Total brood	Eggs	Larvae	Pupae	Active cell provisions
SRA								
Caribbean wet forest								
Min.–max.			1–4	0–5	0–2	0–3	0–4	0–1
All nests		40	1.7	1.2	0.4	0.5	0.4	0.2
Multi-female nests	32.5	13	3	2.7	0.6	1	1.1	0.5
Single-female nests	67.5	27		0.6	0.3	0.2	0.1	0.1
BCI								
Mid-isthmus moist forest								
Min.–max.			1–5	0–8	0–2	0–4	0–4	0–2
All nests		40	1.7	2.5	0.7	1	0.6	0.4
Multi-female nests	32.5	13	3	4.3	1	1.6	1.5	0.5
Single-female nests	67.5	27		1.6	0.6	0.7	0.2	0.4
PNM								
Pacific dry forest								
Min.–max.			1–5	0–9	0–2	0–5	0–4	0–2
All nests		40	1.6	1.4	0.3	0.6	0.5	0.3
Multi-female nests	25	10	3.3	4.3	0.8	1.6	1.8	0.4
Single-female nests	75	30		0.5	0.1	0.3	0.1	0.2
Pooled sites								
Min.–max.			1–5	0–9	0–2	0–5	0–4	0–2
All nests		120	1.6	1.7	0.5	0.7	0.5	0.3
Multi-female nests	30	36	3.1	3.7	0.8	1.4	1.4	0.4
Single-female nests	70	84		0.9	0.3	0.4	0.1	0.2
Collection site effects								
All nests								
Kruskal–Wallis H_2				11.48	11.59	8.86		
Probability			n.s.	0.003	0.003	0.012	n.s.	n.s.
Single-female nests								
Kruskal–Wallis H_2				17.2	12.55	10.4		7.28
Probability			n.s.	<0.001	0.002	0.006	n.s.	0.026

Mean values are provided separately for solitary and multi-female colonies, and on a collection site basis. Non-parametric analyses on the effect of collection site on these metrics are also presented

Body size

There was a highly significant effect of collection site on intertegular width (one-way ANOVA, $F_{2,191} = 14.5$, $P < 0.001$), and post hoc Tukey tests showed that BCI females were significantly larger than females at both SRA ($P = 0.003$) and PNM ($P < 0.001$), whereas females from SRA and PNM did not differ (Fig. 2). Comparing only solitary females again showed a significant effect of site on body size ($F_{2,79} = 15.3$, $P < 0.001$): BCI females were larger than SRA ($P = 0.005$) and PNM ($P < 0.001$) females, with no difference between the latter two sites (Fig. 4).

Mean body size for our populations did not differ over the period of our sampling dates. Independent Kruskal–Wallis tests for each site showed no significant differences between intertegular width as a function of collection week (SRA $H_4 = 4.54$, $P = 0.338$; BCI $H_3 = 1.76$, $P = 0.624$; PNM $H_5 = 5.37$, $P = 0.372$).

Multi-female colonies

Morphometrics for individuals from multi-female colonies are summarized in Table S2. In all but one colony, the female exhibiting the greatest ovarian development was fertilized, and in the majority of cases this individual was

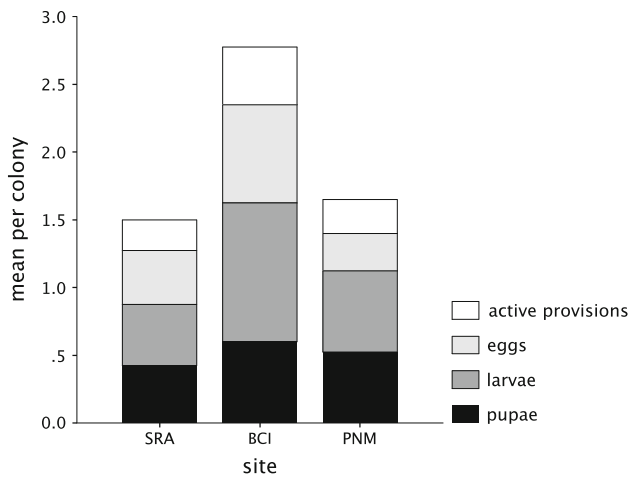


Fig. 1 Brood production summarized by developmental stage. Mean number of open brood cells being actively provisioned (active provisions), and cells with eggs, larvae and pupae per nest are summarized for each collection site: *SRA* Santa Rita Arriba, *BCI* Barro Colorado Island, *PNM* Parque Natural Metropolitano

