

**Impacts of brood parasites, floral abundance, and bee age on
maternal investment in a solitary bee, *Osmia iridis***

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Abstract:

Parents can adjust both the size and number of offspring they produce in order to maximize their own fitness payoff (Smith & Fretwell 1974). Optimal parental investment is dependent in part on likelihood of offspring survival, which is impacted by predation and parasitism. When risk of predation or parasitism is high, it may be adaptive for parents to reduce either the number of offspring they produce or their investment in individual offspring. Brood parasitism by sapygid wasps represents a significant fitness cost for *Osmia* bees. Sapygid wasps enter brood cells during the provisioning or brood cell construction stages, when mother bees are absent collecting pollen and nectar provisions for their larvae or materials for constructing brood cell walls. In order to avoid losing their investment in offspring, *Osmia* bees should reduce provisioning time when parasitism risk is high, providing pollen provisions smaller than what would be optimal for offspring development. Alternatively, instead of investing less per offspring, bees may produce fewer offspring overall when they detect parasites. In this study, we tested these predictions in a naturally occurring population of *Osmia iridis* bees at the Rocky Mountain Biological Laboratory in Gothic, Colorado, measuring the response of *O. iridis* to both spatial and temporal variation in sapygid wasp abundance. In addition, we measured other factors which might impact provisioning efficiency, including floral abundance and bee age, and uniquely marked individuals to control for bee identity. Floral abundance was found to be most predictive of pollen provision size, while *Sapyga* abundance was most predictive of the number of brood cells per nest a bee constructed. These results suggest that bees respond to parasite presence by reducing the number of offspring they produce per nest rather than investing less per offspring. Further research is needed to confirm these findings and to investigate the impacts this investment strategy may have on both lifetime reproductive success and nesting site selection.

Introduction:

Across a broad range of taxa, parents provision resources for their offspring. Optimal Allocation Theory predicts that parents should allocate investment amongst their progeny according to an optimum at which their own (i.e., the parents') fitness is maximized (Smith & Fretwell 1974). However, if risk of predation or parasitism are positively correlated with time spent provisioning offspring, this optimum level of investment may be reduced as parents risk losing either their lives or their investment in progeny the longer they spend procuring resources. These predations have been most studied in birds, which lay smaller clutches when exposed to high predation risk (Doligez and Clobert 2003; Eggers et al. 2006; Zanette et al. 2011; Noreikiene et al. 2021). Among invertebrates, female shield bugs (*Elasmucha ferrugata*) were found to allocate less resources to offspring exposed to higher predation risk. Mothers shield the eggs with their body to protect the brood from predators, resulting in higher rates of predation on eggs located in the periphery of the nest. As a result, *E. ferrugata* produces larger eggs at the center of the nest, since those will have a greater likelihood of survival (Mappes, Mappes, & Lappalainen 1997).

This trade-off between parental care and predation or parasitism risk may be especially pronounced in *Osmia*, a group of solitary bees which provision their offspring prior to hatching with all of the resources required for development. *Osmia* construct nests consisting of a linear series of brood cells in existing gaps and cavities, provisioning each brood cell with loaves of pollen and nectar. Since discrete amounts are provisioned for each larva and parental care ends once a brood cell is sealed, pollen provision size can act as a proxy for maternal investment. However, creating larger provisions requires longer provisioning trips, potentially leaving the brood cells vulnerable to parasitism by brood parasites such as *Sapyga* wasps, which lay eggs in

Osmia brood cells. The wasp larva consumes both the bee larva and the food provision. The longer a brood cell remains open and unguarded during provisioning trips, the greater the probability that a cell will be parasitized (Brockmann & Grafen 1992; Danforth & Visscher 1993; Goodell 2003). Since *Osmia* larvae inhabiting parasitized brood cells typically die, female *Osmia* should be expected to minimize parasitism risk by reducing provisioning time when parasitism risk is high, either by sealing cells before they contain what would otherwise be the optimal amount of pollen for offspring development or by reducing the number of brood cells they produce per nest.

