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Nesting aggregation as a predictor of brood parasitism in mason bees (*Osmia* spp.)

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Abstract. 1. Parasitism can be an important source of mortality for insect populations; however, we know little about the factors influencing vulnerability of wild bees to parasites. Mason bees (genus *Osmia*; Hymenoptera: Megachilidae) are important pollinators of crops and wild plants and are vulnerable to attack by brood parasites. High nest densities may increase rates of brood parasitism by attracting disproportionate numbers of parasites.

2. Three years of field observations from multiple sites were analysed to assess whether mason bee brood parasitism increased with host density. Mason bees were allowed to nest in artificial nesting blocks and establish natural variation in nesting density. Nest cells constructed by bees were checked for the presence of parasite eggs.

3. Parasitism of nest cells strongly increased with the number of actively nesting bees at a nesting block. Mason bees showed no preference for nesting in blocks that were occupied or unoccupied by other mason bees. Parasitism also increased with the number of days a nest was provisioned and decreased over the course of the season. Nest cells constructed last in a nest were significantly more parasitised than inner cells, despite being sealed against invasions.

4. These findings show positively density-dependent parasitism in mason bees. They also suggest that bees terminate parasitised nests, causing parasitised cells to become outermost nest cells – a behaviour that may represent a defence against parasites. Our results have implications for the management of mason bees as agricultural pollinators, as cultivating them at high densities could reduce offspring survival.

Key words. Cleptoparasites, density dependence, native bees, population ecology, Rocky Mountain Biological Laboratory.

Introduction

Parasitism can be a considerable source of mortality in insect populations (Steffan-Dewenter & Schiele, 2008; Klemola *et al.*, 2014), and numerous factors can drive intraspecific variation in parasitism rates (Rosenheim, 1990; Danforth & Visscher, 1993; Antonini *et al.*, 2003; Bischoff, 2003; Goodell, 2003; Seidelmann, 2006; Klemola *et al.*, 2014; MacIvor & Salehi, 2014). Features of the abiotic environment, such as resource availability (Goodell, 2003), and behavioural traits such as foraging efficiency (Danforth & Visscher, 1993; Seidelmann, 2006) can influence the vulnerability of insects to parasite exploitation. Other interacting species can also influence parasitism rates: for example, the release of volatiles by plants in response to herbivore damage can attract parasites of those herbivores (Charleston *et al.*, 2006). Population density and size are frequently important determinants of rates of parasitism, with larger or denser populations sometimes suffering increased parasitism and sometimes being better protected against natural enemies (Rosenheim, 1990; Antonini *et al.*, 2003; Klemola *et al.*, 2014).

Bee populations are thought to be regulated, at least in part, by natural enemies (Wcislo & Cane, 1996). Rates of parasitism in bee species are highly variable among years and between regions (Danforth & Visscher, 1993; Petanidou *et al.*, 1995; Wcislo & Cane, 1996), and nesting aggregation could play a role in this variation. Nesting aggregations are common in solitary Hymenoptera, and may arise for a number of reasons, outlined by Rosenheim (1990): First, limited availability of appropriate nesting locations could cause many individuals to choose to nest in the same area. Similarly, the presence of conspecifics can act

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as an indicator of appropriate nesting locations, thereby concentrating nests in single locations. Finally, philopatry (selection of nesting locations close to the place of birth) could lead to aggregations of related individuals close to their maternal nests.

Previous studies have reported conflicting results regarding the effect of aggregation on parasitism of solitary Hymenoptera nests. While the majority of studies suggest positive density dependence (in which individuals nesting in larger aggregations suffer increased rates of parasitism), instances of negative density-dependent parasitism have also been reported (Wcislo, 1984; Rosenheim *et al.*, 1989; Rosenheim, 1990; Coster-Longman *et al.*, 2002; Antonini *et al.*, 2003). There are a number of proposed explanations for both positive and negative density dependence in parasitism rates. Larger nesting aggregations might deter parasites from attacking because the greater density of potential targets (Wcislo, 1984; Antonini *et al.*, 2003) might cause confusion, making it difficult for the parasite to locate a single target (Jeschke & Tollrian, 2007). Large aggregations can also lead to dilution of risk, as there are a large number of targets for natural enemies to exploit (i.e. the 'selfish herd'; Hamilton, 1971). This effect has been observed in solitary wasps (Coster-Longman *et al.*, 2002). Finally, larger aggregations of nesting individuals may provide increased defence against natural enemies because nest-guarding tasks can be divided among multiple individuals (Thorp, 1969). Conversely, aggregations might attract larger numbers of parasites. Parasites may aggregate in response to high host densities, an effect that has been noted with parasitoid wasps (Rosenheim *et al.*, 1989; Matsumoto *et al.*, 2004). Higher nesting densities may also favour parasite persistence in these areas, and persistently high host densities may allow parasite populations to build up over multiple seasons (Rosenheim *et al.*, 1989).