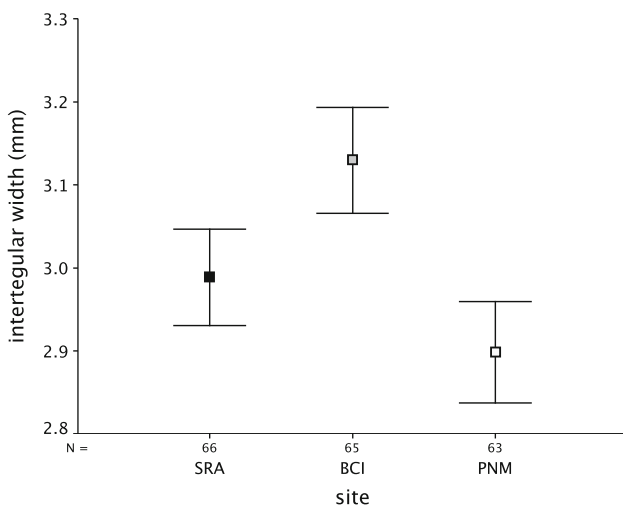


Fig. 2 The mean intertegular width as a measure of body size is summarized by collection site. Error bars represent 95 % confidence intervals. Collection sites are coded: *SRA* black, *BCI* grey, *PNM* white

also the largest female within the nest (pooled sites 73 %; *SRA* 69 %; *BCI* 62 %; *PNM* 91 %). In most (76 %) nests more than one female were inseminated. Nine of 36 (25 %) multi-female colonies contained a sole inseminated female accompanied by un-mated female(s) with lesser ovarian development. In one multi-female nest (*PNM* 8) females were uninseminated, had small ovaries and no wing wear, suggesting that the nest may have been recently orphaned. We statistically assessed the relationships between body size and ovarian development at the population level in greater detail below (see among-site comparisons).

Ovarian development

Body size scaling effects

An ANCOVA on solitary females, with the sum of oocyte lengths as the dependent variable, collection site as the fixed factor and intertegular width as a covariate, indicated no interaction between collection site and intertegular width ($F_{2,74} = 1.17, P = 0.317$). With the interaction removed from the model we found no evidence of a body size scaling effect on ovarian development, while controlling for site ($F_{1,76} = 1.5, P = 0.225$). There was, however, a significant effect of site on ovarian development when the covariate (body size) was controlled for ($F_{2,76} = 5.3, P = 0.007$).

In comparisons among collection sites, *BCI* solitary females had significantly greater ovarian development than solitary females at the other two sites (Fig. 3). A one-way ANOVA (covariate removed) indicated a highly significant site effect ($F_{2,77} = 9.5, P < 0.001$). *BCI* solitary females showed significantly greater mean ovarian development than either *SRA* ($P = 0.002$) or *PNM* ($P < 0.001$) solitary females, and the means of the latter two sites did not differ.

Social female ovarian state

Among-site comparisons There were no differences between sites in ovary size for first rank social females (Fig. 3). For second rank females, however, those of *BCI* had larger ovaries than females from the other two sites, but differences were marginally non-significant with a Bonferroni correction (adjusted $\alpha = 0.025$); *SRA* ($U = 41$,

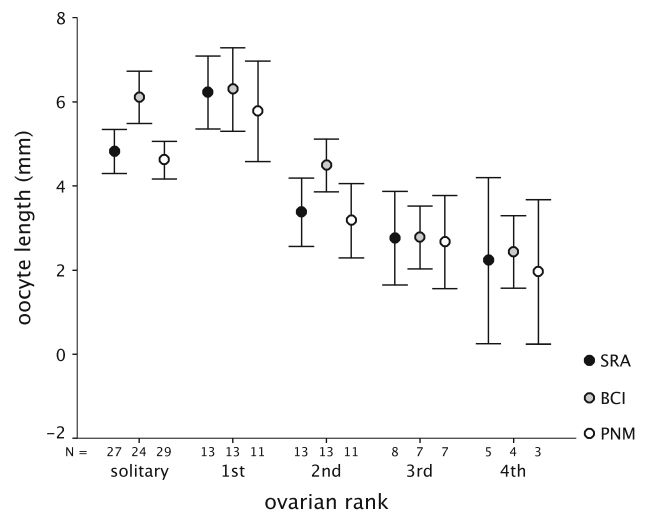


Fig. 3 The mean length of summed oocytes (with 95 % confidence intervals) is given as a function of ovarian rank for females in solitary nests, and those in multi-female colonies: 1st ranks are the putative reproductively dominant queens; 2nd to 4th ranks are supernumerary females. Collection sites are coded: *SRA* black, *BCI* grey, *PNM* white

$P = 0.026$) and PNM ($U = 33$, $P = 0.026$), which is the same pattern as found in solitary females (above). Ovarian development of other lower ranked females did not differ among collection sites.