In addition to parasitism risk, factors such as floral resource availability, bee age, and bee size can impact provisioning time. When exposed to low floral resource environments, *Osmia cornuta* provision their broods with smaller amounts of pollen, resulting in a male-biased sex ratio (since male offspring are smaller and therefore require smaller pollen provisions than female offspring) and a reduction in offspring body size (Bosch 2008). Presumably, bees are unable to compensate for low resource availability by extending provisioning time due to the greater risk of parasitism this would entail. Similarly, provisioning efficiency declines as bees age; over the course of the nesting season, female *Osmia rufa* reduce provisioning time (and, in turn, the body size of offspring) while shifting the sex ratio of their brood towards males (Seidelmann 2006). Finally, large bees are able to collect larger provisions in a shorter amount of time compared to small bees; to “make the best of a bad lot,” small *O. rufa* females will shift the sex ratio of their brood towards males while larger females with greater provisioning efficiency will produce more females, the sex for which fitness is more dependent upon body size (Seidelmann 2010).

In this study, we investigated whether parasitism risk impacts 1) how much a bee provisions individual offspring and 2) how many brood cells per nest a bee constructs in naturally occurring population of *Osmia iridis* (Hymenoptera: Megachilidae), a semivoltine species of solitary bee. When risk of parasitism by *Sapyga* is high, we hypothesize *O. iridis* will reduce investment in offspring by creating smaller pollen provisions or by creating fewer brood cells per nest. We utilized both temporal (across the season) and spatial (between site) variation in *Sapyga* abundance to examine the impacts on *Osmia* maternal investment.

Methods:

Study species

Wood-nesting *Osmia* construct their nests in existing holes in dead wood. Nests consist of a linear series of brood cells containing pollen and nectar provisions, each flanked by walls of masticated plant matter or mud. The outermost cell of a nest (the vestibular cell) is typically empty, acting as a buffer to protect the brood from natural enemies (Seidelmann 1999). Once the nest is sealed, parental care by *Osmia* females is complete. Some *Osmia* species are parasitized by sapygid wasps (Hymenoptera: Sapygidae), which lay their eggs in the provisioned brood cells prior to wall construction or when newly constructed walls are still pliable. When the wasp egg hatches, it consumes both the bee egg and the provision. At our sites in subalpine Colorado, at least three species of wood-nesting *Osmia* which are also parasitized by sapygid wasps occur: *O. iridis*, *O. tersula*, and *O. tristella*. Two morphologically distinct *Sapyga* species occur at our sites, and one of these is a specialist on *Osmia*; however, the identity of the species is unknown. *O. iridis* was identified in the field by Dr. Jessica Forrest based on adult morphology and nest construction. To confirm field identifications, pollen samples were taken from the first and last

brood cell of each nest and will be used to determine whether host plant use is consistent with that of *O. iridis*, a Fabaceae specialist.

Study sites

This study was conducted on the historical homeland of the Ute peoples at what is now the Rocky Mountain Biological Laboratory in Colorado, U.S.A. Data were collected from five sites located at Kebler Pass, Kebler Clearing (Elk Creek, in the Coal Creek drainage), Rosy Point, Brush Creek, and Virginia Basin (Figure 1). Sites were visited twice per week.

Nesting blocks

Nesting blocks consisted of a block of softwood lumber containing 10-16 holes drilled approximately 14 cm deep and between 6.4 and 9.5 mm in diameter. Each hole was lined with a translucent paper straw which could be removed to inspect nest contents and progress. There were 12 nesting blocks at each site, affixed to or near dead trees where *Osmia* would naturally nest. Distance of nesting blocks from the ground was approximately 0.5 m.

