**Solitary floral specialists do not respond to cryptic flower-occupying predators**

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**Abstract**

The impacts of predators on bee foraging behavior are varied, but have been suggested to depend on both the type of predator (namely their hunting strategy) and also risk assessment by the prey (i.e., ability to perceive predators and learn to avoid them). However, nearly all studies have explored these impacts using social bees, despite the fact that solitary bees are extremely diverse, often specialized in their floral interactions, and may exhibit different behaviors in response to flower-occupying predators. In this study, we examined foraging behaviors of wild solitary long-horned bees (*Melissodes* spp.)in response to a cryptic predator, the ambush bug (*Phymata americana*) on the bees’ primary floral host, the prairie sunflower (*Helianthus petiolaris*). We found sex-specific differences in foraging behaviors of bees, but little evidence that ambush bugs affected either pre-landing or post-landing foraging behaviors. Male bees visited flowers three times more often than females but female bees were five times more likely to land than males. Ambush bugs did not reduce visitation in either sex. These results suggest that cryptic ambush bugs are either rarely detected by these solitary bees, or that foraging *Melissodes* do not alter their foraging behavior because of the perceived risk of predation. We discuss the implications of these findings and compare them to other studies of social bees.

**Key words:**

solitary bees, *Melissodes*, *Phymata*, cryptic predator, foraging, risk, avoidance behavior

**Introduction**

The foraging behaviors of bees (Apoidea) can be influenced by the presence of predators (Dukas 2001; Jones 2010; Kacelnik and El Mouden 2013), although bees’ responses to predators could vary depending on a number of factors. Many of the cues bees use for optimizing foraging are also used by predators (Greco and Kevan 1994; Heiling et al. 2004), and many predators occupy flowers as hunting platforms, potentially increasing predation risk to bees while foraging. A recent cross taxa review of floral visitors found that such flower-occupying predators reduced floral visitation by 36% and the duration of visits by 51% compared to flowers without predators (Romero et al. 2011). However, the responses to predators differed across taxa, and specifically suggested there could be differences in response by social and solitary bees (Romero et al. 2011). This highlights the need for more studies addressing the role of flower-occupying predators on different bee taxa, and on bees with varying ecological and life-history characteristics.

The behavioral responses of foraging bees to predators likely depend on the bees’ ability to recognize predators and whether or not they can learn to avoid them. Most behavioral studies have focused exclusively on eusocial bees in controlled laboratory settings using artificial predators. For example, such studies have shown that social bees are risk averse, becoming more discriminating of flowers after experiencing repeated simulated predation attempts (Ings and Chittka 2008; Ings et al. 2012), modulating their exposure to predators by avoiding patches of flowers with high predator densities, scanning flowers before landing on them, and aborting foraging attempts on predator-occupied flowers (Ings and Chittka 2008; Ings and Chittka 2009; Ings et al. 2012). In contrast, the behaviors of solitary bees in response to flower-occupying predators have rarely been studied (though see Reader et al. 2006; Oliveira et al. 2016). Solitary bees’ response to predators could vary greatly depending on a number of factors. On the one hand, if solitary bees are risk averse, they should display pre-landing avoidance behaviors to flower-occupying predators similar to those of eusocial bees. On the other hand, if the cost of predation is greater for solitary bees than social bees, they may exhibit greater responses to flower-occupying predators. Fitness optimization models predict that solitary bees should be more risk averse than social bees (Jones 2010; Rodriguez-Girones and Bosch 2012), given that individual female solitary bees are the sole provisioners of their offspring. Female mortality while foraging would therefore effectively eliminate that individual's reproductive effort, compared to social bees in which foragers contribute indirectly through shared fitness.

