An eagerness for conspecifics: The distribution of nests of the sunflower leafcutting bee, *Megachile pugnata* (Megachilidae), when nesting cavities are in excess

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Abstract. Under unmanaged conditions, many ground-nesting bee species nest in large aggregations. Although cavity-nesters (xylophiles) will also nest in large aggregations when managed in agricultural settings, it is unclear if they do so instinctively or due to opportunity. Here we report that females of the xylophile *Megachile pugnata* Say preferentially aggregate their nests at three measured scales of cavity availability even when nesting cavities are in excess. We also find that they sometimes prefer to initiate nesting at the edges of nesting blocks but that they are generally unresponsive to the position of cavities in nest shelters: aggregations are evident irrespective of location. We propose that an important but previously unemphasized reason that aggregation behavior has been maintained by xylophiles and ground-nesting species, is the genetic benefits realized by having many unrelated mating partners available.

INTRODUCTION

According to recent estimates, over 80% of non-parasitic bee species nest in the ground (Cane & Neff, 2011; Harmon-Threatt, 2020), many in dense aggregations, some of which are transitory, others of which can last for many years (Michener, 1974; Danforth *et al*., 2019). Indeed, all of the many examples of aggregations catalogued by Danforth *et al*. (2019) are ground-nesters. The proximate reasons offered for such aggregations include attraction to conspecifics to obtain information about food and nest-sites, limited acceptable substrate that forces females to cluster their nests, protection from enemies, and philopatry (Rosenheim, 1990).

Many of the remaining 20% of species are xylophiles, species, mostly in the Megachilidae, that nest in cavities in wood or twigs. In contrast to ground-nesting species, few, if any, megachilid aggregations have been reported from natural situations, perhaps because densely distributed cavities in wood that facilitate nesting aggregations are uncommon (Michener, 1974; Morato & Martins, 2006). Nevertheless, many megachilids are facultative gregarious nesters in agricultural settings when presented with thousands of artificial nesting cavities grouped closely together in bee shelters. The preeminent example, the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius) (Pitts-Singer & Cane, 2011), has now been joined by a growing number of other xylophiles in the genera *Megachile* Latreille and *Osmia* Panzer currently being managed or developed for crop pollination (Osterman *et al*., 2021).

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In such agricultural situations many thousands of bees are released *en masse,* and it is difficult to discern the sequence in which nest cavities are adopted and how aggregations form. Do females begin by scattering nests and only later, when almost all cavities have been filled, appear to have aggregated them or do they aggregate their nests from the beginning? Any tendency of xylophiles to aggregate is likely to be a characteristic retained from their ground-nesting ancestors (Michener, 1964; Eickwort *et al.*, 1981; Litmann *et al.*, 2011; Sedivy *et al.*, 2013), some of which probably nested gregariously. It is unlikely that xylophiles evolved from non-aggregating ground-nesting species and then independently developed aggregating behavior when nest sites in wood conducive to doing so are rare.

Here, we examine the nesting choices of females of the xylophile, *Megachile pugnata* Say, sunflower leafcutting bee (SLCB), when presented with nest cavities in excess. Information on how females initially distribute their nests helps us to evaluate the various hypotheses proposed to explain aggregations. 1) Do females aggregate or scatter their nests when their numbers are greatly exceeded by the number of available nesting cavities? 2) Do females prefer to nest at the edges of nest blocks as has been shown for *M. rotundata* (Fauria *et al.*, 2004; Guédot *et al.*, 2006)? 3) Do all females practice the same nest spacing behavior or do some aggregate and others isolate their nests, perhaps indicating that the population contains both individuals attracted to conspecifics (joiners) and others that are either repelled by or unresponsive to them (loners)? 4) Recognizing that there are likely to be several factors that intertwine to promote nesting aggregations, does a description of the development of SLCB nest-spacing behavior enable us to evaluate the likely importance of those factors?

The SLCB is a large bee (13–18 mm length) that occurs throughout the U.S. and southern Canada; it specializes in collecting pollen from plants in the Asteraceae and was previously considered as a potential commercial pollinator of sunflowers (*Helianthus* L. species; Parker & Frohlich, 1985). Females readily nest in artificial cavities. Leaf material and soil are typically used for brood cell partitions although brood cells are not lined with leaves. Typically, one cell is completed per day with another day used to plug nests (Frolich (*sic*) & Parker, 1983). Nests placed in cavities the size of those employed here contain an average of between 3.5 and 3.8 cells (Tepedino & Frohlich, 1982; Parker & Frohlich, 1985). Depending upon location and weather, SLCBs may have more than one generation per year. For additional information see Tepedino & Frohlich (1982), Frolich (*sic*) & Parker (1983), and Parker & Frohlich (1985).