***Sapyga* abundance and activity**

Sapyga abundance and activity was measured observationally and by counting *Sapyga* eggs within *O. iridis* nests. Each tree with a nesting block containing an *O. iridis* nest was observed for fifteen minutes whenever a site was visited, and the number of individual *Sapyga* wasps seen within one meter counted. To avoid counting the same individual wasps multiple times, a second wasp was only counted if both wasps were seen simultaneously or if wasps were observed at least one minute apart. Sites were visited between 10 AM and 4 PM twice per week when weather conditions were amenable to insect activity (<50% cloud cover and >16° C, per Forrest & Chisholm 2017). Each brood cell was dissected once the next brood cell in the sequence (or the entire nest) had been completed, and the presence of *Sapyga* eggs assessed. A small window into each brood cell was cut using a scalpel and microscissors, and the contents examined with a hand lens. The opening was taped closed after determining whether *Sapyga* eggs were present, and the straw was re-inserted into the nesting block. Number of *Sapyga* wasps seen per 15 minutes of observation was averaged across two visits to a site to approximate *Sapyga* abundance during the period in which any new brood cells were being constructed. Number of *Sapyga* eggs found in brood cells constructed between those two dates were also averaged.

***Osmia* provision size**

Provision size was found by measuring the length of the provision through the straw with calipers each time a new brood cell was constructed. Provisions were measured at the longest point. Pollen provision length and the diameter of the straw the bee was nesting in were used in a formula for the volume of a cylinder to find pollen provision volume. Provision volume was halved to account for the pooling of pollen at the bottom of the straw, which resulted in wedge-shaped pollen balls (Equation 1). If multiple brood cells were constructed between two visits to a site, the mean pollen provision volume was calculated.

$$V = \frac{\pi \left(\frac{d}{2}\right)^2 l}{2}$$

Equation 1. Pollen provision volume . d = straw diameter, l = length of pollen provision.

Floral resources

O. iridis is a pollen specialist on legumes (Fabaceae, tribe Fabeae), of which two species occur at our study sites: *Lathyrus leucanthus* and *Vicia americana*. *L. leucanthus* and *V. americana* flowers were counted in successive circles from a flag at the center of a site (as in Forrest & Chisholm 2017). A flag was planted once 100 of each of *O. iridis*' host plants were counted and the distance of the flag from the center of the circle measured in order to approximate floral density (Equation 2). Floral density was averaged across two visits to a site to obtain the mean floral density during the period in which any new brood cells had been constructed.

$$\text{Floral density} = \frac{100}{\pi * \text{distance}^2}$$

Equation 2. Floral density. Distance = distance from the center of the site to the point where 100 flowers were counted.

Bee identity and bee age

Nesting bees were captured, uniquely marked with paint, and released so that individual identity could be accounted for. The day a bee began nesting was recorded as day 0 to approximate bee age, assuming that bees likely emerged not long before they began nesting. If bees were encountered after they had already made progress on a nest, the number of brood cells already constructed was used to approximate age as *O. iridis* typically completes a maximum of two brood cells per day (personal communication, Jessica Forrest). As a conservative estimate, one day was subtracted from the current date for each completed brood cell.

Data analysis

Data were analyzed in Program R using the lmerTest package for performing tests in linear mixed effects models (R Core Team 2021; Kuznetsova, Brockhoff, and Christensen 2017). Mean pollen provision volume, mean floral density, and bee age were log transformed to normalize their distributions. To check for collinearity, linear regressions were performed between bee age and mean floral density and *Sapyga* seen per 15 minutes of observation and mean floral density.

A mixed linear model was created to evaluate the relationship between mean pollen provision volume and mean *Sapyga* seen per 15 minutes across each observation period in which brood cells were being constructed (two consecutive dates when a site was visited), accounting for variation in floral resource availability and bee age. Mother bee and site identity were included as random factors.

A separate linear model was used to examine the relationship between the number of cells in a nest at completion, mean number of *Sapyga* seen per 15 minutes, and mean floral density across each observation period. Bee age was not included due to complications in extracting the age a bee was when each nest was begun from the dataset. Random effects such as bee identity were not included to avoid overfitting the model.