However, the impacts of predators likely also vary based on their predatory behavior. For example, visually cryptic predators should only induce avoidance behaviors in bees that have experienced predation attempts (Ings et al. 2012), whereas obvious predators may reduce the likelihood of visiting a flower regardless of experience (but see Dawson and Chittka 2014). If the consequences of not detecting a predator are rarely realized by foragers (i.e., an extremely low chance of attack), ignoring the predator and visiting as many flowers as quickly as possible would maximize a bee’s foraging efficiency (Burns 2005). Moreover, while female solitary bees forage for nectar and pollen on flowers, males forage mainly for nectar, which takes less time, enabling males to visit flowers more frequently in search of mates, and ultimately leading to differential effects on the pollination of the flowers they visit (Ne'eman et al. 2006). However, nearly nothing has been reported on the response of both male and female solitary bees to cryptic flower-occupying predators. Understanding how solitary bees respond to predators and in more natural settings would provide important insights, not only into bee foraging strategies, but also their implications for pollination.

To test whether a cryptic predator would affect the foraging behaviors of solitary bees, we experimentally manipulated predator presence on flowers in an experimental common garden and observed freely foraging solitary bee behaviors. The ambush bug, *Phymata americana* Melin, (Reduviidae) is one of the most common predators found on sunflowers across the plains of Colorado. While nothing is known about natural predation rates, we observed ambush bugs attacking the most common sunflower visitors, solitary long-horned bees, *Melissodes* spp. Latreille (Apidae) in 2014. To determine whether ambush bugs would affect the foraging behavior of long-horned bees we manipulated ambush bug presence on prairie sunflowers (*Helianthus petiolaris* (Nuttall), Asteraceae), and recorded the foraging behavior of bees while visiting predator occupied versus control flowers. Given that male and female bees exhibit sex-specific foraging behaviors, we also explored sex-specific responses of foraging bees to predators. In addition, we measure the spectral reflectance of ambush bugs and sunflowers, to assess their degree of crypsis. We predicted that if solitary long-horned bees were risk averse, they would respond to predators by modifying their foraging behaviors on predator occupied flowers. Specifically, we predicted that solitary bees would respond to flowers with predators by 1) visiting those flowers less frequently, 2) spending less time per visit, and 3) being less likely to collect pollen and nectar when visiting. In addition, if exposure to predators varied between male and female bees based on foraging behavior, we expect to observe 4) sex-specific behavioral responses to predators.

**Materials and Methods**

*Study System*

Sunflowers (*Helianthus* spp., Asteraceae) are a dominant flower across the plains of North America and the primary food resource for at least 31 species of bee (Hurd et al. 1980), including many long-horned bees, members of the solitary bee genus *Melissodes.* The prairie sunflower, *Helianthus petiolaris,* is especially abundant in sandy disturbed soils found throughout urban and rural environments (Heiser 1947; Hurd et al. 1980) and along roadsides on the plains of Colorado, where densities of plants can be as high as four plants per meter (Ntransects = 32, mean ± SE = 22 ± 8.96 plants/ 50 m, Schwantes, *unpub. data*). Each plant has multiple flowers borne on a highly branched stem. In Colorado, *Melissodes agilis* (Cresson) is one of the most frequent visitors to *H. petiolaris* and in the height of summer, females can be found collecting both nectar and pollen to provision offspring with while males forage for nectar and occur at even higher densities while patrolling for potential mates (A. Carper personal observation). Moreover, *M. agilis* is a reported oligolege of *Helianthus* (LaBerge 1961) with specialized morphology and behaviors to collect pollen (Cane 2017; Parker et al. 1981). Other species of *Melissodes* also frequently visit *H. petiolaris* in Colorado, including *M. coreopsis* (Robertson), *M. communis* (Cresson), and *M. comptoides* (Robertson), though determinations to species in the field or even by photo are rarely possible.

In addition to bees, the flowers of *H. petiolaris* in CO are also frequently occupied by the jagged ambush bug, *Phymata americana*, which can be easily observed on the discs of flowers (Fig. 1). Ambush bugs are common, occurring on roughly 40% of flowering plants in August and September, with predators on approximately 1 in 10 blooms (average density of 0.08 ± 0.03 ambush bugs per flower head, Ntransects = 32). They are cryptic predators, preferentially occupying yellow flowers (Greco and Kevan 1994), which match their bright yellow color; are capable of capturing prey many times their own size; and have been reported preying on at least18 different species of bees(Balduf 1943; Elliott and Elliott 1994; Mason 1977). Moreover, ambush bugs also consume nectar from the flowers on which they hunt, allowing them to survive longer periods of time without prey (Yong 2003), and enabling them to be very effective sit-and-wait predators.