Mason bees (*Osmia* spp.; Hymenoptera: Megachilidae) are important pollinators in natural and agricultural systems (Bosch & Kemp, 1999; Ward *et al.*, 2010; Schindler & Peters, 2011). They are effective pollinators for multiple crops, including almond, pome, and stone fruit (Ward *et al.*, 2010; Schindler & Peters, 2011). Mason bees will readily nest in artificial housing (Kemp & Bosch, 2001; Taki *et al.*, 2004; Gruber *et al.*, 2011), including cardboard tubes, reeds, and paper straws, that enable them to be transported and cultivated in agricultural settings. Mason bees, similar to other solitary bees, are attacked by a variety of brood parasites, often other Hymenoptera or Diptera (Torchio, 1979; Wcislo, 1984; Rosenheim *et al.*, 1989). These parasites are typically either cleptoparasites, which oviposit within bee nests, their larvae consuming both the host bee's food provision and the host egg or larva (Torchio, 1979; Danforth & Visscher, 1993), or parasitoids, which lay eggs within or adjacent to bee larvae or eggs, and whose larvae develop and consume the bee larva as a host (Rosenheim *et al.*, 1989). Parasites also have varying strategies for accessing bee nests: some oviposit only in exposed (incomplete or abandoned) brood cells (Torchio, 1979; Danforth & Visscher, 1993), as they lack an ability to penetrate brood cell walls; whereas others penetrate completed brood cells by destroying or piercing cell walls using varying strategies such as long ovipositors (MacIvor & Salehi, 2014) or digging through cell walls with specialised front legs (Ordway, 1964). Factors thought to affect parasitism rates in megachilids include nest architecture (Munster-Swendsen & Calabuig, 2000), resource availability (Goodell, 2003), foraging efficiency, age of the mother bee (Seidelmann, 2006), and choice of nesting material (MacIvor & Salehi, 2014); however, the influence of population density on parasitism has received little attention.

We conducted an observational field study in 2014 with artificial nesting blocks specifically to assess the effects of nesting aggregation in *Osmia* bees on brood parasitism by cleptoparasites. We also used a separate dataset, collected for other purposes in the same study region in 2013–2015 (Forrest & Chisholm, 2017), to determine if the relationships observed in the 2014 dataset held over a broader spatial and temporal extent. As the most common mason bee species in our study area (*Osmia iridis* Cockerell and Titus, 1902, *Osmia tersula* Cockerell, 1912, and *Osmia tristella* Cockerell, 1897) do not show overt aggression towards intruders, we expected these bees not to benefit from shared nest defence in larger aggregations. We also suspected that parasite confusion at high host densities (an effect of aggregation sometimes observed in mobile animals (Jeschke & Tollrian, 2007)) would be unlikely, due to the immobility of mason bee nests. We expected instead that larger aggregations of nesting bees would provide brood parasites with more easily located targets for oviposition, leading to disproportionately higher levels of brood parasitism in areas with larger numbers of nesting bees. Furthermore, the most abundant cleptoparasites in our study area (*Sapyga* spp.; Hymenoptera: Sapygidae) typically oviposit in incomplete bee nests and can penetrate only recently completed brood cell walls (Goodell 2003; J. Forrest, pers. obs.), probably making responses to bee activity important to the success of these parasites. In addition, to determine whether the mason bees in this study naturally formed nesting aggregations, we assessed the first nesting choices of all mother bees. We used these data to test whether bees chose to construct nests in nesting blocks occupied by other conspecifics more or less than expected by chance.

Materials and methods

Study species

Osmia spp. in subalpine Colorado, where we conducted our study, emerge between late May and early July and live as adults for 3–4 weeks. Females of several species construct nests in existing holes in dead wood (Michener, 2000). Nests consist of a linear series of individual brood cells, each containing a single egg and a food provision of pollen and nectar, separated by walls of mud or masticated leaf material. Nests are normally constructed with an empty vestibular cell in front of the brood cells (Seidelmann, 1999), followed by a thick outer nest seal that limits access to completed nests by natural enemies. The latter is added when a mother bee terminates a nest (Tepedino *et al.* 1979; Seidelmann, 1999). Female mason bees only construct one nest at a time, but they may construct multiple nests sequentially in their lifetimes. Once nest cells are closed, there is no contact between mother bees and offspring. Species of wood-nesting *Osmia* recorded at our study locations include *O. coloradensis*, *O. iridis*, *O. montana*, *O. subaustralis, O.*

tersula, and *O. tristella*, although only *O. iridis*, *O. tersula*, and *O. tristella* are attacked by sapygid wasps, the most common parasites in our study area (Spear *et al.*, 2016).

Sapygid wasps (Hymenoptera: Sapygidae) are brood parasites of mason bees. Sapygid females enter mason bee nests after female bees have finished provisioning a cell and have laid an egg. Sapygids oviposit into newly completed cells either before the cell wall is completed or while the wall is still malleable, in which case they oviposit through the wall (Goodell 2003; J. Forrest, pers. obs.). The sapygid egg hatches before the mason bee egg, and the larva consumes the unhatched bee egg and the food provision within the cell (Torchio, 1979). The only sapygid species recorded from *Osmia* nests at our study area is *Sapyga pumila* (Forrest & Thomson, 2011).