Within-site comparisons When each collection site was analyzed independently, we found a significant population level effect of within-nest ovarian rank on mean oocyte length (SRA $H_2 = 21.5$, $P = 0.001$; BCI $H_2 = 23.9$, $P = 0.001$; PNM $H_2 = 14.9$, $P = 0.002$). Within each site, first-ranked females from SRA and BCI had significantly larger ovaries than all other (second to fourth rank) supernumerary females (Mann–Whitney, Bonferroni adjusted $\alpha = 0.0083$: SRA $P \leq 0.003$; BCI $P \leq 0.005$); while first-ranked females from PNM had larger ovaries than second and third rank females only ($P \leq 0.004$). A comparison of supernumerary females revealed that BCI second rank females displayed significantly different ovary development than BCI third rank females ($U = 8.5$, $P = 0.003$), but no other pairwise tests were significant.

Re-sampled pairs to account for reproductive asynchrony Among females sharing the same nest, observed pairwise differences in oocyte length were not significantly different from re-sampled distributions, where females were paired at random (Table S3). At BCI observed differences ranged across the mid-level percentiles (44th–63rd) of the re-sampled distribution, while at SRA and PNM observed differences placed into the upper percentiles of the re-sampled distribution (SRA 73rd–96th percentile range; PNM 69th–77th percentile range). Results did not differ greatly among procedures that either (1) replaced or (2) excluded previously sampled pairs from the sampling pool.

Ovarian state of first-ranked females versus solitary females Within each site, we also compared ovary size between first rank females of multi-female colonies and those of solitary females. Analyses showed that first rank females from colonies at SRA and PNM, respectively, had more developed ovaries than solitary females at SRA ($U = 84.5$, $P = 0.007$) and PNM ($U = 90.0$, $P = 0.035$). On BCI, however, ovary size of solitary females did not statistically differ from first-ranked females from multi-female colonies (Fig. 3).

Ovarian differentiation and body size

We tested for evidence of size-dependent reproductive differentiation, comparing differences in mean body size (intertegular width) among ovarian ranks of multi-female colonies (Fig. S2), conducting analyses independently for each site. To present these findings in a clearer manner, we grouped all supernumerary females and compared this mean with that of first-ranked females, the putative queens (Fig. 4). BCI colonies showed no significant difference in

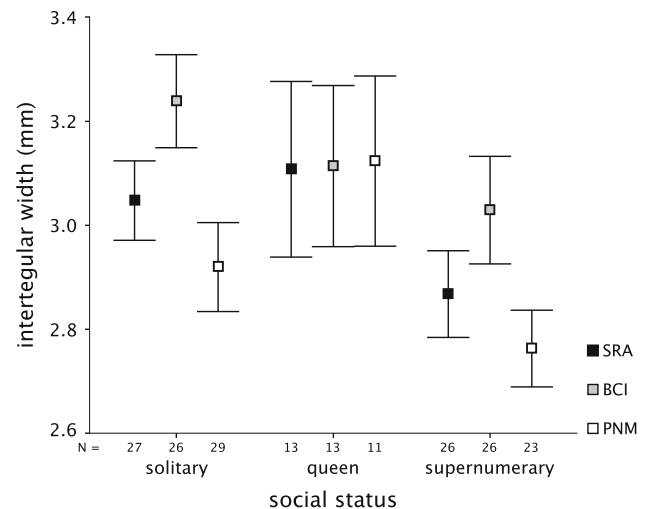


Fig. 4 Body size as a function of social status. Mean intertegular width (with 95 % confidence intervals) as a measure of body size, given as a function of nesting habit and ovarian rank for females in ‘solitary’ nests, and those in multi-female colonies: 1st ranks are the putative reproductively dominant ‘queens’; 2nd to 5th ranks are designated as ‘supernumerary’ females (mean body size for all supernumerary ranks are provided in Fig. S2). Collection sites are coded: SRA black, BCI grey, PNM white

mean body size among ovarian ranks. SRA colonies showed a marginal effect of ovarian rank ($F_{3,35} = 2.9$, $P = 0.047$), but post hoc tests did not indicate any pair-wise differences between ranks; although a t test comparing queens with pooled supernumerary females was significant ($t_{37} = 3.04$, $P = 0.004$). There was a significant difference in body size among ovarian ranks only for PNM colonies ($F_{3,28} = 8.8$, $P < 0.001$), such that first-ranked females were significantly larger than all other ovarian ranks (all post hoc Tukey comparisons with $P \leq 0.035$); this finding was substantiated by a t test when supernumerary females were pooled and compared with queens ($t_{32} = 5.01$, $P < 0.001$).