*Experimental Design*

We conducted this experiment and all observations in a common garden of *Helianthus petiolaris* at the University of Colorado’s 30th Street greenhouse (Boulder, Colorado, USA). Plants were grown from seeds collected from wild plants growing in 32 sites across eastern Colorado during September of 2013. Seeds from all sites were mixed before storage to reduce any impacts of local phenotypes. In March of 2014, seeds were germinated in petri dishes and then planted in groups of five seedlings into 10cm x 10cm pots in the Ramaley Biology Building greenhouse. In May, individual sunflowers were transplanted into outdoor flowerbeds, spaced 60 cm apart to mimic natural plant densities, and watered regularly throughout the summer. Plants bloomed for two weeks prior to experimental trials to ensure they were attractive to local bees and continued to bloom several weeks after the trials ended. Before the experiments took place, no predators were observed on any experimental plants.

To determine how foraging bees responded to ambush bugs, we manipulated ambush bug presence on individual sunflower inflorescences. We used two digital single lens reflex (DSLR) cameras to record bee foraging behaviors (see below) over a three day period, and paired treatment and control flowers when recording to control for phenology of flowers, display size, and daily visitor activity. We assigned identification numbers to 40 undamaged, recently opened inflorescences (hereafter flowers) that were accessible for filming, and randomly assigned each to one of two treatments: occupied or control. This resulted in 20 flowers receiving ambush bugs and 20 left as unoccupied controls. Individual flowers received the same treatment throughout the three day experiment but we randomly paired treatment flowers with control flowers on each day, so that pairings were not constant over the three day period to reduce bias due to observation time or position in the garden.

We conducted all experimental observations from August 15th to 17th 2014. We collected 75 individual ambush bugs haphazardly from patches of sunflowers growing along roadside edges in and around Boulder, Colorado. While ambush bugs were collected up to 5 km away from the location of the experimental array, sunflowers, *Melissodes*, and ambush bugs, are all ubiquitous along roadsides and natural areas around Boulder, and have been observed along trails nearby (A. Carper, personal observation), making it unlikely that the presence of ambush bugs is a novel stimulus at our study area. Each ambush bug was stored in an individual container inside of a growth chamber (Percival LLVL) maintained at 25˚C during the day and 20˚C at night with a 14-hour day length. Ambush bugs were not fed during storage, to increase the likelihood of predatory behavior. At the start of each observation day, any arthropods greater than 1mm were removed from experimental flowers and ambush bugs were placed on treated flowers and recording began. If an ambush bug left the flower, it was replaced and the trial was continued. If it left undetected, any data collected after it left were not included in analyses. At the end of the observation period, all ambush bugs were re-collected from flowers and replaced in the growth chamber over-night for possible use the next day. All experimental flowers were observed in the order of their random draw positions for 10 minutes. Observations were recorded using two high definition DSLR cameras (one Canon EOS 10D and one Nikon D5200). Cameras were placed 40 cm from blooms and recorded at 30 frames per second with a resolution of 1920×1080 pixels. Using digital recordings allowed for frame-by-frame analysis of visits with a temporal resolution of 0.03 seconds. Observations began at 07:40 and finished when all pairs had been observed (approximately four hours).

*Bee Foraging Behaviors*

To assess bee foraging behaviors, we reviewed 1200 minutes of recorded observations and scored pre and post landing bee behaviors at all flowers. (1,200 minutes in total) and recorded a number of behaviors which could represent different components of foraging behavior and potentially different risk avoidance behavior (or lack thereof). For instance, if ambush bugs are truly cryptic predators, foraging bees may be unable to perceive them from a distance and may not be able to distinguish between control and occupied flowers until after visiting them. If so, avoidance behaviors may not be expected for visitation *per se*, but may be more likely after initial inspection of the flower. For each experimental flower, we recorded the total number of bees that visited (i.e., flew into frame in front of the flower, inspected it, and either landed or left) as it may reflect detection of ambush bugs from a distance, and the number that landed, as it represented a decision based potentially on the perceived risk of predation. We then calculated the proportion of visiting bees that landed on flowers to determine if ambush bugs deterred visitors once close enough to a flower to detect them.