Study sites

The 2014 study was conducted at the Rocky Mountain Biological Laboratory in Colorado, U.S.A., from the beginning of the nesting period of mason bees until past the peak nesting period (30 May to 21 July). We selected three study sites, each separated by at least 200 m from the others, at the margins of aspen (*Populus tremuloides*) stands (Appendix S1). Sixteen nesting blocks (described in the following section) were placed at each site, approximately 0.5 m above the ground, each attached to an aspen trunk with metal strapping. Nesting blocks were positioned close to or on dead or dying aspens if possible, to be close to areas where mason bees would naturally nest. Each block was placed at least 15 m from the other blocks within the site.

Nesting blocks

Nesting blocks were constructed from sections of untreated pine using methods similar to Taki *et al.* (2004). Each block measured $15 \times 15 \times 10$ cm and had 16 6.4-mm-diameter holes drilled through lengthwise. Half the nest blocks were placed on aspen trunks in groups of two attached together ($15 \times 15 \times 20$ cm; 32 nest holes in total); the other half were placed on trunks individually to encourage diversity in nesting density. The front of each block was scorched with a blowtorch. The blocks were treated with water-based polyurethane and the back was covered in foil tape. The sides adjacent to the nest entrances were covered in burlap. A 4.8-mm-diameter paper straw was inserted into each hole to allow removal and observation of bee nests (Appendix S2). Nesting blocks were not seeded with mason bee pupae before they were placed in the field; all occupancy stemmed from naturally occurring bees colonising the blocks.

Assessing parasitism rates

All nesting blocks were checked for the presence and progress of mason bee nests by removing the paper straws from the nests and observing them at the maximum every 2 days. Nest occupants other than bees were removed. Locations of nests within blocks, numbers of cells constructed, and dates of nest start and completion were recorded. Because of the frequent monitoring, we could estimate with high confidence the day on which each nest cell was constructed. Nesting female bees were marked on the dorsal side of the mesosoma with enamel paint (Testors, Vernon, IL, U.S.A.) of differing colours in order to track any individuals that constructed more than one nest within the blocks (bees were immobilised without harm between pieces of screen and foam within a plastic vial to mark them). To assess whether nest cells had been parasitised, straws were removed from nesting holes during observations, and a small window was cut into each completed nest cell without contacting the cell contents (food provision and egg). Each nest cell was checked for the presence of a parasite larva or egg (Forrest & Chisholm, 2017). Straws were repaired with tape after examination and returned to their nest holes (mother bees were typically away foraging or sitting at the front of the nest during these observations so disruption to their behaviour was minimal). Straws containing completed (sealed) nests were collected at the end of the summer and placed in incubators to allow bee development to adulthood and subsequent species identification.

Naturally established variation in nest density was used to assess the effects of number of nearby active bees on rates of parasitism. When nest cells were checked for the presence of sapygid eggs, we recorded the number of in-progress *Osmia* nests present within the nesting block on the estimated day of cell construction (i.e. including species known not to be vulnerable to sapygid parasitism; later analyses were conducted both with and without these species). We conducted behavioural observations of bees and wasps to ascertain the mechanisms for any variation in brood parasitism rates; however, only two sapygid incursions were observed at nesting blocks during these observations, so these data were not analysed.

We also analysed data on *Osmia* brood parasitism rates from nests at eight additional sites (Appendix S1) near the Rocky Mountain Biological Laboratory between 2013 and 2015. These data were collected using similar methodologies to the 2014 field experiment, except that blocks were visited only twice per week throughout the *Osmia* nesting season (early June to early August; Forrest & Chisholm, 2017). Blocks were similar to those described earlier, except that each block contained only 10 holes, and these holes varied in diameter (4×6.4 mm, 4×7.9 mm, 2×9.5 mm). The 14–24 blocks at each site were located 0.5–10 m from one another.

Analysis

2014 study. We used generalised linear mixed-effect models with binomial error distributions to analyse nest-cell parasitism as a function of the number of concurrently active nests. We used the lme4 package (Bates *et al.*, 2015) in R (R Core Team, 2015). Individual nest cells were the replicates in these models, and whether the cell was parasitised or not was the dependent variable. Nest cells constructed by species not vulnerable to sapygids (*O. montana*, *O. subaustralis* and *O. coloradensis*) were excluded from the analyses, as were those constructed before the first instance of brood parasitism in the season (27

June for the 2014 data). Number of active *Osmia* nests within a block on the date of cell construction was the primary fixed factor of interest. We included four additional fixed factors as covariates in the model: site location (a blocking variable with only three levels), the day of year of cell construction ('date'), number of occupied nest holes within a block (both currently occupied and previously occupied), and whether or not a cell was the outermost cell in its nest (hereafter 'cell position'). Nest identity was included as a random effect in the models. Mother bee was not included as an additional random factor, as it did not improve model fit, and mothers were unknown for 25% of nests in the combined dataset (described later). We calculated variance inflation factors (VIFs) with the R package USDM (Naimi, 2015) to ensure there was not excessive collinearity among predictor variables (i.e. that VIF values were < 2). Significance of fixed effects was assessed using likelihood ratios of models with and without the factor of interest. We did not include host species as an explanatory factor because 4.8% of brood cells could not be assigned to a species (because offspring failed to emerge). However, we repeated the analysis excluding 16 cells that could not be identified to species, and included species (as a fixed effect with three levels) in this model. The latter analysis yielded identical conclusions about the main factors of interest (Appendix S3). We also analysed the data at the level of whole nests, asking what factors predicted whether a nest was parasitised at least once. The maximum number of concurrently active bees within a block during construction of the focal nest, life span of the nest (defined as the number of days a mother bee provisioned a nest), site, number of occupied nests (current and previous) at the start of nest construction, and date on which nest construction began (date) were included as fixed predictors; nest block identity was included as a random effect. Similar to the cell-level analyses, we excluded nests that were completed prior to the first instance of parasitism.