Mean body size and confidence intervals of first rank females (queens) from all sites were virtually the same (Fig. 4), despite highly significant site effects on body size at the population level (see Fig. 2). However, the population level trends of mean body size at each collection site were paralleled in both (1) mean body size of solitary females and (2) supernumerary females (compare Figs. 2; 4). The general trend is that BCI females were biggest, followed by SRA, and PNM females were always smallest.

Raw data scattergrams plotting intertegular width against summed oocyte length are provided in the ESM (Fig. S3). These plots indicate that there were substantial overlaps in body size ranges both among populations and behavioural categories (solitary, queens and supernumerary females). Only five supernumerary females were smaller than the smallest queens (compare Figs. S3c and S3d).

Supplementary results

Data concerning nest architecture (Table S4), colony size (Fig. S1), dissection data (Table S2), resampled ovarian differentiation between nest-mates (Table S3), site effects on body size and ovarian development (Figs. S2, S3), wing wear (Fig. S4) and sex ratio are provided in the ESM.

Discussion

Environmental effects on social organization

Halictines are among the most frequently studied social bees with regard to environmental factors and their influence in shaping the expression of social behaviour (Michener, 1990; Wcislo, 1997, 2000; Richards, 2004; Schwarz et al., 2007; Field et al., 2010). In our study there were no differences in either the proportion or the size of multi-female colonies, across a rainfall gradient. At a finer spatial scale (BCI 50-ha forest plot), Smith et al. (2012) found there was no correlation between the spatial distribution of floral resources and the spatial distribution of *M. genalis* social or solitary nests, indicating that both solitary and social nests have access to the same resources at this spatial scale. Such observations suggest that resource availability is not a decisive factor in the expression of social nesting in *M. genalis*. Interpreting the among-site behavioural differences in the context of local variation in resource availability is complicated by the fact that the sites differ significantly in tree species composition (Condit et al., 2005), and hence are predicted to have somewhat different phenologies due to phylogeny (Wright and Calderon, 1995), regardless of local variation in rainfall patterns.

Variation in local climate at the same site across annual cycles has been shown to influence sociality in a temperate zone halictine. Increased rainfall during brood rearing periods of temperate *Halictus ligatus* (Halictidae: Halictini) is associated with production of smaller and fewer workers, as compared to drier brood rearing seasons when there are more workers that are larger, mated and actively reproducing (Richards and Packer, 1995, 1996; Richards et al., 1995, Richards, 2004). While the proportions of *M. genalis* multi-female colonies did not differ across our sites, colonies were most productive at the mid-point (BCI) of our rainfall transect (Fig. 2) and supernumerary females (putative workers) were smallest at the driest site (with the longest dry season), PNM on the Pacific coast (Fig. 4).

Environmental effects on colony productivity and body size

Results suggest that *M. genalis* may show evidence of seasonal difference in brood productivity and body size at

different sites that vary in rainfall. Studies of two populations of *L. umbripennis* in Costa Rica demonstrated an influence of seasonality on colony size and degree of worker-queen body size differences (Wille and Orozco, 1970; Eickwort and Eickwort, 1971). In contrast, different populations of *M. genalis* showed no observable differences in colony size and there is no indication of diapause in any of our samples. The reproductive dominants and supernumerary females in our study differed most significantly in body size in nests from the Pacific site (PNM, Fig. 4), but the greatest observed differences in nest-mate ovarian development were found among colonies at the Caribbean site (SRA, Fig. 3), which is the least seasonal site in terms of rainfall.