Once bees landed, ambush bugs are presumably detectable by visiting bees, and foraging may then be contingent upon perceived risk while foraging for nectar or pollen. Alternatively, if bugs are cryptic or bees simply do not encounter them while foraging on the flower head, they may not respond. Subsequently, for each individual bee recorded, we calculated the duration of each landing visit (measured as the number of seconds making contact with the flower), and also scored individual foragers for both nectar and pollen collection. If a bee inspected a flower but did not land, we calculated the time it took to inspect a flower and leave. Bees were scored as collecting nectar if they placed their head into a disk flower or if their proboscis was visibly extended into a disk flower. Similarly, bees were scored as collecting pollen when they passed over anthers and moved pollen to their scopal hairs. Because our study involved focal animals in the field, we were unable to implement blinding methods when recording and analyzing data. To reduce observer bias, C. Schwantes therefore scored all visitation behaviors to help standardize observations. All bee visitors were sexed and identified to genus from video with the help of V. Scott at the University of Colorado Museum of Natural History Entomology Collection.

*Spectral Reflectance*

Given that bee response to ambush bug presence likely depends on the cryptic ability of ambush bugs on sunflower, we measured the color of both ambush bugs and sunflowers to determine the degree of crypsis that ambush bugs achieve. We collected a single head from 10 haphazardly chosen sunflower plants and 8 associated ambush bugs in September 2018. Inflorescences were all similarly sized and in mid-bloom, having dehiscent anthers in the majority of the disc flowers. We measured the spectral reflectance of bugs and flowers using a JAZ ILX-511B spectrometer and OceanView version 1.6.7 software (Ocean Optics Inc., FL, USA). We measured reflectance from 300 -700 nm (within the bee visible light spectrum) at a 45° angle of reflectance. For sunflowers, we measured both ray and disc florets, given the contrast between the two. Since disc florets are much smaller than ray flowers, we dissected the corollas from disc florets and flattened between 10 and 15 corollas together to ensure an adequate area for reflectance measurements. Since ambush bugs exhibit patches of dark and light color, we measured the reflectance of patches of both light and dark areas of cuticle. Measurements of dark coloration were taken from the nota (dorsal thorax) wile light coloration was taken from the abdominal sterna.

*Statistical Analyses*

All statistical analyses were run in R version 3.4.3 (R Core Team 2017). As expected, *Melissodes* were by far the most common visitor (see Table 1) and statistical analyses were restricted to bee visits from the genus. While marking and following individual bees would have been ideal, it could have elicited a negative response from bees visiting the array and skewed results. Thus, wby *Melissodes* We compared visitation (pre-landing) behavior to ambush bug-occupied versus control flowers using linear mixed effect models (lme4 and lmerTest packages, Bolker 2017; Kuznetsova et al. 2017, respectively), with the sex of the visiting bee, the presence of ambush bugs, and their interaction as fixed effects. Given that we had visits by both male and female bees to the same experimental flowers but included sex as a fixed effect,we to avoid pseudoreplication given two measures of each response (i.e., male and female bee behavior). Also, we excluded males from the pollen collection analysis since only female bees collected pollen, and only had the main effect of ambush bug presence as a fixed factor and no random effect of flower ID. To meet the assumptions of normality, we log10 transformed the total number of visits to flowers, log10(+1) transformed the total number of bees that landed on flowers, and logit-transformed the proportion of visits that landed on flowers, collected nectar, or collected pollen (Warton and Hui 2011).

