J Insect Behav
https://doi.org/10.1007/s10905-019-09736-7

Small Body Size Is Associated with Increased Aggression in the Solitary Sweat Bee *Nomia melanderi* (Hymenoptera, Halictidae)

Adam R. Smith · Timothy DeLory · Makenna M. Johnson · Anna C. Figgins · Mallory A. Hagadorn · Karen M. Kapheim

Received: 5 June 2019 / Revised: 10 December 2019 / Accepted: 15 December 2019

Abstract Modifying ancestral regulatory mechanisms can be a source of evolutionary novelty. Bees in small-colony, dominance-based societies typically show a link between size and aggression: larger bees are more aggressive. This led to the hypothesis that this size-aggression link is a characteristic of ancestral solitary bees that has acquired a novel function in the evolutionary transition from solitary to social behavior. Here we test the central prediction of this hypothesis, that size is linked to aggression in an ancestrally solitary bee. We use the sweat bee *Nomia melanderi* (Hymenoptera, Halictidae) in the subfamily Nomiinae, which is sister to the social sweat bees, all of which are in the subfamily Halictinae. We measured aggression using a standardized behavioral assay (circle tube) in which two bees were placed together and allowed to interact. We used three treatments: size matched small bees, size matched large bees, and one large bee paired with one small bee. We found no link between ovary size and aggression, but because we used only reproductively active bees we may not have had sufficient variation to detect such a link. Across treatments, body size negatively correlated with aggression. There were no differences between large and small bees in the matched treatments, but in the mixed treatment, small bees were more aggressive than large bees. This supported our prediction of a size-aggression link, although the link was in the opposite direction seen in social bees. Moreover, our data show that even solitary bees can modulate their aggression in response to social context and highlight the importance of studying related solitary species to understand the evolutionary origins of sociality.

Keywords Alkali bee · dominance · circle tube · social evolution

A. R. Smith (✉)
Department of Biological Sciences, George Washington University, 800 22nd St NW, Washington, DC, USA
e-mail: adam_smith@gwu.edu

T. DeLory · M. M. Johnson · A. C. Figgins · M. A. Hagadorn · K. M. Kapheim
Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322, USA

Introduction

The social insects in the order Hymenoptera (bees, ants, and wasps) are characterized by the presence of a reproductive queen and non-reproductive workers (Wilson 1971). Small colony species representative of the presumed early steps in the evolutionary origins of social cooperation are characterized by dominance-based societies (Michener 1990; West-Eberhard 1996; Bourke 2011). Thus, understanding how aggressive dominance behaviors evolved from solitary ancestors is important to understanding the origins of sociality. The range of social behaviors expressed in bees, from solitary nesting to small groups with a queen and worker to large colonies numbering in the thousands, makes them excellent organisms for comparative studies of social evolution (Michener 1974; Schwarz et al. 2007; Kocher and

Published online: 20 December 2019

Paxton 2014; Kapheim et al. 2015; Rehan and Toth 2015; Toth and Rehan 2017; Kapheim 2019).

The sweat bees (Halictidae) are especially useful because the family has two independent origins of sociality from a solitary ancestor (Gibbs et al. 2012). Previous studies of sweat bees have shown queens are larger than workers in most, but not all species (Michener 1990; Richards and Packer 1994). Replacement queens (workers that become reproductive after the original queen's death or removal) also tend to be larger than other workers (Michener 1990; Mueller 1993). Larger body size is also associated with higher levels of aggression in behavioral assays of *Lasioglossum pauxillum, L. malachurum* and *Halictus ligatus* (Smith and Weller 1989; Pabalan et al. 2000). In recent studies of the facultatively eusocial (sensu Batra 1966) or solitary sweat bee *Megalopta genalis* we have shown that queens are larger than workers (Smith et al. 2008, 2009; Kapheim et al. 2012, 2013) likely because they provide worker-destined daughters with less pollen for larval development (Kapheim et al. 2011). Upon emergence, worker-destined daughters are aggressively dominated by queens (their mothers) (Kapheim et al. 2016). Queens who are similarly sized, or smaller than their workers are more likely to be usurped by those workers (Kapheim et al. 2013). Thus, behavioral studies across the halictids seem to suggest that size-based aggression is an important feature of the earliest stages of eusocial evolution in this family of bees.

There is a rich history of studying aggressive behavior in sweat bees using a classic experimental paradigm known as the circle tube assay (Breed et al. 1978; reviewed by Pabalan et al. 2000; Packer 2006; Dew et al. 2014). In this standardized behavioral assay, two bees are placed into a transparent tube which is then joined into a circle, forcing the bees to interact. In previous studies comparing *M. genalis* queens and workers in circle tubes, queens exhibited dramatically higher levels of aggressive behaviors than workers (Smith et al. 2018, 2019), consistent with in-nest observations of behavior (Kapheim et al. 2016) as well as in-nest behavioral observations of other sweat bee species (reviewed in Michener 1990; Dalmazzo and Roig-Alsina 2018a, b). This caste-based difference in aggression was seen whether individuals were matched for caste, but from different nests (Smith et al. 2018), or were a queen and worker from the same nest (Smith et al. 2019). In both studies body size was the best predictor of aggression in *M. genalis*: larger bees were more aggressive. Even among workers, who were much less aggressive than queens or solitary reproductives, larger workers were more aggressive than smaller workers (Smith et al. 2018, 2019). This suggests that size and aggression may be linked via physiological mechanisms, independent of social context.