Combined dataset. The 2013–2015 dataset was added to the 2014 data for a global analysis, again excluding cells from non-vulnerable species and from before parasites became active in a given year. This yielded a total of 1760 nest cells. For the cell-level analysis, number of active bees, cell position, day of year of construction (date), number of occupied nest holes (current and previous), and year of construction were included as fixed effects, with site and nest identity as random effects. For the nest-level analysis, which was conducted on 440 nests, maximum number of active bees, life span of nest, year, number of occupied nest holes (current and previous) at nest start date, and nest starting date were included as fixed effects, with site and nest-block identity as random effects.

To further test the robustness of our findings, we repeated the analyses of the combined cell-level dataset: (i) with species included as a factor, and unidentifiable bees excluded; (ii) only considering parasitism by sapygids (other parasites excluded); (iii) excluding the 2014 study data; (iv) including data from only *O. iridis*, the most abundant species in the dataset; and (v) only counting nests of vulnerable *Osmia* species in the numbers of concurrently active nests (i.e. ignoring any concurrently nesting *O. coloradensis*, *O. montana*, and *O. subaustralis*). We also conducted additional analyses with (vi) number of unoccupied nest holes within a block (i.e. holes without current or previous bee activity from any species, at the time of nest or cell initiation) added as a fixed factor for both the cell and nest levels to determine if the number of holes per block influenced the likelihood of parasitism. Because our conclusions were unchanged by these modifications and the added fixed factors were not significant predictors of parasitism, we report them only in Appendix S3.

Nesting preferences. To determine whether bees preferentially chose to nest in blocks that were concurrently occupied by other mason bees, we used data on nest selection from the 2014 study and the separate 2013–2015 dataset, combined. We tabulated the number of available nesting holes within nesting blocks at a site that were occupied or unoccupied by mason bees, for each occasion that an individual bee began to nest in a particular block. Nest holes unoccupied by mason bees were considered available for occupancy. (Other nesting bee and wasp taxa constituted only 5.1% of observed occupancy in all nesting sites and tended to be active later in the season than *Osmia* spp.; i.e. after most mason bee nesting choices had been made.) From this, the likelihood of each bee's observed choice of nesting hole (either within an occupied block or in an unoccupied block) was calculated. For example, imagine a site with 14 nesting blocks, each containing 10 holes. If an individual bee constructed a nest in a block occupied by another bee, while two total blocks at the site were occupied by one mason bee each, the likelihood of its specific choice (an occupied block) would be as follows:

$$\frac{2 \text{ blocks} \times 9 \text{ holes per block}}{\begin{array}{c}(2 \text{ blocks} \times 9 \text{ holes per block}) \\ + (12 \text{ blocks} \times 10 \text{ holes per block})\end{array}} = 0.13$$

We assumed that subsequent nests constructed in the same block did not represent independent choices; these were not considered. The likelihoods for all bees were then combined using Fisher's method to generate a χ^2-value with 702 degrees of freedom (2×351 observations of bee first choices). This analysis was repeated for *O. iridis* only (190 first choices), and counting only the very first nest choice for each individual bee (excluding nests from unknown mothers; 194 first choices).

Results

Nesting statistics

A total of 78 mason bee nests comprising 330 individual nest cells were constructed within the three sites in the 2014 study, 304 of which (within 69 nests) were constructed after the first instance of brood parasitism of bee species known to be vulnerable to brood parasitism. Of these 304 cells included in the analysis, 30 were parasitised by sapygids; no other parasites were detected. *Osmia iridis* was the most common host species (73.0% of nests). Other occupants were *O. tersula* (14.8% of nests), *O. tristella* (4.2%), and *O. montana* (3.2%).

Table 1. Results of models of brood parasitism for individual nest cells from sites used in the 2014 study ($n = 304$ cells) (a) and the combined dataset ($n = 1760$ cells) (b). Models were generalised linear mixed-effect models with binomial error, and significance of each term was assessed using likelihood-ratio tests of nested models. Variables in the models: (a) 2014 data: number of active bees, whether a cell was the last in its nest, day of year of construction (Date), site, number of occupied nests, and nest identity; and (b) combined data: number of active bees, whether a cell was the last in its nest, date of construction, year of construction, number of occupied nests, site, and nest identity.