We compared the duration of visits (in seconds) with linear mixed effect models and log10-transformed all responses. Given that nearly all non-landing visits were male, we only analyzed males for non-landing visit duration, measured as the time spent inspecting flowers before leaving. We used each recorded visit as our unit of replication, and included treatment as a fixed effect, and a unique flower ID as a random effect to account for multiple measures of bee visit duration across the three observation days. Since individual flowers (our unit of experimental replication) were only recorded for 10 minutes, once per day, it is unlikely that any single bee visited the same flower twice during an observation period. Still, it is possible that bees were recorded more than once throughout the experiment and thus, our responses should be construed as general response of foraging *Melissodes* to ambush bug presence, and not of individually tracked bees. We analyzed the duration of landing visits using the sex of bee, treatment, and their interaction as fixed effects, and flower ID as a random effect. A significant effect of ambush bug presence on visitation would suggest that bees can perceive them from a distance, while a significan effect of ambush bug presence on the duration of a landing visit would suggest alternative modes of detection. An interaction between sex and ambush bug presence would suggest that either male and female bees perceive ambush bugs differently, or that the perceived risk of visiting flowers is different for male and female bees.

We visually compared the colors of sunflower and ambush bug parts by fitting reflectance curves. Given that bees only perceive color through their visual system we then explored perception through a bee vision model. While no vision model exists for *Melissodes*, methods have been well developed for honeybee (*Apis mellifera* L.) vision and both are in the Apidae family. Moreover, photoreception appears to be quite conserved in bees (Briscoe and Chittka 2001), suggesting that the honeybee model is likely an appropriate approximation of *Melissodes* vision. We used the ‘pavo’ package (Maia et al. 2013) to fit a honeybee vison model to the spectral data of both sunflowers and bugs, and plotted the results using the hexagonal color space model (see Chittka 1992).

**Results**

Video footage revealed that ambush bugs tended to remain stationary on the periphery of the floral disk, staying in place for 8 minutes and52.2 seconds (­SE + 28.7 seconds) on average. Overall, we observed 704 individual bees from 14 genera visiting sunflowers during recording bouts (Table 1). As expected, long-horned bees were by far the most frequent visitors, making up 87% of all recorded visits (Table 1). Of the other genera that visited, only three, *Bombus*, *Apis*, and *Halictus* (entirely *Halictus ligatus)*, include species known to be eusocial. Those eusocial bees accounted for just 5 % of all recorded visits. Of the 360 total recorded visits to ambush bug occupied flowers, only one predation event was recorded, representing just 0.15% of visits to occupied flowers. In this attack, the foraging honeybee landed on top of the ambush bug with its head and abdomen oriented in the same direction as the predator. As the bee moved towards the center of the flower, the ambush bug grasped it with its forelegs and inserted its beak in the membrane between the head and thorax. The bee struggled and attempted to sting for approximately 25 seconds but was unable to free itself (Supplementary Material). No other attempted predation events were observed.

Summed across all three days, we recorded 613 total visits from *Melissodes* to 17 control flowers and 18 occupied flowers. We found a significant effect of sex on visitation, with male *Melissodes* visiting flowers three times more frequently than females (F1,31.9 = 27.21, P < 0.001), with females making up just 24% of visits (147 compared to 466 male visits). We found no significant effect of ambush bug presence on total visitation (F1,32.3 = 0.00, P = 0.963), and no interaction between the sex of long-horned bee visitor and ambush bug presence (F1,31.9 = 3.28, P = 0.080, Fig. 2a). Of the 613 total visits from *Melissodes* recorded during the experiment, only 222 bees landed on flowers. We found no effect of ambush bug presence on the number of bees that landed on flowers (F1,32.6 = 0.48, P = 0.493) and no interaction between ambush bug presence and bee sex (F1,32.2 = 0.160, P = 0.692, Fig. 2b). However, twice as many female bees landed on experimental flowers than males (F1,32.2 = 11.7, P = 0.002). Moreover, the proportion of female bees landing after visiting a flower was 5 times greater than male bees (92% vs 17% respectively, F1,62 = 110.03, P < 0.001, Fig. 2c), although the interaction between ambush bug presence and sex again was not significant (F1,62 = 3.38, P = 0.071) and there was no overall main effect of ambush bug presence (F1,62 = 0.14, P = 0.706).