Smith et al. (2018) hypothesized that size may be mechanistically linked to aggression. If so, queens' nutritional manipulation of their worker daughters' larval provisions might exploit existing ancestral regulatory mechanisms linking size and aggression to pre-dispose the daughters to subordinate worker behavior. In this case, manipulating a daughter's nutrition would not only influence her size, but also her expression of aggressive behavior. A prediction of this hypothesis is that ancestrally solitary sweat bees should also show a correlation between aggression and body size. Many solitary sweat bees are not ancestrally solitary, but instead secondarily solitary, as they evolved from social ancestors (Wcislo and Danforth 1997; Danforth et al. 2003). The only ancestrally solitary halictid studied to date, *Xeralictus bicuspidariae* (Halictidae: Rophitinae) showed no effect of body size on aggression, but that study was not designed to explicitly test for such an effect (Richards and Packer 2010). A second bee from the same subfamily, *Penapis toroi*, has also been studied in circle tubes, but without testing for a relationship between body size and aggression (Packer 2005, 2006). McConnell-Garner and Kukuk (1997) found no association between size and aggression in two species of secondarily solitary *Lasioglossum* sweat bees. In a forced-competition assay for a single nesting site, larger females of the solitary bee *Megachile rotundata*, which is in a different family (Megachilidae) than the sweat bees, out-competed smaller females, although aggression was not directly measured (Fischman et al. 2017). While no study has linked experimental manipulation of nutrition to social behavior in sweat bees, such experimental reductions of developmental nutrition decrease aggression in the bee *Ceratina calcearta* (family Apidae; Lawson et al. 2017). Together, these studies suggest that a regulatory relationship between body size and aggression may be conserved among bees.

A related hypothesis is that ovary size may affect aggression. In *M. genalis* there was little correlation between ovary size and aggression in circle tube trials between non-nestmates, but there was such a correlation between nestmate queens and workers, even after accounting for the caste-based differences in ovary size

(Smith et al. 2018, 2019). Even though all queens had larger ovaries than the workers in their nests, the queens with relatively larger ovaries were relatively more aggressive as well (Smith et al. 2019). This pattern of an ovary size influence on aggression in individuals in social groups, but not solitary nests, is also seen when comparing individuals from social species to solitary species (Richards and Packer 2010). Smith et al. (2018) suggested that this resulted from the shared developmental history of the queen and worker, in which body size (via maternal manipulation of nutrition), ovary size (via aggressive suppression of worker ovaries by the queen) and aggressive behavior were all linked. Richards and Packer (2010) suggested that the correlation between ovary size and aggression may result from reproductive females aggressively defending their eggs from possible replacement rather than a direct link between ovary size and behavior. Either hypothesis predicts that ovary size would not affect aggressive behavior in a solitary bee.

Here we use the ancestrally solitary sweat bee *Nomia melanderi* (Halictidae: Nomiinae) to test for a pre-existing link between size and aggression that may have been exploited in the evolution of sociality. *Nomia melanderi* has been used to test for other ancestral links between physiology and behavior (Kapheim and Johnson 2017a, b), and is in a different subfamily (Nomiinae) than the two ancestrally solitary halictid species previously tested. Our study differs from previous studies of ancestrally solitary halictids (Packer 2005, 2006; Richards and Packer 2010) in that it is specifically designed to test for an effect of body size on aggression. To explicitly test for effects of size, we chose relatively large and relatively small bees from the same population. From this pool, we created three treatments: large vs. large dyads, large vs. small dyads, and small vs. small dyads. This allowed us to separately test for effects of absolute and relative size on behavior. Based on previous studies, we predicted that larger bees would be more aggressive than smaller bees. We also dissected females after each trial to measure ovary size. Based on previous studies of solitary bees, we predicted that ovary size would not be associated with behavior.

Methods

Bees were collected between 28 May – 02 July 2018 in Touchet, WA. All females were actively provisioning nests at the time of the study, as indicated by the presence of pollen on their hind legs at the time of collection. Females were collected with a hand net and chilled for 10 min to permit marking and measurement with digital calipers. Both females were marked on the thorax between the tegula with a randomly chosen color of Testors brand enamel paint (green, yellow, red, or white) applied using a toothpick. We marked both females to prevent marking effects biasing our results (Packer 2005). After measurement, bees were assigned to a size category. Size categories were determined from the size distribution of bees collected in 2016 (mean intertegular width = 2.46 mm; 95% CI = 2.45–2.48). Bees in the top 40% of this distribution were considered large and bees in the bottom 40% of the distribution were considered small. Preliminary sampling of bees collected midway through the 2018 season suggested the size distribution was similar to that of 2016 (mean = 2.45, 95% CI = 2.44–2.49, t-test: $p > 0.7$). We recorded videos of 19 large-large (LL) pairs, 20 large-small (LS) pairs, and 20 small-small (SS) pairs. Females were placed in 2 ml mini centrifuge tubes and allowed to acclimate for 10 min prior to the start of each trial. Trials were conducted in an open-sided field laboratory at ambient temperature. The bees were then released into plastic tubing (30 cm long, 8 mm diameter). A bee was inserted at each end, and the ends were joined in a circle with a piece of wider tubing. Trials ran for 15 min and were videotaped by a Logitech c900 camera attached to a computer. Videos were scored using an ethogram based on previous studies (Dew et al. 2014; Kapheim et al. 2016; Smith et al. 2018), with some modifications (Table 1).