Factor†	Coefficient‡	d.f.	χ^2	Pr(> t)*
(a) 2014 dataset				
Active bees	0.852	1	13.18	**0.0003**
Last cell	1.019	1	4.93	**0.0262**
Date	0.269	1	0.93	0.3338
Site	1.388	1	5.31	0.067
Occupied nests	0.135	1	1.29	0.2543
Nest ID	0.559	–	–	–
(b) Combined data				
Active bees	0.412	1	9.60	**0.0019**
Last cell	0.699	1	16.16	**5.8 e^{-5}**
Date	−0.194	1	1.60	0.2054
Year	−0.298	1	0.74	0.6904
Occupied nests	0.236	1	0.13	0.7153
Site	0.944	–	–	–
Nest ID	2.08	–	–	–

*P-values in bold denote significance ($P < 0.05$).
†Italics denote random factors.
‡Variance was used as the coefficient for random factors.

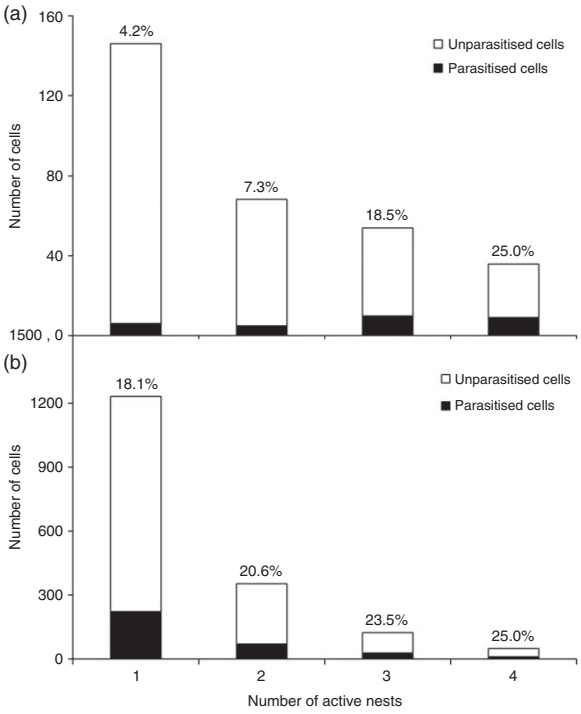

Fig. 1. Numbers of parasitised and unparasitised *Osmia* nest cells in the 2014 study ($n = 304$ cells) (a) and the combined dataset ($n = 1760$ cells) (b) versus the number of active bees within a nesting block during construction of each cell. Percentage of parasitised cells in each condition is included above each column.

For the 2013–2015 dataset (excluding the data from the 2014 study, described earlier), 518 bee nests comprising 2013 individual nest cells were constructed within the eight sites, 1456 of which (within 371 nests) were constructed after the first instance of brood parasitism by vulnerable species; these cells were included in analysis. In all, 307 of these cells were parasitised (96.4% by sapygids; other parasites included chrysidid and pteromalid wasps). *Osmia iridis* was again the most common occupant (42.6% of nests). Other occupants were *O. tersula* (3.9%), *O. tristella* (12.2%), *O. coloradensis* (13.7%), *O. montana* (5.7%), and *O. subaustralis* (4.1%).

Predictors of parasitism

Up to four bees were observed constructing nests within a block at one time. Nest cells constructed with one bee present at the block (the mother of that nest) constituted the largest proportion of nest cells constructed in both the 2014 study and the combined dataset (Fig. 1). As the number of active bees at a block increased, the proportion of nest cells parasitised increased, with the effect being more pronounced in the 2014 study (Table 1, Fig. 1). In addition, the outermost cells of nests were significantly more likely to be parasitised than other cells (Table 1, Fig. 2), with 20.0% of outermost cells being parasitised, compared with only 6.8% of other cells, in the 2014 data; and 27.1% of outermost cells parasitised, compared with 16.6% of other cells, in the combined dataset.

These two factors (number of active bees and cell position) remained significant predictors of parasitism in all alternative analyses of the data (2014 data only with no unknown species; combined data including only vulnerable species in the number of concurrently active bees; combined data with no unknown species; combined data with sapygid parasitism only; combined data with *O. iridis* only; and combined data with the 2014 study excluded; Appendix S3). The combined data excluding the 2014 study differed slightly from the other datasets in the relationship between parasitism and number of active nesting bees, in that the three-active-nest condition had the highest proportion of cells parasitised (active nests: 1, 20% parasitised; 2, 24%; 3, 28%; 4, 25%). When we considered only vulnerable bee species as contributing to the number of concurrently active bees, we observed little variation in parasitism rates except for an increase in the four-active-nest condition (active nests: 1, 19% parasitised; 2, 20%; 3, 19%; 4, 24%). Nevertheless, the significant positive coefficient for number of concurrently active nests was consistent across all analyses. None of the other fixed factors considered in the models for any analyses (site, date, species identity, number of occupied or unoccupied nest holes within a block) was a significant predictor of cell parasitism in either the 2014 or combined study data (Table 1).