Only 14 female bees inspected flowers without landing compared to 377 males, making statistical comparison between sexes difficult. In general, male bees that did not land spent very little time at flowers, likely just inspecting them in search of mates. Males briefly visited flowers (mean ± SE = 1.75 seconds ± 0.64) and regularly visited flowers occupied by other bees. On average, male bees spent 45% more time inspecting flowers before deciding to leave than female bees (0.36 vs 0.20 seconds, respectively) but ambush bugs had no effect on the duration on non-landing male visits (F1,24.1 = 1.68, P = 0.207, Fig. 3a). Summed over the three days of observation, 133 female and 89 male *Melissodes* visits landed on flowers. The amount of time bees spent on flowers averaged 6.4 seconds, though some bees that landed spent an exceptionally long time on flowers (up to 46 seconds, apparently simply resting and not foraging). We therefore identified 12 extreme outliers that were above the 95th percentile and excluded them from analyses. Once landed, female bees spent 2.5 times longer on flowers than male bees (F1,199.7 = 24.95, P < 0.001). While ambush bug presence tended to reduce the duration of visits by some 30% (13.4 % in females vs 57.6% in males) the effect was not significant (F1,23.45 = 1.48, P = 0.236) and there was no interaction between the sex of bee and ambush bug presence (F1,199.7 = 0.00, P = 0.956, Fig. 3b) on visit duration. The proportion of females nectaring once landed was 37% higher than males (F1,52 = 13.54, P < 0.001) but there was no effect of ambush bug presence on nectaring (F1,52 = 3.79, P = 0.057), no interaction with sex (F1,52 = 1.75, P = 0.191), and no effect of ambush bug presence on the proportion of females collecting pollen during visits (F1,29 = 0.95, P = 0.339).

Spectrometry showed ray flowers had much higher reflectance in the yellow to red range of the visual spectrum (570-700) than disc flowers or either measure of ambush bug coloration; The reflectance of the ambush bug nota were also much darker and similar to disc flowers, exhibited little reflectance in the same ranges (Fig. 4a). Plotting the spectral data in the honeybee color space indicated nearly complete overlap in both ray flower and ambush bug notal coloration, though ray petals and sternal color of ambush bugs were different, exciting visual receptors more in the green and bluegreen sectors, respectively.

**Discussion**

Overall, our results suggests that cryptic predatory ambush bugs in this study had little impacts on the foraging behaviors of long-horned bees and may not be a significant factor driving *Melissodes* foraging decisions. Bees visiting ambush bug occupied flowers spent similar amounts of time on those flowers compared to those visiting unoccupied blooms and there was little effect of the presence of ambush bugs on other bee foraging behaviors. This appears driven by cryptic coloration of ambush bugs, which are likely not perceived against the dark ray flowers while hunting on sunflowers. However, we did detect sex-specific differences in bee foraging behaviors, with males visiting more frequently but for shorter periods of time. Given the differences in foraging behavior recorded between sexes, this could suggest that male and female bees vary in exposure to flower-occupying predators. These finding are novel, given the paucity of studies evaluating solitary bee foraging behavior in response to predators, and suggest that much of what we know about predator risk assessment and avoidance in social bees may not necessarily apply to solitary bees, given differences in both their ecology and natural history, and the different strategies of flower-occupying predators.

Our findings contrast what others have found studying social bee response to flower-occupying predators. For example, honeybees foraging on *Solidago odora* Ait. (Asteraceae)spent significantly less time foraging on flowers with *Phymata americana* present, although total visitation did not vary between occupied and unoccupied flowers (Elliott and Elliott 1994). However, most other studies have used predator models or non-cryptic predators (mostly spiders), and often see effects of predators after repeated exposure. For example, in experiments where eusocial honeybees and bumble bees learn to recognize predators, they only become wary of predator-occupied flowers after repeatedly experiencing simulated attacks (Dukas 2001; Ings et al. 2012). In our experiments, in only one visit to occupied flowers did an ambush bug attack a bee and then it consumed it, meaning we observed no opportunities for bees to learn to recognize ambush bugs. Solitary bees may therefore have less information about the risk posed by ambush bugs, given the low, but successful, attack rate; although, more studies are need that explore learning potential of solitary bees in general.