We used previous studies of bees in circle tubes to designate tolerant and avoidance behaviors (Dew et al. 2014; Lawson et al. 2017; Gonzalez et al. 2018). We designated 'Pass', 'antennate', 'mandible touch', and 'head–head' as tolerant behaviors. 'Back up' and 'reverse' were designated avoidance behaviors. For aggressive behaviors, we included 'Push', 'nudge', 'bite', 'nip', and 'nip-nudge'. We did not include 'back into' as an aggressive behavior because it correlated negatively with expression of push, nudge, and bite (see Results, below). Our previous studies with *M. genalis* (Kapheim et al. 2016; Smith et al. 2018, 2019) suggested that C-posture may be defensive rather than aggressive because it was displayed by subordinate bees in response to aggressive behavior, so we also analyzed that separately (see Results, below), even though previous authors have treated it as an aggressive behavior (Dew et al. 2014).

Table 1 Ethogram of behaviors used in the study

Behavior	Description
Pass	Bees pass each other so that there is room for each.
C-Posture	Bee curls metasoma under its body, presenting the sting to the other bee.
C-Posture 2	Bee curls metastoma under its body, presenting the sting to the other bee; this is accompanied with rapid forward motions of the c-postured bee into the other bee's body
Mandible Flare	Bee stands with its mandible open; if the bee closes and opens the mandible after a mandible flare within 1 s, this is not considered an additional mandible flare
Nip	One bee closes mandibles <1 cm from the other without touching
Bite	One bee completely closes mandibles around the body part of another bee.
Nudge	Quick head contact with the other bee, forward and backward movement in the same motion.
Nip-Nudge	Quick head contact with other bee with a forward and backward movement observed at an angle where mandible visibility of the bee making the nudge-like motion is obscured
Push	One bee applies force to another with its head (not "quick and back" like nudge).
Back up	One bee moves quickly away from an interaction without turning around.
Reverse	One bee moves quickly away from an interaction, but turns around first.
Head-Head	Both bees have heads touching the other, but do not move aggressively against each other.
Head-Abdomen	One bee touches their head to the other bee's abdomen.
Antennate	One bee touches their antennae to the head of the other bee.
Follow	One bee follows another bee after it either backs or reverses out of an interaction.
Back-Into	One bee backs up, pushing the other with the end of its abdomen
Mandible Kiss	Both bees touch their mandibles together

All females were preserved in 70% ethanol following the trial, and later dissected for ovarian measurement. The dorsal tergites were removed and the ovaries photographed through a dissecting microscope, and the area of the ovaries measured from the photographs following Smith et al. (2009) and Kapheim et al. (2012). We also assigned an ovary development score using a scale from 1 to 5 in which scores of 4 and 5 represent reproductive ovaries with mature or nearly mature oocytes (Michener 1974, 1990).

Statistics

We used non-parametric statistics throughout (except for comparisons of body size between treatment groups) because the behavioral data were counts with many zeros. Because behavior data were counts, we had to control for variation in overall activity. Also, because the activity levels of one bee (measured as total recorded behaviors) correlated with the activity level of the other bee in its dyad ('tubemate'), we had to control for the activity of the other bee in the tube. Thus, all our correlational analyses are non-parametric Spearman's rank-based partial correlations, controlling for total activity and nestmate total activity (Conover 1999). For within-individual analyses $N = 118$ bees. For between-individual (tubemate) analyses, $N = 59$ pairs. For comparisons of small and large bees from the LS trials, we used Wilcoxon pair-rank tests to account for the non-independence of bees in the same tube. All statistics were performed in SPSS 25.

Results

Overall Activity

There were no significant correlations between either body size, relative body size (the ratio of a bee's thorax width to the thorax width of the other bee in the circle

tube), ovary size, or relative ovary size (the ratio of a bee's ovary area to the ovary area of the other bee in the circle tube) with overall activity, measured as the total number of behaviors recorded for each bee (all p values >0.50). There were also no significant effects of these variables on the overall activity of the other bee in the dyad (all p values >0.50). There was no correlation between body size and ovary size (rho = −0.04, $p = 0.71$). All females had reproductive ovaries, and there was no difference in ovary size between large and small bees (large average ± SD = 4.27 ± 0.73 mm^2; small = 4.27 ± 0.71 mm^2). There was, however, a correlation between overall activity of the two bees in the dyad (rho = 0.44, $p < 0.001$).