Results:

Twenty-six individual *O. iridis* females were monitored across five sites, and 123 brood cells dissected overall. Floral density had a significant, positive correlation with pollen provision size (Table 1, Figure 2C). Bee age and *Sapyga* abundance had no effect on pollen provision size (Figure 2A and 2B). *Sapyga* abundance was marginally, significantly correlated with the number of brood cells in a nest at completion (Table 2, Figure 3B), while floral density had no effect

(Figure 3A). Bee age and floral density were significantly positively correlated ($P = 0.008$, Figure 4A). There was no relationship between *Sapyga* seen per 15 minutes of observation and floral density ($P = 0.20$, Figure 4B).

Discussion:

Floral abundance was most predictive of pollen provision size, while *Sapyga* abundance was most predictive of the number of brood cells per nest a bee produced. These results indicate that, when *Sapyga* abundance is high, bees produce fewer offspring per nest rather than investing less per offspring. Similar patterns were found by Goodell (2003), where *Osmia pumila* nests in flight cages with high *Sapyga* abundance produced significantly fewer brood cells per nest. Floral abundance has also been shown to strongly impact provision size in *Osmia cornuta*, with low floral abundances leading to a reduction in pollen provision size (Bosch 2008), although the non-independence of bee age and floral density may have impacted the significance of floral density's correlation with pollen provision size in this study (Figure 4A). Bees could theoretically compensate for low floral abundances by spending longer foraging for pollen and nectar; however, bees may be unable to increase foraging times adequately due to the higher risk of parasitism this would entail (Seidelmann 2006). The longer a bee spends provisioning, the longer a brood cell remains open and unguarded, increasing the probability that it will be parasitized (Brockmann & Grafen 1992; Danforth & Visscher 1993; Goodell 2003).

If provision sizes decline at low floral abundances, varying floral abundance may impact the sex ratios of *Osmia* broods. *Osmia*, like all Hymenoptera, can control the sex of their offspring via haplodiploid sex determination. Fertilized eggs become females, while unfertilized eggs develop into males. Because female *O. iridis* construct nests and provision brood cells, their reproductive success is more dependent upon body size (Seidelmann 2010). Therefore, under low resource conditions, bees might be expected to skew the sex ratio of their brood towards males, which require smaller amounts of provision, as was seen in Bosch (2008). To investigate these questions, bees emerging from nests monitored this year could be captured in emergence traps during the summer of 2022. If bees adjust the sex ratios of their broods based on floral abundance, we would expect more male-biased broods to emerge from nests that were constructed at sites or times when floral abundance was low during the summer of 2021.

In this study, we found no correlation between bee age and provision size. However, Seidelmann (2006) found that, as bees aged over the course of the nesting season, they provisioned less for offspring, resulting in a reduction in offspring body size and broods that were male-skewed. The effects of age on provisioning may not have been detectable in this study because of small sample size or poor estimation of bee age; bees may have been active long before they were marked or constructed brood cells at rates faster or slower than the two-per-day rate used to estimate when a bee began nesting. Finally, longer observation periods or only conducting observations under more stringent conditions might have increased the detectability of *Sapyga* and resulted in stronger correlations, as their activity seemed to be highly temperature dependent. *Sapyga* were only seen on hot days in the afternoon, but some sites were consistently visited in the morning when it may not have been warm enough yet for *Sapyga* to be active.

To make more robust conclusions, a larger sample size is needed. Further research is needed to confirm the relationship between *Sapyga* abundance and the number of brood cells in a nest at completion. Future studies might investigate the impacts of early nest completion on a bee's lifetime reproductive success: do bees construct more few-celled nests to compensate? Goodell (2003) found that *Osmia pumila* in flight cages with high parasite abundance had lower

reproductive output. Parasite detection may also lead to changes in nest selection: do bees construct subsequent nests in the same area, or do they move to a new site where parasite abundance may be lower? To investigate these questions, *Sapyga* abundance could be tracked along with the nesting locations and reproductive output of individual bees to see if there is any association between *Sapyga* abundance, 1) how many brood cells a bee completes over the course of the whole season, and 2) how far away separate nests are from each other, taking into account floral abundance, which likely plays a large role in nesting site selection, as well.