For the analysis conducted at the level of whole nests, none of the included factors was a significant predictor of parasitism for the 2014 data (Table 2). Life span of the nest and nest start date were significant predictors of parasitism in the combined

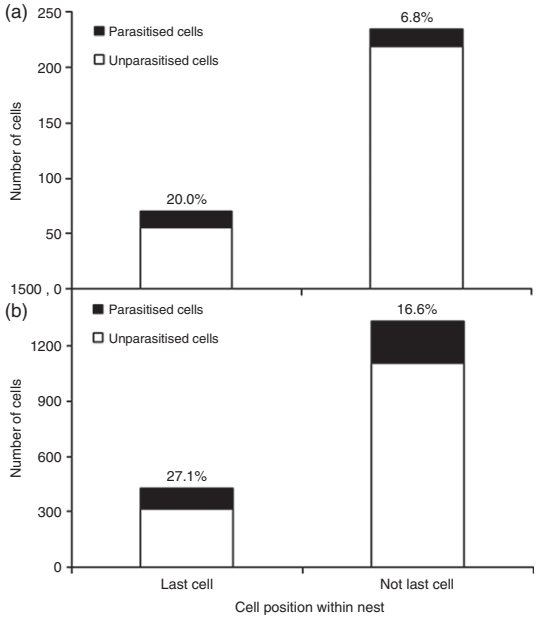

Fig. 2. Numbers of parasitised and unparasitised nest cells in the 2014 study ($n = 304$ cells) (a) and the combined dataset ($n = 1760$ cells) (b) as a function of cell location within the nest [whether the last (outermost) cell or not]. Percentage of parasitised cells in each condition is included above each column.

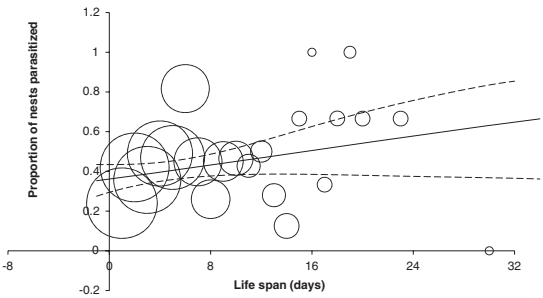

Fig. 3. Proportion of nests parasitised in the combined dataset versus the life span (in days) of the nest ($n = 440$ nests). The size of each data point corresponds to the number of individual nests constructed with a particular life span (number of nests represented by a point ranges from 1 to 30). Binomial line of best fit (McFadden's pseudo-$R^2 = 0.079$) and 95% confidence intervals are presented.

dataset (Table 2): unsurprisingly, nests with longer life spans had a greater probability of being parasitised at least once (Fig. 3), and nests initiated later in the season had a smaller probability of parasitism (Fig. 4). However, these factors explained little of the variation in parasitism rates (life span, McFadden's pseudo-$R^2 = 0.079$; start date, pseudo-$R^2 = 0.034$).

Nesting preferences

On their first choice of a nesting hole at a block, a slightly larger proportion of vulnerable bees chose unoccupied nest blocks than expected by chance (81.9% of bees chose nest holes

Table 2. Results of models of brood parasitism for individual nests from sites used in the 2014 study ($n = 69$ nests) (a) and the combined dataset ($n = 440$ nests) (b). Models were generalised linear mixed-effect models with binomial error, and significance of each term was assessed using likelihood-ratio tests of nested models. Variables in the models: (a) 2014 data: maximum number of active bees, life span of nest, start date of nest, site, and nest block; and (b) combined data: maximum number of active bees, life span of nest, start date of nest, year, site, and nest block.

Factor†	Coefficient‡	d.f.	χ^2	Pr(>t)*
(a) 2014 data				
Max. active bees	0.085	1	0.075	0.7834
Life span	0.137	1	1.094	0.2011
Start date	−1.632	1	4.402	0.1109
Site	−0.223	1	0.438	0.5079
Nest block	0.211	–	–	–
(b) Combined data				
Max. active bees	0.216	1	2.660	0.1029
Life span	0.625	1	11.636	**0.0006**
Start date	−0.357	1	6.872	**0.0087**
Year	−0.207	1	0.507	0.7760
Site	0.521	–	–	–
Nest block	0.721	–	–	–

*P-values in bold denote significance ($P < 0.05$).
†Italics denote random factors.
‡Variance was used as the coefficient for random factors.

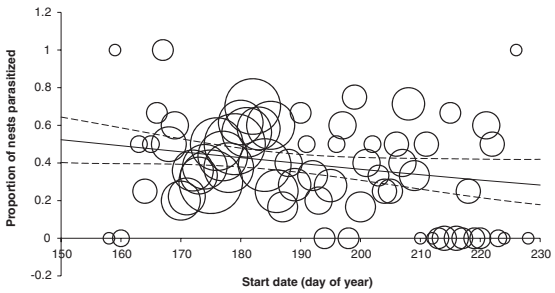

Fig. 4. Proportion of nests parasitised in the combined dataset versus the day of year on which the nest was initiated ($n = 440$ nests). The size of each data point corresponds to the number of individual nests started on a particular date (number of nests represented by a point ranges from 1 to 26). Binomial line of best fit (McFadden's pseudo-$R^2 = 0.034$) and 95% confidence intervals are presented.

in unoccupied blocks as their first choice compared with an expected 77.1%); however, the difference from expected values was not significant (Fisher's method, $\chi^2 = 309$, d.f. = 702, $P = 1$). This pattern varied little and was non-significant for the alternative analyses of nest choices, i.e. (i) *O. iridis* only: 74.9% chose unoccupied nests vs. 78.3% expected; (ii) very first nest choices only: 84.3% unoccupied nests vs. 86.6% expected.