MATERIAL AND METHODS

Two wood shelters were placed about 3 m apart in the midst of a field of blooming commercial sunflowers (*Helianthus annuus* L.) in Esparto/Madison, Yolo Co., California, USA. Each shelter comprised three attached panels that contained 4, 20, and 6 wood nesting blocks, respectively. Panels contained different numbers of wood blocks (Fig. S1) to explore the possibility that cavity adoption was influenced by the numbers of closely adjacent blocks. Each nesting block contained 42 (7 rows × 6 columns), 49 (7 × 7) or 56 (7 × 8) equally spaced cavities. Each shelter offered ≥1500 cavities lined with paper straws (7 mm diam, 15 cm length).

Bees were obtained from trap blocks (Krombein, 1967), whose cavities had been lined with paper straws, that had been placed at several sites in Cache and Rich Counties, northern Utah, USA, in 2001. Straws containing bees were maintained overwinter in a cooler at ~ 3 °C and then brought to California under refrigeration in early June while bees were still in diapause. Straws were incubated at 27 °C until males began to emerge in late June at which point they were transferred to a shaded emergence box next to the shelters. Females soon began emerging and were nesting by 30 June. Because the objective was to discover how females distributed their nests initially, when cavities greatly exceeded female number, all straws with unemerged bees were removed on 1 July.

The number of nesting females was estimated on June 30 by examining the paper straws in each block along the two diagonal rows, *i.e.*, the rows from top left to bottom right and from top right to bottom left. The percentage of occupied straws in the diagonals was then multiplied by the total number of holes in the block to approximate the number of nesting females. On 3 July, the plugged nests in all blocks were mapped and counted. Straws were extracted with forceps on both dates and examined for evidence of use (completed or partially completed cells, leaf material) and quickly returned to the same cavity, taking care to maintain original orientation.

Aggregation of nests was tested in several ways and at different spatial scales. First, we examined aggregation across each shelter using the 30 nesting blocks as the units of aggregation; aggregation was manually evaluated using Morisita's commonly employed Index of Dispersion (Krebs, 1999):

$$I_d = n\{\sum x^2 - \sum x/(\sum x)^2 - \sum x\,]$$

where n= the number of nest blocks, and x = the number of plugged nests within a block. Dispersion is indicated by values < 1 and aggregation values >1. I_d was tested using Chi-square test statistic where:

$$X^2 = I_d(\sum x-1) + n - \sum x\,, (d.f.=n-1).$$

The Morisita Index was also used to test for aggregation of nests within each of the six panels. In addition, we used least squares linear regression (Sokal & Rohlf, 1969) to examine the effect of the estimated number of bees nesting per block on 30 June (independent variable) on the number of plugged nests in that block (dependent variable) on 3 July.

Next, we tested aggregation within blocks by comparing the observed nearest-nest distances to those based on a null model simulating the same numbers of nests per block but with locations assigned randomly (following Krebs, 1999). Nearest-nest distances were calculated for 99 randomizations for each block. Significance at $\alpha = 0.01$ was tested by comparing rank of the observed distance within the set of randomizations. For example, a rank of 10th out the 100 total would equate to $P = 0.1$. We did not adjust alpha value to account for tests across multiple blocks.

In addition to examining aggregation within blocks, we also asked whether nest-seeking females tended to choose cavities on the four edges of the blocks, which could produce a degree of aggregation unique to our experimental setup. For each shelter we grouped blocks by cavity number and then tallied the total number of nests made in edge or inner cavities. For example, in 7 × 7 blocks there are 24 edge cavities and 25 inner cavities; for 7 × 8 blocks there are 26 and 30, respectively. Nests were combined for all equal-size blocks and then expected versus observed cavities were compared using Chi-Square tests.

Using Pearson correlation analysis, we also tested for a nest position effect, *i.e.*, did females tend to locate their nests in blocks at specific locations in the shelters. The number of nests in each of the 30 blocks in Shelter 1 was compared with its exact positional counterpart in Shelter 2.

Finally, we examined the attraction of females to the different numbers of blocks in the panels in each shelter. We asked if females distributed their nests in proportion to the number of blocks in a panel. Thus, we expected 4/30 or 13.3% of nests to be made in panels with four blocks, etc. We tested these expectations for each shelter using Chi-Square.