Analysis of body size shows that our treatment groups differed as intended. Large bee average ± SD thorax width was 2.58 ± 0.08 mm ($N = 58$), small bee average was 2.30 ± 0.09 mm (N = 58). This difference was statistically significant ($t_{116} = 18.49$, $p < 0.001$). Bees in the LL and SS circle tubes were similarly sized. The average ratio of largest to smallest individual in each circle tube in the LL treatments was 1.03 ± 0.03. The average in the SS treatments was 1.05 ± 0.05. However bees in the LS treatments were not similarly sized (average largest to smallest ratio = 1.12 ± 0.04. The difference in relative size ratios was significant (Kruskal-Wallis H = 26.05, $p < 0.001$. LL vs. LS pairwise comparison $p < 0.001$, SS vs. LS $p < 0.001$, LL vs. SS $p = 0.41$).

Aggressive Behaviors

We examined associations between possibly aggressive behaviors in order to avoid a priori classifications that did not fit our species' behavior (Dew et al. 2014). Partial correlations controlling for total activity and tube-mate total activity showed that 'back into' correlated negatively with 'push', 'nudge', and 'bite', (push: rho = − 0.26, $p = 0.005$, nudge: rho = −0.29, $p = 0.002$, bite: rho = 0.23, $p = 0.01$) so we did not include 'back into' as an aggressive behavior. C-posture correlated with 'nip' after controlling for total activity and tubemate total activity (rho = 0.29, $p = 0.02$) and 'C-posture 2′ (rho = 0.20, $p = 0.03$), but not any of the other aggressive behaviors. 'C-posture 2′ did not correlate with any of the other aggressive behaviors. Given the ambiguous results of the two C-posture behaviors and previous studies suggesting that it may be a defensive behavior in the sweat bee *M. genalis,* we combine the two C-posture behaviors, referred to as 'C-postures', but treat them separately from aggressive behaviors, below.

Relative Expression of Behaviors

Avoidance behaviors were the most common (34.43% of all behaviors), followed by aggression (25.51%), tolerant behaviors (15.89%), and C-postures (8.81%); the percent values do not add up to 100 because not all behaviors were assigned to one of the larger categories (see Methods, above). If C-postures were included in the aggression measure, then aggression would total 34.32% of all behaviors. Figure 1 shows the distribution of expression of each type of behavior.

Within Individual Behavioral Correlations

Individuals with higher levels of tolerant and avoidance behavior were less likely to express C-postures or aggressive behaviors. Within individuals, expression of tolerant behaviors correlated negatively with expression of C-postures, after controlling for total activity and tube-mate activity (rho = −0.19, $p = 0.04$) and also correlated negatively with aggression (rho = −0.30, p = 0.002). Avoidance behavior correlated negatively with C-postures (rho = − 0.25, $p = 0.01$) and aggression (rho = −0.48, $p < 0.001$). However, avoidance and tolerance did not correlate with each other (rho = − 0.05, $p =$

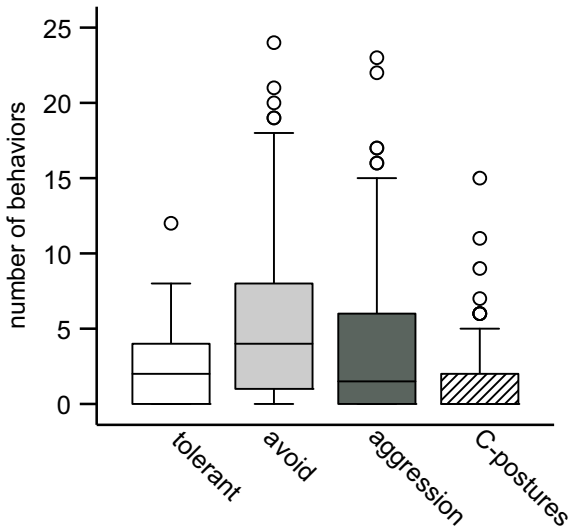

Fig. 1 Rates of expression of different types of behaviors (N = 118 bees). Avoidance behaviors are shown in light gray, tolerant behaviors in white, aggressive behaviors in dark gray, and C-postures in hatched boxes. For all boxplots, horizontal lines show the median, boxes the interquartile range (IQR), and whiskers up to 1.5*(IQR). Open circles represent data >1.5*(IQR) from the median

0.61). C-postures did not correlate with aggression (rho = 0.08, $p = 0.39$).

Between-Individual Behavioral Correlations

Expression of tolerant behaviors correlated strongly with tubemate expression of tolerant behavior (partial correlation controlling for activity and tubemate activity, rho = 0.66, $p < 0.001$). There was a negative correlation between tolerant behavior and tubemate aggressive behavior, but this was not statistically significant (rho = −0.23, $p = 0.10$). Avoidance behavior and tubemate avoidance behavior were negatively correlated (rho = 0.40, $p = 0.003$). As with tolerant behavior, there was a negative correlation between avoidance behavior and tubemate aggressive behavior, but this was not statistically significant (rho = − 0.25, $p = 0.07$). C-postures expression correlated with tubemate aggression (rho = 0.27, $p = 0.047$).