Discussion

The goal of this study was to elucidate the effects of nesting aggregation on vulnerability of mason bees to brood parasitism. Overall, we found that higher natural mason bee nesting

densities were associated with increased brood parasitism in both an initial study and a separate 3-year dataset. We also found that the last cell within a mason bee nest was significantly more likely to be parasitised than were inner nest cells, suggesting that bees may terminate nests after brood parasite attack. We found no evidence that mason bees avoid or select nesting locations near other nesting bees. Mason bee nests with shorter life spans and those initiated later in the season were less likely to be parasitised.

Predictors of parasitism

We observed a strong positive association between bee nest density and brood parasitism (primarily by sapygid wasps), with the number of concurrently active *Osmia* nests significantly predicting brood-cell parasitism in all analyses. Furthermore, vulnerability to parasites increased quickly with increasing host density: brood parasitism rates rose from 18% in otherwise unoccupied nest blocks (in the combined dataset) to 25% at the maximum host-bee density we observed (four active bees per nesting block). However, in contrast to the cell-level analysis, the maximum number of concurrently active nests within a block over the course of a single nest's construction was not a significant predictor of parasitism (Table 2). These results suggest that sapygid wasps respond to host-bee aggregations on a short-term basis, perhaps using actively nesting individuals as indicators of opportunities to oviposit and aggregating in these locations during the day (cf. Rosenheim *et al.*, 1989). This result, along with the finding that overall nest activity (number of occupied or previously occupied holes within a block) did not influence likelihood of brood parasitism at either the cell or nest level, makes other proposed mechanisms of positive density-dependent parasitism, such as locating and continuing to exploit previously successful locations (Rosenheim *et al.*, 1989; Rosenheim, 1990), seem less likely. Still, to rigorously assess this assertion, behavioural observations of sapygids would have to be conducted over multiple days. If individual sapygids respond to bee density on a day-to-day basis, rather than returning preferentially to nesting blocks where they had previously detected high bee activity, short-term responses to host density would be supported. Interestingly, the relationship between number of active bees and parasitism rate was stronger when we included all *Osmia* species in the active bee count, as opposed to only the species known to be vulnerable to sapygid parasitism. This suggests that the parasites respond in a generalised way to mason bee numbers, irrespective of whether those bees are actually suitable hosts. Parasites may be unable to recognise vulnerable nests – or host species – from a distance.

Our results also suggest a behavioural response by host bees to parasite attack. Cells that were the last to be constructed in their respective nests (i.e. cells that were outermost) were significantly more likely than inner (earlier-constructed) cells to be parasitised (Table 1, Fig. 2) – despite the fact that parasitism rates generally decreased over the course of the season (Table 2). It is important to note that parasitised nests were virtually always still completed by mother bees, having the final nest seal constructed at the entrance to the nests; thus, outer cells were not simply more exposed and therefore vulnerable to parasitism than inner cells. This finding probably reflects a tendency for bees to terminate nests once they detect parasite intrusion (ceasing construction of additional cells within the nest, while still constructing the outer nest seal), such that a parasitised cell is more likely than an unparasitised cell to become the last cell in a nest.

Terminating nests that have been parasitised could represent a defensive response of bees to brood parasitism. Continuing to construct a nest that has been parasitised and is vulnerable to subsequent parasitism could be more costly than initiating a new nest elsewhere. Nest abandonment is a strategy that has been observed in both vertebrates and invertebrates for avoiding continued costs from brood parasitism (Polidori & Andrietti, 2006; Guigueno & Sealy, 2011). If mason bees do abandon their nests more readily in response to brood parasitism, it suggests that they have the ability to recognise oviposition by parasites, by either olfactory or visual cues. This possibility remains to be tested experimentally.

At the entire nest level, nests that were active for longer were more vulnerable to being parasitised, with nest life span a significant (though weak) predictor of parasitism in the combined dataset. This result suggests that mason bee nests being open and vulnerable to parasitism (with new cells being constructed, and before construction of the outer seal) for longer increases the likelihood that a parasite will find and oviposit in the nest at least once. This finding also corresponds well with observations in other bee species, in which older individuals that complete their nests more slowly are more vulnerable to parasitism (Danforth & Visscher, 1993; Seidelmann, 2006).

In our study, nests constructed later in the season were less likely to be parasitised, with start date being a weak but significant predictor of parasitism (again, only in the combined data). The mechanism for this effect cannot be confirmed, due to a lack of independent information on sapygid phenology, but it suggests that mason bees may have a partial phenological escape from parasitism. Similar patterns have been observed in other Hymenoptera (Godfray *et al.*, 1994; Strohm *et al.*, 2001), with hosts nesting late in the season avoiding the active period for the majority of brood parasites. Late activity probably comes with other costs, however – most notably a shorter season for brood provisioning and offspring development. It should also be noted that our analyses excluded the earliest mason bee nests (those constructed before the appearance of parasites) for a more conservative test of the influence of nesting density. Thus, an early escape from brood parasitism may also be possible in our study system, although it could not be detected in our analyses.