<div align="center">RESULTS</div>

The estimated number of bees nesting on 30 June was: Shelter 1 – 198, Shelter 2 – 205. The number of plugged nests per block on 3 July was significantly related to the

estimated number of bees nesting there on 30 June for both shelters (Shelter 1, $F_{1,28}$ = 127.8, r^2 = 0.82, $P < 0.001$, y = 0.67x – 0.155; Shelter 2, $F_{1,28}$ = 57.1, r^2 = 0.67, $P < 0.001$, y = 0.51x + 1.35).

Although available cavities greatly outnumbered nesting bees, SLCB females significantly aggregated their nests at the level of blocks in both shelters (Table 1, Fig. S2). In Shelter 1, 23 of 30 blocks had 5 or fewer plugs – of these, 12 had no plugs, whereas the 4 blocks with more than 10 plugs had 72 of the total 127 plugs – 56.6%. The tally in Shelter 2 was similar: 22 of 30 blocks had 5 or fewer plugs while only 6 blocks had more than10 containing a total of 93 of 144 plugs – 64.6%. Nests also were aggregated within each of the three panels in each shelter (Table 1).

Table 1. Number of sunflower leafcutting bee (*Megachile pugnata* Say) nest plugs found on 3 July in two bee shelters for each of three panels (A, B, C), along with values of Morisita's Index of Dispersion (I_d) and the p-value based on X^2 for each shelter and each panel. Nests in both shelters and all panels significantly aggregated among blocks.

SHELTER 1: 127 plugs (I_d = 3.30, $P < 0.001$) 1505 cavities

Panel A (I_d = 1.72, $P < 0.001$)		Panel B (I_d = 3.30, $P < 0.001$)					Panel C (I_d = 2.97, $P < 0.001$)	
30 plugs		71 plugs					26 plugs	
Plugs per block								
18	8	8	2	22	2	10	2	18
0	4	1	0	3	0	0	0	0
		0	2	0	3	0	2	4
		14	4	0	0	0		

SHELTER 2: 144 plugs (I_d = 2.29, $P < 0.001$) 1512 cavities

Panel A (I_d = 1.33, $P \sim 0.03$)		Panel B (I_d = 2.54, $P<0.001$)					Panel C (I_d = 1.71, $P<0.001$)	
19 plugs		70 plugs					55 plugs	
Plugs per block								
0	6	1	3	0	13	5	1	13
4	9	15	0	0	4	1	19	3
		1	0	5	2	1	0	19
		1	0	1	14	3		