Effects of Body Size

Larger bees were less aggressive. Body size (thorax width) correlated negatively with aggression (partial correlation controlling for activity and tubemate activity, rho = −0.25, p = 0.01). Relative body size also correlated negatively with aggression, but the effect was not statistically significant (rho = −0.15, $p = 0.12$). Larger bees were also more likely to avoid their tubemates (size-avoidance behavior partial correlation rho = 0.19, $p = 0.05$; relative size-avoidance behavior partial correlation rho = 0.29, p = 0.003). There were no significant partial correlations, controlling for activity and tubemate activity, of body size on tubemate behaviors (tolerance rho = 0.04, $p = 0.78$; avoidance rho = −0.06, $p = 0.66$; aggression rho = −0.06, $p = 0.67$). Nor were there any significant partial correlations of relative size on tubemate behaviors (tolerance rho = 0.04, p = 0.78; avoidance rho = −0.17, $p = 0.22$, aggression rho = 0.15, $p = 0.30$).

Effects of Size Treatment

Above we presented correlations using all bees. Our size-matched and size-contrasted treatments let us test for differences between large and small bees when matched against similarly sized individuals (the LL and SS treatments) and differently sized individuals (the LS treatment). There were no differences in overall expression of avoidance, tolerant, aggressive, or C-posture behaviors across the large-large (LL), large-small (LS) or small-small (SS) treatments (Kruskal-Wallace test for aggression: H = 0.37, $p = 0.54$, C-posture: H = 0.99, $p = 0.32$, tolerance: H = 1.18, $p = 0.28$, avoidance H = 0.04, $p = 0.84$). Within the LS treatment pairs ($N = 20$), small bees showed significantly more aggression (Wilcoxon paired-rank test W = 2.05, p = 0.04). There were no statistically significant pairwise differences in the other behavior categories (avoidance W = 1.38, $p = 0.17$, tolerance W = 0.54, $p = 0.59$, C-posture W = 0.47, $p = 0.64$).

Effects of Ovary Size

There was no effect of ovary size on the behaviors we measured. Partial correlations controlling for activity and tubemate activity between ovary size and behavior expression were not significant (aggression rho = −0.06, $p = 0.55$, tolerance rho = 0.002, $p = 0.99$, avoidance rho = 0.16, $p = 0.09$, C-postures rho = − 0.03, $p = 0.77$). Nor was there a statistically significant effect of relative ovary size on behaviors (partial correlations controlling for activity and tubemate activity, aggression rho = − 0.04, p = 0.67, tolerance rho = −0.12, $p = 0.21$, avoidance rho = 0.01, $p = 0.90$, C-postures rho = 0.08, $p = 0.44$). There was also no effect of ovary size on tubemate behavior (partial correlations controlling for activity and tubemate activity: aggression rho = −0.06, $p = 0.69$, tolerance rho = 0.14, $p = 0.33$, avoidance rho = 0.03, $p = 0.82$, C-postures rho = − 0.16, $p = 0.25$). Similarly, there was no effect of relative ovary size on tubemate behavior (partial correlations controlling for activity and tubemate activity, aggression rho = − 0.04, $p = 0.79$, tolerance rho = 0.17, $p = 0.23$, avoidance rho = −0.08, p = 0.55, C-postures rho = 0.01, $p = 0.94$).

Discussion

In colonies of social bees, body size and aggression are strongly correlated, with large females typically being dominant over smaller females. Previous research has suggested that this pattern may be derived from an ancestral regulatory relationship between the mechanisms governing body size and behavior (Smith et al. 2018, 2019). This would suggest that in the process of evolving sociality, ancestral regulatory mechanisms of behavior were re-purposed for social interactions: by

creating smaller daughters, queens could have workers that were easier to dominate not only due to their size, but also their relative lack of aggression (West-Eberhard 1987, 1996). We tested this hypothesis by examining the relationship between body size and aggression in a solitary halictid bee, representative of the ancestor that gave rise to sociality in sweat bees. We predicted that in *N. melanderi*, larger bees would be more aggressive than smaller bees. Our data do not support our prediction, although we did find evidence that even these solitary bees can adjust their behavior in response to changes in the social environment.

Our primary finding was that small bees were more aggressive than large bees, which is opposite to what we predicted based on the behavior of social sweat bees. The large bees in our study were 12% larger than the small bees. This magnitude of difference affects social interactions in social species. For instance, in nests of *M. genalis,* queens are 8.5–13.5% larger than workers, depending on the collection (Smith et al. 2008, 2018; Kapheim et al. 2012). In this study, *N. melanderi* body size correlated negatively with aggressive behavior. Comparing bees in the LS treatment to the bees in the LL and SS treatment shows that the differences were greatest when bees were not size matched (Fig. 2). Both large and small bees expressed similar levels of aggression when in matched size treatments (LL and SS). However in the mixed treatment (LS), small bees expressed more aggression than large bees. Thus, smaller bees tended to be more aggressive, and these solitary bees were able to adjust their behavior based on perceptions of conspecifics and variation in the social environment.