We found no other significant predictors of sapygid parasitism. Risk of parasitism did not differ significantly between years in the combined data or between the mason bee species known to be vulnerable to parasitism. We did not investigate attributes of the nest location, such as insolation or local vegetation characteristics, which might have explained some of the remaining variation in parasitism risk; however, most sites in our study were quite similar in habitat, selected to maximise occupancy of bees. Among-habitat variation in parasitism risk has been observed in other groups, including great reed warblers (*Acrocephalus arundinaceus*) parasitised by cuckoos (Moskat & Honza, 2000) and

caterpillars parasitised by *Trichogramma* wasps (Romeis *et al.*, 2005). To our knowledge, however, there is little information available on which, if any, abiotic variables are associated with levels of parasitism in bee populations.

Do mason bees avoid forming aggregations?

Mason bees (including *O. iridis*) showed no preference with regard to constructing their first nests in blocks that contained other mason bees or not. We did not observe any behaviour of individual mason bees that either promoted or prevented the formation of nesting aggregations. These results are in contrast to the observed tendency of another mason bee species (*O. lignaria*) to nest gregariously (Torchio, 1984). However, our study was conducted in a natural setting where other nesting locations were presumably available, unlike the agricultural setting of the previous study (Torchio, 1984). Furthermore, the observational nature of our study leaves open the possibility that uncontrolled variation in nest-block quality (as perceived by the bees) could have obscured a tendency for mason bees to avoid nesting near other bees (if nest-block quality were uniform).

Nevertheless, our results suggest that *Osmia* bees lack behaviours to avoid nesting near one another, despite the potential risks of increased parasitism – perhaps because selection to avoid aggregations in natural settings is weak. As most brood cells in our study were constructed when there was only one bee present (the mother), most bees were not at high risk of density-related parasitism. It is possible that bee nesting densities naturally tend to be low, so there is no need for active avoidance of aggregation. An alternative explanation is that if nesting sites are highly limiting in nature, then bees normally have no choice but to nest in high densities. Regardless, our results suggest that mason bees are likely to be vulnerable to parasitism if nesting sites are limited or if populations are large, which could be the case in managed populations in agricultural settings.

Implications

One of the potential benefits of animals developing sociality is increased defence against natural enemies, the presence of multiple individuals in a location deterring predators or parasites, or increasing the effectiveness of defence against them (Rosenheim, 1990). However, the formation of (small) nest aggregations in mason bees clearly does not provide this benefit, at least with respect to sapygid parasitism in this natural setting. This study suggests that natural enemies would be unlikely to drive selection for social behaviour in mason bees.

Positive density-dependent brood parasitism also suggests the possibility of bee population regulation by top-down control. If nesting locations are limited and patchily distributed, large populations of mason bees would inevitably form aggregations, which would increase parasite attack and consequently decrease population growth. As brood parasites can significantly reduce the reproductive output of bees (Seidelmann, 2006; Forrest & Chisholm, 2017), the patterns of brood parasitism observed in this study suggest that parasites could have a limiting effect on mason bee populations.

Given the significance of mason bees as pollinators and as managed agricultural species (Ward *et al.*, 2010; Fliszkiewicz *et al.*, 2011; Gruber *et al.*, 2011; Schindler & Peters, 2011; Jauker *et al.*, 2012), understanding factors regulating their populations is important. The high levels of parasitism we observed even in response to small nesting aggregations represent a potential problem if small numbers of large, artificial nest structures are used for cultivating mason bees in agriculture. In such conditions, mason bees are likely to be vulnerable to exploitation if parasites are present (Torchio, 1979; Kemp & Bosch, 2001). The increased vulnerability of high-density mason bee populations to parasitism suggests that an overhaul of artificial bee nesting structures may be beneficial. The use of multiple smaller nesting blocks in agriculture should help to avoid high levels of brood parasitism in areas where sapygid parasitism is a risk. Our findings support recent suggestions that artificial 'bee hotels' may not in fact benefit native bee populations (MacIvor & Packer, 2015). High parasitism rates have been observed in these 'hotels' (MacIvor & Packer, 2015), perhaps because of the high nesting densities they encourage. Our results are also in accordance with the finding that *O. lignaria* retain nests for longer when they are provided many small nest boxes rather than a few large ones (Artz *et al.*, 2013). Finally, our results reinforce the benefits of supplementing artificial nesting structures with natural nesting habitat (e.g. woody hedgerows) if the goal is to support bee populations (Williams & Kremen, 2007; Morandin & Kremen, 2013), as abundant nesting habitat should diminish the likelihood of aggregations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12484

Appendix S1. Coordinates of study sites.

Appendix S2. Images of nesting blocks from study sites.

Appendix S3. Results of supplementary analyses.

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