While females aggregated their nests among blocks within shelters, evidence for nest aggregation within blocks was mixed. Twenty-three of 33 blocks combined for both shelters showed greater aggregation of nests than expected when compared with randomly placed nests of the same numbers within blocks (Fig. 1). However, only four of these comparisons were significant (Table S1). Conversely, if we analyze only the 13 blocks with between 2 and 10 plugs (chosen because they are more likely to show aggregation than blocks with larger numbers of plugs), in Shelter 1, 38 of the 54 plugs (70.4%) were immediately adjacent to or only one cavity away from another plug. This despite there being between 38 and 52 vacant cavities in each of these 13 blocks (median ~ 45). The results for Shelter 2 were similar. Forty of the 44 plugs (90.9%) in the 10 qualifying blocks were immediately adjacent to or only one vacant cavity away from another plug; there were between 40 and 53 vacancies in these blocks (median ~ 46).
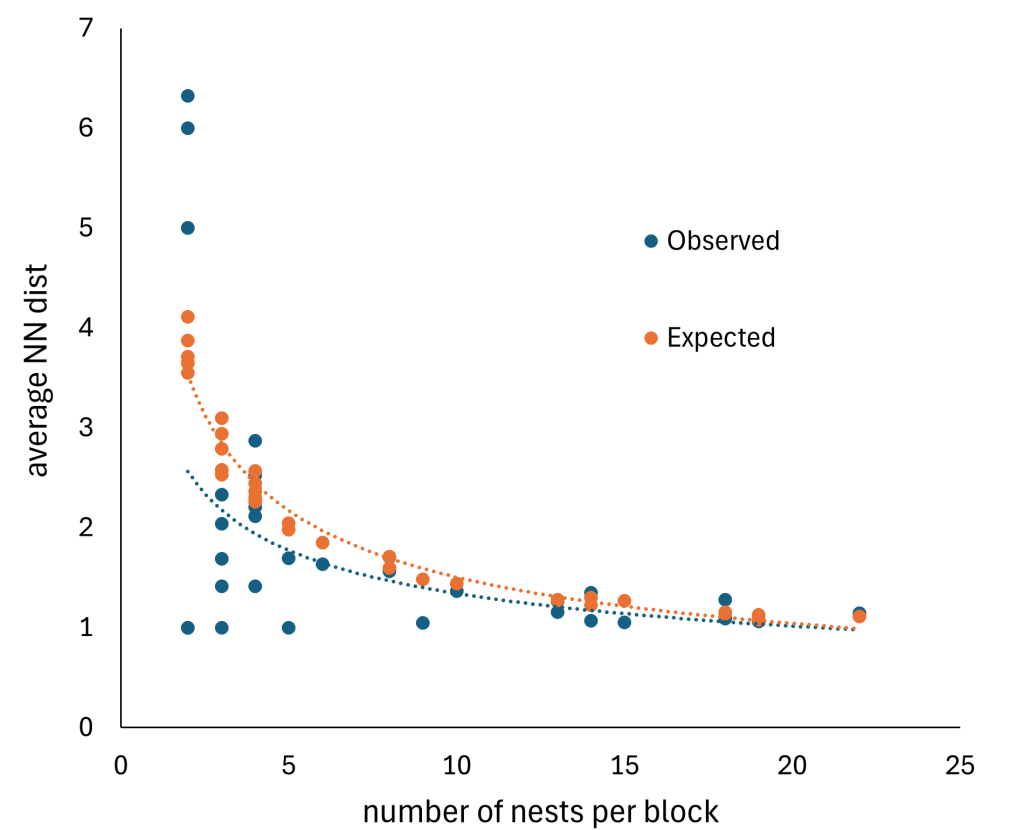
Figure 1. Average observed nearest nest distances (cm) among sunflower leafcutting bee (*Megachile pugnata* Say) nests within blocks as a function of the number of nests within the blocks.

Females were inconsistent between shelters in their attraction to edges (Table 2). Those nesting in Shelter 1 significantly preferred edge cavities in both 7 × 7 and 7 × 8 blocks but females nesting in Shelter 2 expressed no such preference. We found no evidence for a strict position effect: females did not consistently prefer to nest at any particular position within the shelters (r = - 0.08, *P* = 0.66). Blocks in one shelter that contained numerous plugs were commonly vacant or mostly ignored in their exact counterpart cavities in the other shelter.

Neither did females distribute themselves proportionately across panels, nor was their distribution the same in the two shelters. In Shelter 1, a larger than expected proportion of females nested in the panel with four blocks (Table 2). In Shelter 2 a larger than expected proportion of females nested in the panel with 6 blocks. In both shelters many fewer than expected females nested in the panel with 20 blocks.

Although most females aggregated their nests, there was a small percentage (7.7%) that nested at least four vacant cavities from the nearest conspecific ($\bar{x}$= 5.9, median = 6). Seven of these 21 "loners" (33.3%) were alone in their chosen blocks. Only 8.4% of cavities in Shelter 1 and 9.5% of cavities in Shelter 2 were occupied.

DISCUSSION

Our results show that the probable ancestral characteristic to aggregate nests found in ground-nesting bees (Michener, 1964, 1974; Eickwort *et al.*, 1981; Sedivy *et al.*, 2013) is present in at least one derived xylophile (and perhaps many others) even though situations conducive to aggregating are uncommonly encountered in nature

Table 2. Distribution of sunflower leafcutting bee (*Megachile pugnata* Say) nesting plugs by block size (A) and blocks/panel (B) for each shelter. A compares the number of plugs in edge versus inner cavities, B compares the number of plugs in panels with different numbers of blocks. Comparisons made using X^2 statistic.

	Shelter 1		Shelter 2	
A				
Block Size	7 × 7	7 × 8	7 × 7	7 × 8
Total Plugs	75	48	95	28
Expected Edges	36.7	32	46.5	13
Observed Edges	50	22.3	45	9
X^2 (df=1)	4.8, $P<0.05$	4.2, $P<0.05$	0.05, $P>0.80$	1.23, $P>0.20$
B				
Blocks/Panel	Expected	Observed	Expected	Observed
4	17	30	19	19
6	25	26	29	55
20	85	71	96	70
X^2 (df=2)	12.3, $P < 0.01$		30.4, $P<0.001$	

(Michener, 1974; Morato & Martins, 2006). In the presence of essentially unlimited artificial cavities, many SLCB females "chose" to aggregate their nests at multiple scales: in each shelter, in each panel within each shelter, and to some extent within individual blocks (Table 1). The possibility that our results, recorded over a three-day span, were due, in part, to females constructing second nests adjacent to their initial ones is unlikely in most instances. Typically, one cell is completed per day with another partial day used to plug nests (Frolich (*sic*) & Parker, 1983). Nests placed in cavities the size of those employed here, and with similar resource availability, contained an average of between 3.5 and 3.8 cells (Tepedino & Frohlich, 1982; Parker & Frohlich, 1985). Thus, at least four days are required to complete the typical nest.