Megalopta genalis were physically larger and more fecund at the mid-isthmus site (BCI) than at either extreme of the isthmian transect, in our samples from an 11-week period spanning the transition from dry to rainy seasons. BCI was the only site where the ovarian development of reproductive dominants (ovarian first rank) from social nests was not significantly greater than that of solitary females, at the population mean level (Fig. 3). This finding contradicts results from previous studies of *M. genalis* from BCI (Smith et al., 2008, 2009; Kapheim et al., 2012), in which social queens displayed greater ovarian enlargement than solitarily nesting females and workers in social nests. Kapheim et al. (2012) set up artificial observation nests collected from roughly the same period of the same season as our study, although these nests were seeded with newly eclosed callow females that might or might not have founded nests in their own right. Our results also differ from modified natural nests observed by Smith et al. (2009), but these were collected earlier in the dry season (February–March 2004). It is possible that some of our solitary nests may have eventually developed into social nests, and that this is masked by our sampling procedures; however, the same site-related patterns of ovarian development observed among solitary females are also maintained among second ovarian-rank females from social nests (Fig. 3).

Reproductive dominants from BCI social colonies were not physically larger than supernumerary females, when assessed at the population level (Fig. 4). Again, this differs from results of the previously mentioned studies of *M. genalis* from BCI (Smith et al., 2009; Kapheim et al., 2012). Size-based reproductive hierarchies in these bees are determined relative to the body size of nest-mates (Smith et al., 2008, 2009; Tierney et al., 2008a; Kapheim et al., 2012), rather than absolute size. The similarity of mean body size (and variances) of queens across all collection sites (Fig. 4) raises the possibility that a certain threshold is required to maintain a dominance hierarchy; dominant females face an increased risk of nest supersedure if their daughters are equal or nearly equal to them in body size

(Kapheim et al., [in press](#)). By comparison, the site-related patterns of body size in solitary and supernumerary females imply that there are localized site effects on phenotype. Given that our data are snap-shots of colony phenology we do need to consider the possibility that (1) some supernumerary females may disperse to found their own nests, (2) some solitary nests may develop into social colonies, and (3) some foundresses may have died or been superseded (Kapheim et al., [in press](#)). Nevertheless, site-related trends in body size are consistent across all supernumerary females ranks (Fig. S2, summarized in Fig. 4).

The question remains as to whether these site-related phenotypic differences represent a plastic response to environmental variation, as demonstrated for *H. rubicundus* (Field et al., 2012). In central Panama, the dry season begins earlier and is longer at the Pacific end of the transect (Condit et al., 2000, 2004), so it may be that at the time of sampling BCI offered higher quality or more abundant floral resources than the other sites. However, detailed phenological data across sites are unavailable to test this hypothesis. Times of peak brood rearing and resource availability may vary both spatially and temporally. For example, a study of the BCI population showed that both bee productivity and diversity of pollen within cells was higher in dry season collections (February and March 2007) than in wet season ones (May and July 2007) (Smith et al., 2012).

Pollen protein quality supplied to *Lasioglossum* bee larvae is more critical than pollen quantity in determining adult body size (Roulston and Cane, 2002), but there is no evidence that bees can judge quality when collecting pollen. Seasonal studies on a tropical stingless bee, *Nannotrigona perilampoides* (Apidae: Meliponini), show that worker body size and mass directly correspond to seasonal pollen protein content (Quezada-Euán et al., 2011), and that size variation is lowest among cohorts of the same age as opposed to individuals collected across the year. This implies that variance in bee body size is partly determined by temporal variance in resource quality, but our study cannot distinguish pollen use at different sites.

Translocation experiments are an appropriate way to tease apart the relative influence of environment on population phenotypes in facultatively social bees (Cronin, 2001; Field et al., 2010, 2012). While constraints of genetic lineage may need to be considered (Plateaux-Quénu et al., 2000; Soucy and Danforth, 2002; but see Zayed and Packer, 2002), the overlap in body size ranges among populations and behavioural castes of *M. genalis* presented here suggests social polyphenism.

Conclusions

Based on censuses of nest collections, there were no differences in the frequency of social nesting in populations of

Megalopta genalis at three different sites across a rainfall gradient in central Panama. There were, however, significant differences among some sites in body size, ovarian development and brood productivity for some classes of females. Spatial and temporal variation in an environmental factor such as rainfall may partly determine flowering phenology, and hence resource availability for bees. In turn, quantity and quality of available resources may ultimately affect size-based reproductive differentiation and social organization. Most population level studies lack detailed phenological information, and data on resource utilization. The site-related differences identified in this study should provide an incentive to collect comparative data on flowering phenology and quality and quantity of floral resources, to better understand the role of resource availability on bee social organization.

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