Most other research on solitary bees has focused on crab spiders. In one of the few other studies using solitary bees, Reader *et al* (2006) found no effect of crab spider presence on visitation by the solitary bee *Eucera notata* (Apidae) to flowers of crimson spot rockrose, *Cistus ladanifer* L. (Cistaceae)*,* even though honeybees avoided both inspecting and landing on flowers when crab spiders were present (Reader et al. 2006). In contrast, models of non-cryptic spiders altered behavior of both males and females of the solitary bee *Ptilothrix fructifera* (Holmber) (Apidae), altering male territorial perching behavior, and reducing female visitation and foraging time (Oliveira et al. 2016). However, these studies used either non-cryptic predators or lumped cryptic and non-cryptic spider species together, and differences between these and our study suggest that some unique mechanisms may be driving solitary bee response to cryptic predators. For instance, strong impacts of ambush bugs on sunflowers could be buffered by their cryptic coloration and behavior (Romero et al. 2011). Results from our spectral analyses suggests that ambush bugs are likely cryptic when hunting on the disc flowers of sunflowers, which is a common strategy. In our study, male bees tended to land and collect nectar less often during visits to ambush bug occupied flowers, suggesting that they likely perceive ambush bugs to some degree; although, females showed no response to ambush bugs and the interaction was not significant. One possible explanation could be differences in exposure to predators between sexes. While both male and female *Melissodes* are typically most active in the early morning, females rest in burrows in the afternoons and overnight while males often gather in sleeping aggregations on sunflower heads, sheltering under ray flowers until morning (Parker et al. 1981). This could to lead to males having greater risk exposure, and therefore add to their potential assessment of risk when visiting predator occupied flowers. More studies are needed to verify if there are indeed sex-specific differences in risk-assessment in solitary bees.

The lack of discernable response to predators in our study could also be due to the small spatial scale and high predator density of the experimental garden. The capacity for social bees, such as *Bombus*, to learn has been shown to create spillover effects, where predator presence on flowers can indirectly lead to reduced visitation to all flowers, including those not occupied by predators (Ings and Chittka 2009). Thus, in order to avoid cryptic predators, bumble bees exhibit reduced foraging behaviors to all flowers in a patch and increase discriminating behavior (Ings and Chittka 2008). Our experimental garden was relatively small (~25m2) and the effects of predators may have been diffuse over the entire flowering patch. That we did not find statistically significant impacts of cryptic ambush bugs in our results could therefore suggest that solitary bees exhibited similar avoidance behaviors at the patch level, regardless of the floral treatment. Manipulating patch-level ambush bug presence was beyond the scope of this study but warrants further investigation.

An alternative explanation for the lack of effects of predators in this study could be driven by the constrained feeding habits of *Melissodes*. Unlike generalist social bees, which can change floral hosts depending on predator presence, *Melissodes* are sunflower specialists and may be constrained to one or only a few pollen host species during their life at any given location. Thus maximizing foraging, at least for pollen by females, may be the best strategy to mitigate combined risk of predation and resource competition. Therefore, ignoring other floral occupants altogether, both before and after landing, may be the best foraging strategy for female bees, as they gain access to more floral resources (Burns 2005) without losing time required to discriminate other occupants. This could also make intuitive sense, given that sunflowers are very large, and often occupied by many other types of insects simultaneously, such as other bees, beetles, flies, butterflies, and also predators. The relative importance of competitive vs predatory interactions at flowers is relatively unstudied, but could play an important role in foraging decisions, especially for specialist solitary bees.

In summary, our data suggest sex-specific differences in foraging behaviors of solitary bees, but little evidence that ambush bugs affected foraging behavior. These results suggest that these predators are cryptic and either rarely detected by these solitary bees, that foraging *Melissodes* do not alter their foraging behavior because of the perceived risk of predation, or that attacks may be too rare to have an impact on foraging behavior in the field. The best foraging strategy for female solitary bees may thus be to largely ignore floral occupants when assessing floral characteristics and respond to floral occupants as necessary after landing. For males, tradeoffs between mate searching and foraging likely drive visitation frequency and duration more so than the presence of flower-occupying predators. Future work to disentangle flower versus patch level effects of flower-occupying predators could provide further insight into the effect of predator distribution on foraging behaviors of solitary bees.