The location of initiation and growth of aggregations was variable. In some instances, aggregations were associated with edge cavities but not consistently so. We detected no block position effect, *i.e.*, females did not consistently prefer certain areas in the shelters, panels or blocks. Instead, aggregations coalesced from single-nest nuclei which could be initiated, seemingly randomly, in any part of the shelter and then expanded as other females joined.

Only one other study of which we are aware has tracked distribution of nest cavities. Groulx & Forrest (2018) followed six species of *Osmia* (Megachilidae) in subalpine Colorado (USA). They found that "mason bees showed no preference for nesting in blocks that were occupied or unoccupied by other mason bees", *i.e.*, no consistent formation of aggregations. These differing results may be accounted for by two factors: 1) the number of available cavities – over 3,000 in adjacent shelters in an agricultural field in our study – versus 256 cavities (16 cavities per each of 16 dispersed blocks) at each of three natural sites (Groulx & Forrest, 2018); and 2) colonization by females of the shelters and blocks. In our study, blocks were colonized, and nests aggregated, by hundreds of females mass-released from a single location between a plethora of convenient and obvious cavities in the shelters; Groulx & Forrest (2018) examined colonization by naturally occurring *Osmia* females of several species, likely dispersing from multiple natural nests. We suspect that because nest sites for xylophiles are probably limited in natural situations (Morato & Martins, 2006; Steffan-Dewenter & Schiele, 2008; but see Roulston & Goodell, 2011), searching female xylophiles, as in

Groulx & Forrest (2018), will accept the first promising cavity encountered, whether other bees are nesting nearby or not, rather than expending additional effort in search for an uncommon aggregation.

Hypotheses to account for the costs and benefits of aggregating compared to scattering have been reviewed by Rosenheim (1990; see also Parrish & Edelstein-Keshet, 1999; Bengtsson, 2008). Although this study does not address experimentally any of these hypotheses, the behavior of the SLCB under cavity surfeit suggests that certain factors may be categorized as subsidiary for both xylophiles and ground-nesting species. The philopatry hypothesis emphasizes the advantage of nesting at the same sites from which a female emerges, *i.e.*, "if it was good enough to support my development, it's probably good enough to support that of my progeny". Another hypothesis stresses the potential advantage gained by the time and energy saved by re-use of old burrows (Hefetz & Tengo, 1992). However, in our study neither philopatry nor reuse of old burrows were necessary for the SLCB aggregations to develop. This is not to deny the importance that they may play under other circumstances but serves to demonstrate that neither is necessary for aggregations to form. The "limited substrate" hypothesis proposes that females are forced to aggregate their nests because acceptable nesting substrate is localized and limited. Limited substrate, while documented for some ground-nesting species (*e.g.*, Potts & Willmer, 1997), fails for many others (Michener, 1974; Rosenheim, 1990) and obviously does not apply here where nesting cavities were unlimited.

The remaining hypotheses advanced to explain the benefits of aggregating can be grouped under the Allee effect (Stephens & Sutherland, 1999), which highlights the reproductive fitness gained by individuals that congregate with conspecifics. One hypothesis proposes that searching females cue to the presence of conspecifics, or perhaps even to other species, at "information centers" where they may improve their progeny production rate by learning the location of nest sites and floral resources from other females. While cues from conspecifics to shelter and food were likely rendered superfluous by the proximity to, indeed the obviousness of abundant cavities and flowers, the information center hypothesis remains viable because of the atypical situation of our study. Under more natural conditions information could indeed be passed between aggregated bees (Loukola *et al.*, 2020; Chittka & Rossi, 2022).