The other behavior that correlated (negatively) with body size and relative body size was avoidance, suggesting that large bees may be trying to avoid the more aggressive smaller bees. Within individuals, we found that tolerance and avoidance correlated negatively with C-postures and aggression. This suggests that these two sets of behaviors are alternatives: either tolerate or avoid the other bee in the tube, or react with aggression or C-postures. Between-individual results supported this interpretation as well: when one bee was tolerant, the other was as well. When one bee expressed high avoidance, the other bee did not, presumably because the first bee

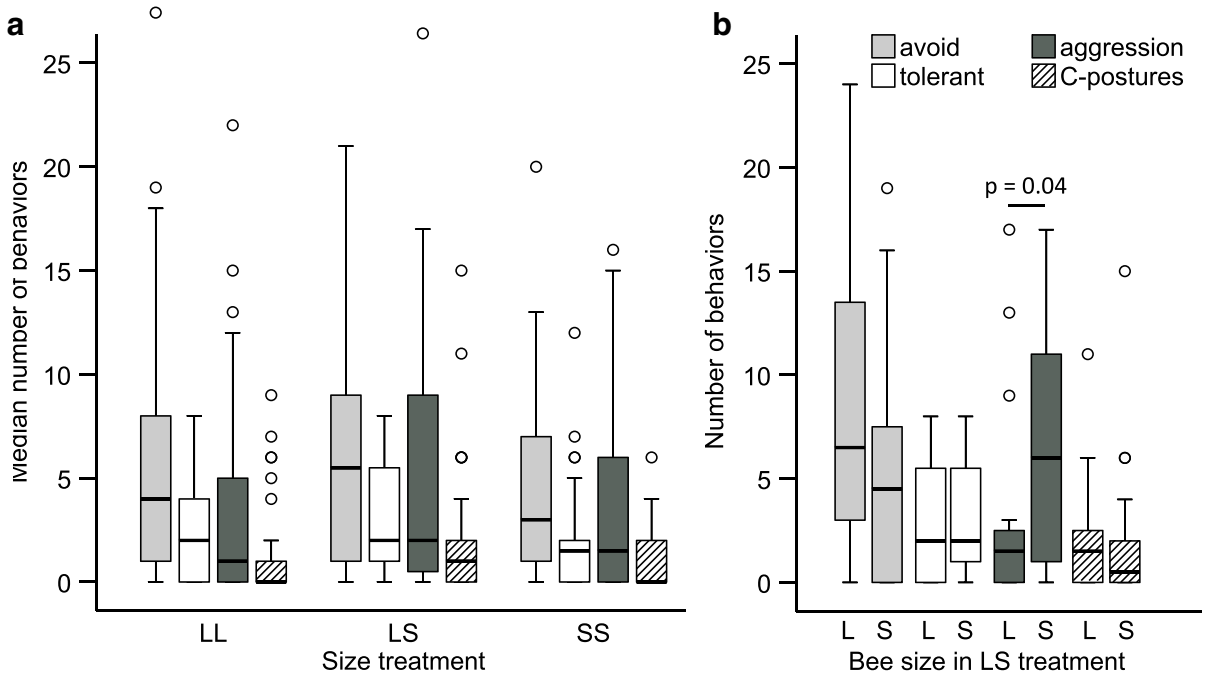

Fig. 2 **a** Behaviors expressed in the three different treatments: large-large (LL), large-small (LS), and small-small (SS). **b** Behaviors expressed by large (L) and small (S) bees from the LS treatment. In both panels, avoidance behaviors are shown in light gray, tolerant behaviors in white, aggressive behaviors in dark gray, and C-postures in hatched boxes. For all boxplots, horizontal lines show the median, boxes the interquartile range (IQR), and whiskers up to 1.5*(IQR). Open circles represent data >1.5*(IQR) from the median

was already avoiding interaction. C-postures correlated with tubemate aggression, suggesting that it may be a defensive behavior in reaction to aggressive acts. This modulation of behavior in response to the behavior of the other bee in the tube again suggests that even solitary bees can evaluate their social environment and adjust their behavior accordingly.

Aggression was rare in *N. melanderi* (median = 1.5 aggressive behaviors per bee per 15 min trial, Fig. 1) compared to the facultatively eusocial *M. genalis* (worker median = 3, solitary female = 4, queen = 7; Smith et al. 2018). Eusocial bees generally show more aggression than avoidance in circle tubes, while solitary bees show more avoidance than aggression (Packer 2006; Richards and Packer 2010). *Nomia melanderi* showed higher rates of avoidance than aggression (Figs. 1 and 2). The only exception is the small bees in the LS treatment, which showed slightly more aggression than avoidance. However, if C-postures had been included with aggressive behaviors, as it was in most previous studies (Packer 2006; Richards and Packer 2010), the rates of avoidance and aggressive behaviors would have been similar.