**Table 1**. Bee visitation and behavior by genus during the experiment. “Visits” is the total number of occurrences of each genus during the experiment. “Pollen” and “Nectar” are the number of pollen and nectar collection events recorded for each genus. “Male” and “Female” refers to the number of visits by each sex. “Ambush” and “Control” are the numbers of visits to flowers with or without ambush bugs, respectively

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Genus** | **Sociality** | **Visits** | **Pollen** | **Nectar** | **Male** | **Female** | **Ambush** | **Control** |
| *Agapostemon* | Solitary | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Andrena* | Solitary | 18 | 3 | 5 | 14 | 4 | 8 | 10 |
| *Anthidium* | Solitary | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Apis* | Eusocial | 6 | 0 | 2 | 0 | 6 | 3 | 3 |
| *Bombus* | Eusocial | 8 | 2 | 5 | 1 | 7 | 6 | 2 |
| *Colletes* | Solitary | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Diadasia* | Solitary | 5 | 1 | 3 | 2 | 3 | 3 | 2 |
| *Eucera* | Solitary | 2 | 1 | 1 | 0 | 2 | 0 | 2 |
| *Halictus* | Eusocial | 22 | 2 | 4 | 8 | 14 | 11 | 11 |
| *Lasioglossum* | Social | 5 | 1 | 1 | 1 | 4 | 3 | 2 |
| *Lithurgus* | Solitary | 19 | 5 | 9 | 4 | 15 | 11 | 8 |
| *Megachile* | Solitary | 1 | 1 | 1 | 0 | 1 | 0 | 1 |
| *Melissodes* | Solitary | 613 | 45 | 148 | 466 | 147 | 313 | 300 |
| *Perdita* | Solitary | 2 | 0 | 0 | 0 | 2 | 2 | 0 |

**Table 2.** Summary of *Melissodes* foraging behavior during the experiment. “Treatment” refers to whether or not there was an ambush bug present. “Visits” are the total number of bees that either inspected of landed while visiting. “Landings” refers to the number of bees that landed. “Inspection” refers to the average amount of time in seconds non-landing bees spent before leaving. “Duration” refers to the average length of landing visits in seconds. “Pollen” and “Nectar”” refer to the number of events in which pollen or nectar was collected

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Visits** | **Landings** | **Inspection (s)** | **Duration (s)** | **Nectar** | **Pollen** |
| control | 300 | 112 | 0.46 | 6.89 | 84 | 24 |
| female | 80 | 72 | 0.18 | 8.18 | 59 | 23 |
| male | 220 | 40 | 0.47 | 4.58 | 25 | 1 |
| ambush | 313 | 110 | 0.26 | 5.94 | 64 | 21 |
| female | 67 | 61 | 0.22 | 7.87 | 44 | 20 |
| male | 246 | 49 | 0.26 | 3.54 | 20 | 1 |
| **Total** | **613** | **222** | **0.35** | **6.42** | **148** | **45** |

**Figure Legends**

**Fig. 1** A photograph of the jagged ambush bug, *Phymata americana*, a) resting on the disk of a *Helianthus petiolaris* inflorescence, and b) against the ray petals of an inflorescence while feeding on a captured *Melissodes* spp. Photos courtesy of Travis Bildahl.

**Fig. 2** Effects of ambush bug presence on a) the total number of *Melissodes* visits per flower, b) the number of landing visits per flower, or c) the proportion of visiting bees that landed on flowers

**Fig. 3** Effects of ambush bugs on a) non-landing and b) landing visit durations of *Melissodes*

**Fig. 4** While there was variation in a) spectral reflectance curves of flower and ambush bug parts, when run through a honeybee visions model b) the darker color of the ambush bug notum is similar to the dark disc flowers of sunflowers.

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