Females may also form aggregations for reasons other than information exchange. The "selfish herd" hypothesis proposes that aggregation affords protection from predators and parasitic insects because large, concentrated numbers of individuals may confuse, discourage or even attack potential enemies and thereby decrease offspring mortality (Hamilton, 1971; Wcislo, 1984; Rosenheim, 1990). Although enemies were not a factor here, under more natural situations, the SLCB is vulnerable to several species including the beetle *Trichodes* Herbst (Cleridae) and the eulophid wasp *Melittobia* Westwood (Tepedino & Frohlich, 1982) and may well benefit from aggregating. Vulnerability to parasites and disease has also been used as an argument for isolating nests (Rosenheim, 1990; Groulx & Forrest, 2018). Clearly, additional studies are needed to compare the reproductive success of aggregating versus scattering females in this and other solitary bee species.

A final potentially important but commonly overlooked reason to aggregate nests is the genetic benefit females gain by providing their progeny with the opportunity to mate with an individual from a large pool of unrelated individuals rather than with sibs. Such diverse mating will reduce inbreeding and the incidence of inviable diploid males and generally increase the fitness of resultant offspring (Zayed, 2009). This genetic benefit is unavailable to bees emerging from solitary nests because they are forced to either mate with sibs or to disperse in search of unrelated receptive partners, an action whose success is surely less likely than finding a receptive mate at an aggregation site. While consanguineous intranidal mating has been recorded

for one communally-nesting ground-nesting species (Paxton *et al.*, 1996; Paxton, 2005) it has never been reported for cavity-nesting megachilds nor is it likely because cells are produced in tight-fitting linear arrays in wood and males emerge first from outer nest cells several days before inner-cell occupying females (Danforth *et al.*, 2019). Sib-mating is also unlikely because early emerging males cruise either or both the entire nest site or flowers in search of mating opportunities with any emergent females. The fitness advantage that accrues from diverse mating opportunities may also explain why evidence for aggregation within a nest block was consistent among blocks within shelters, but not as strongly within blocks. There may be little fitness advantage to nesting within 1 cm versus 20 cm of a conspecific as both would increase access to diverse matings. Thus, nesting in aggregations should be favored because the incidence of inbred offspring is lowered.

A small percent of nesting females (7.7%) founded their nests in relative isolation (at least four vacancies from the nearest conspecific nest). Whether this represents simple random variation in nest placement behavior or an actual behavioral polymorphism of joiners and loners is unclear. The recorded number of loners is also quite possibly an underestimate because we have no idea how many females dispersed from the site in search of other less crowded nesting opportunities. Several behavioral polymorphisms have been reported for bees (Richards *et al.*, 2003; Kocher *et al.*, 2018) and one for nest placement would not be surprising (*e.g.*, Wuellner, 1999). Indeed, there are potential benefits to scattering nests. For example, scattering might increase female fitness by reducing interference between, and agonistic interactions among females, including the chances of a nest lost to usurpation (Hefetz & Tengo, 1992; Tepedino *et al.*, 1994; Guédot *et al.*, 2006). Scattered nests are also likely to be less attractive to enemies and might also decrease the likelihood of disease transmission (Rosenheim, 1990; Bengtsson, 2008). For example, Groulx & Forrest (2018) found higher rates of parasitism in blocks with larger numbers of nesting females suggesting that parasites, in this case the wasp *Sapyga* sp., were attracted to higher bee activity.

We have shown that, despite the limited opportunities to nest in aggregations in situations under which they evolved, nesting in proximity to conspecifics appears to be the preferred behavior of many females of one species of xylophile. Based on the evidence of aggregated nesting by other species of megachilids in agricultural settings summarized in Osterman *et al.* (2021), we propose that this will prove to be the preferred behavior for many other species of xylophiles. Unresolved are competing explanations for aggregation behavior although our results suggest that the hypotheses of philopatry, limited substrate and re-use of old burrows be downgraded in importance. While they may sometimes play a role in the formation of aggregations, our results show that they are not necessary. More important reasons are likely to be some amalgam of mating opportunities, protection against enemies, and information exchange. The benefits gained by protection and information exchange will vary with the type and number of enemies present and the need for direction to flower patches. In contrast, the benefit gained by the likelihood of non-consanguineous mating is ever-present.

SUPPLEMENTAL MATERIAL

Figure S1. Three-panel nesting shelter with positioned nest blocks. Each block contained drilled cavities lined with paper straws.

Figure S2. Percentage of plugs in each of 4 groups for Shelters 1 (plain) and 2 (hatched).

Table S1. Complete list of observed nearest neighbor distances (cm) for each nest block, average nearest neighbor distances from 99 randomized nest placements for the same block.

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