We also showed that there was no link between ovary size and aggression. However, our study only included reproductive bees, which all have enlarged ovaries. If ovary development correlates with aggression, either through physiological links, such as with juvenile hormone levels (West-Eberhard 1987, 1996), or by predisposing females who just laid eggs to defend them (Richards and Packer 2010), comparisons of bees that all have enlarged, reproductive ovaries may not show this. Future studies comparing reproductive and non-reproductive females would be more powerful for detecting ovary development influences on behavior (e.g. Kapheim et al. 2012; Smith et al. 2013).

Within the social sweat bees, there is a general, but not absolute, association of size with reproductive dominance (Michener 1990; Mueller 1993; Richards and Packer 1994; McConnell-Garner and Kukuk 1997; Pabalan et al. 2000; Smith et al. 2009; Kapheim et al. 2013).

Modifying existing regulatory mechanisms of behavior can be a source of evolutionary novelty (West-Eberhard 1987, 1996). In social species, queens manipulate the nutrition of their daughters to make them smaller (Kapheim et al. 2011; Lawson et al. 2016), and in the one experimentally tested species, less aggressive as well (Lawson et al. 2017). This association between size and aggression led to our hypothesis that ancestral links between size and aggression may have been exploited in the evolution of sociality in order to help create a larger dominant queen caste and smaller, subordinate workers. We found a link between size and aggression, but it was in the opposite direction than we predicted: smaller bees were more aggressive. Moreover, size-based expression was dependent on the social context: small bees were more aggressive when matched against large bees than when matched against other small bees. This shows that even solitary bees can modulate their expression of aggression in response to social context. It also suggests that in the evolutionary transition from solitary to social behavior bees may have had to reverse the directionality of a pre-existing negative association between size and aggression. Future experimental studies on other solitary species (e.g Richards and Packer 2010; Fischman et al. 2017) would be useful for testing for the generality of this observation.

Acknowledgements This work was funded by NSF grant #17-1028536545 to ARS and KMK and a USDA-APRI grant to KMK. Hunter Barr measured ovaries.

References

Batra SW (1966) Nests and social behavior of halictine bees of India (Hymenoptera: Halictidae). Indian J Entomol 28:375

Bourke AF (2011) Principles of social evolution. Oxford University Press, Oxford

Breed MD, Silverman JM, Bell WJ (1978) Agonistic behavior, social interactions, and behavioral specialization in a primitively eusocial bee. Insect Soc 25:351–364

Conover W (1999) Practical nonparametric statistics, John Wiley & Sons. INC, New York

Dalmazzo M, Roig-Alsina A (2018a) Dominant-subordinate social interactions and subordinate behavioral responses in the primitively eusocial sweat bee *Augochlora phoemonoe* (Hymenoptera: Halictidae). Apidologie 49:852–861

Dalmazzo M, Roig-Alsina A (2018b) Primitively eusocial behavior observed in colonies of *Augochlora amphitrite* (Hymenoptera: Halictidae) reared in laboratory. Sociobiology 65:773–776

Danforth BN, Conway L, Ji S (2003) Phylogeny of eusocial *Lasioglossum* reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). Syst Biol 52:23–36 doi: QKDHRNCHYDLR6VXB [pii]

Dew RM, Gardner MG, Schwarz MP (2014) The problems of a priori categorisation of agonism and cooperation: circle-tube interactions in two allodapine bees. Ethology 120:551–562

Fischman BJ, Pitts-Singer TL, Robinson GE (2017) Nutritional regulation of phenotypic plasticity in a solitary bee (Hymenoptera: Megachilidae). Environ Entomol 46:1070–1079

Gibbs J, Brady SG, Kanda K, Danforth BN (2012) Phylogeny of halictine bees supports a shared origin of eusociality for

Halictus and *Lasioglossum* (Apoidea: Anthophila: Halictidae). Mol Phylogenet Evol 65:926–939

Gonzalez V, Patton R, Plascencia M, Girişgin A, Çakmak I, Barthell J (2018) High levels of tolerance between nestmates and non-nestmates in the primitively eusocial sweat bee *Halictus scabiosae* (Rossi) in Turkey (Hymenoptera: Halictidae). Insect Soc 65:339–343

Kapheim KM (2019) Synthesis of Tinbergen's four questions and the future of sociogenomics. Behav Ecol Sociobiol 73:186

Kapheim KM, Johnson MM (2017a) Juvenile hormone, but not nutrition or social cues, affects reproductive maturation in solitary alkali bees (*Nomia melanderi*). J Exp Biol 220:3794–3801. https://doi.org/10.1242/jeb.162255

Kapheim KM, Johnson MM (2017b) Support for the reproductive ground plan hypothesis in a solitary bee: links between sucrose response and reproductive status. Proc Biol Sci: 284. https://doi.org/10.1098/rspb.2016.2406

Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT (2011) Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, *Megalopta genalis* (Halictidae). Behav Ecol Sociobiol 65:1179–1190

Kapheim KM, Smith AR, Ihle KE, Amdam GV, Nonacs P, Wcislo WT (2012) Physiological variation as a mechanism for developmental caste-biasing in a facultatively eusocial sweat bee. Proc Biol Sci 279:1437–1446. https://doi.org/10.1098/rspb.2011.1652

Kapheim KM, Smith AR, Nonacs P, Wcislo WT, Wayne RK (2013) Foundress polyphenism and the origins of eusociality in a facultatively eusocial sweat bee, *Megalopta genalis* (Halictidae). Behav Ecol Sociobiol 67:331–340

Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T, Robertson HM, Hudson ME, Venkat A, Fischman BJ et al (2015) Genomic signatures of evolutionary transitions from solitary to group living. Science 348:1139–1143. https://doi.org/10.1126/science.aaa4788

Kapheim KM, Chan T, Smith A, Wcislo WT, Nonacs P (2016) Ontogeny of division of labor in a facultatively eusocial sweat bee *Megalopta genalis*. Insect Soc 63:185–191

Kocher SD, Paxton RJ (2014) Comparative methods offer powerful insights into social evolution in bees. Apidologie 45:289–305

Lawson SP, Ciaccio KN, Rehan SM (2016) Maternal manipulation of pollen provisions affects worker production in a small carpenter bee. Behav Ecol Sociobiol 70:1891–1900

Lawson SP, Helmreich SL, Rehan SM (2017) Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). J Exp Biol 220:4456–4462. https://doi.org/10.1242/jeb.160531

McConnell-Garner J, Kukuk PF (1997) Behavioral interactions of two solitary, halictine bees with comparisons among solitary, communal and eusocial species. Ethology 103:19–32

Michener CD (1974) The social behavior of the bees: a comparative study. Harvard University Press, Cambridge MA

Michener CD (1990) Reproduction and castes in social halictine bees. Engels W. Social Insects. Berlin, Springer, pp 77–121

Mueller UG (1993) Haplodiploidy and the evolution of facultative sex ratios in a primitively eusocial bee. Dissertation, Cornell University

Pabalan N, Davey K, 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. J Insect Behav 13:627–650

Packer L (2005) The influence of marking upon bee behaviour in circle tube experiments with a methodological comparison among studies. Insect Soc 52:139–146

Packer L (2006) Use of artificial arenas to predict the social organisation of halictine bees: data for fourteen species from Chile. Insect Soc 53:307–315

Rehan SM, Toth AL (2015) Climbing the social ladder: the molecular evolution of sociality. Trends Ecol Evol 30:426–433

Richards MH, Packer L (1994) Trophic aspects of caste determination in Halictus ligatus, a primitively eusocial sweat bee. Behav Ecol Sociobiol 34:385–391

Richards MH, Packer L (2010) Social behaviours in solitary bees: interactions among individuals in *Xeralictus bicuspidariae* Snelling (Hymenoptera: Halictidae: Rophitinae). J Hymenopt Res 19:66–76

Schwarz MP, Richards MH, Danforth BN (2007) Changing paradigms in insect social evolution: insights from halictine and allodapine bees. Annu Rev Entomol 52:127–150

Smith BH, Weller C (1989) Social competition among gynes in halictine bees: the influence of bee size and pheromones on behavior. J Insect Behav 2:397–411

Smith AR, Wcislo WT, O'Donnell S (2008) Body size shapes caste expression, and cleptoparasitism reduces body size in the facultatively eusocial bees *Megalopta* (Hymenoptera: Halictidae). J Insect Behav 21:394

Smith AR, Kapheim KM, O'Donnell S, Wcislo WT (2009) Social competition but not subfertility leads to a division of labour in the facultatively social sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Anim Behav 78:1043–1050

Smith AR, Kapheim KM, Pérez-Ortega B, Brent CS, Wcislo WT (2013) Juvenile hormone levels reflect social opportunities in the facultatively eusocial sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). Horm Behav 63:1–4

Smith A, Simons M, Bazarko V, Seid M (2018) The influence of sociality, caste, and size on behavior in a facultatively eusocial bee. Insect Soc:1–11

Smith A, Simons M, Bazarko V, Seid M (2019) Queen-worker aggression in the facultatively social and solitary bee *Megalopta genalis*. Insect Soc 66:479–490. https://doi.org/10.1007/s00040-019-00712-0

Toth AL, Rehan SM (2017) Molecular evolution of insect sociality: an eco-Evo-Devo perspective. Annu Rev Entomol 62: 419–442

Wcislo WT, Danforth BN (1997) Secondarily solitary: the evolutionary loss of social behavior. Trends Ecol Evol 12:468–474

West-Eberhard MJ (1987) Flexible strategy and social evolution. Ito Y, Brown L, Kikkawa L. Animal societies: theories and facts. Tokyo, Japan Scientific Societies, pp 35-51

West-Eberhard MJ (1996) Wasp societies as microcosoms for the study of development and evolution. West-Eberhard MJ, Turillazzi S. Natural history and evolution of paper wasps. Oxford, Oxford University Press, pp 290-317

Wilson EO (1971) The insect societies. Cambridge, Massachusetts, USA, Harvard University Press