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Appendix:

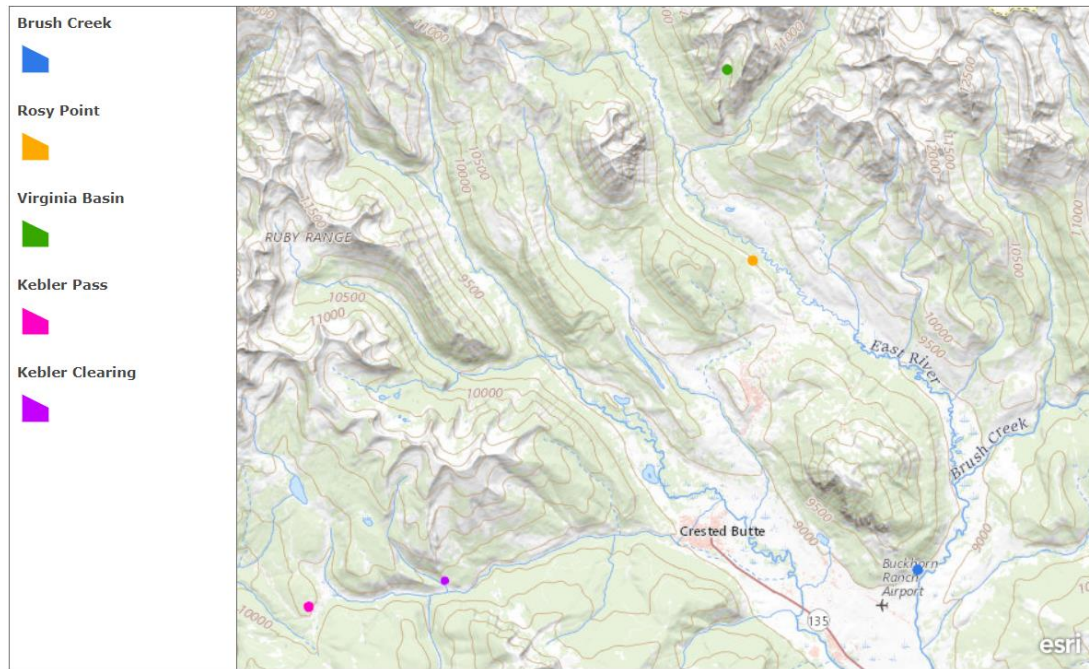


Figure 2. Map of study site locations in Gunnison Valley.

TABLE 1. Mixed linear model and ANOVA with mean provision volume as the response variable, bee age, mean floral density, and *Sapyga* seen per 15 minutes of observation as fixed effects, and bee identity as a random effect. Mean provision volume, bee age, and mean floral density were log transformed to create normal distributions. * $p < 0.05$.

Variable	P-value	Coefficient	t value	df
log(Mean floral density)	0.02*	10.52	2.40	20.20
log(Bee age)	0.92	-0.52	-0.09	21.86
Mean <i>Sapyga</i> seen per 15 minutes	0.52	6.97	0.65	34.49

TABLE 2. Linear model and ANOVA testing the impacts of floral density and *Sapyga* abundance on the number of brood cells in a nest at completion. Mean floral density and number of brood cells in a nest at completion were log transformed to create normal distributions. $N = 23$ *Sapyga* observations conducted at *O. iridis* nests, with floral densities for each date observations were conducted.

Variable	P-value	Coefficient	t value
log(Mean floral density)	0.85	-0.01	-0.095
Mean <i>Sapyga</i> seen per 15 minutes	0.07	-0.22	-1.87

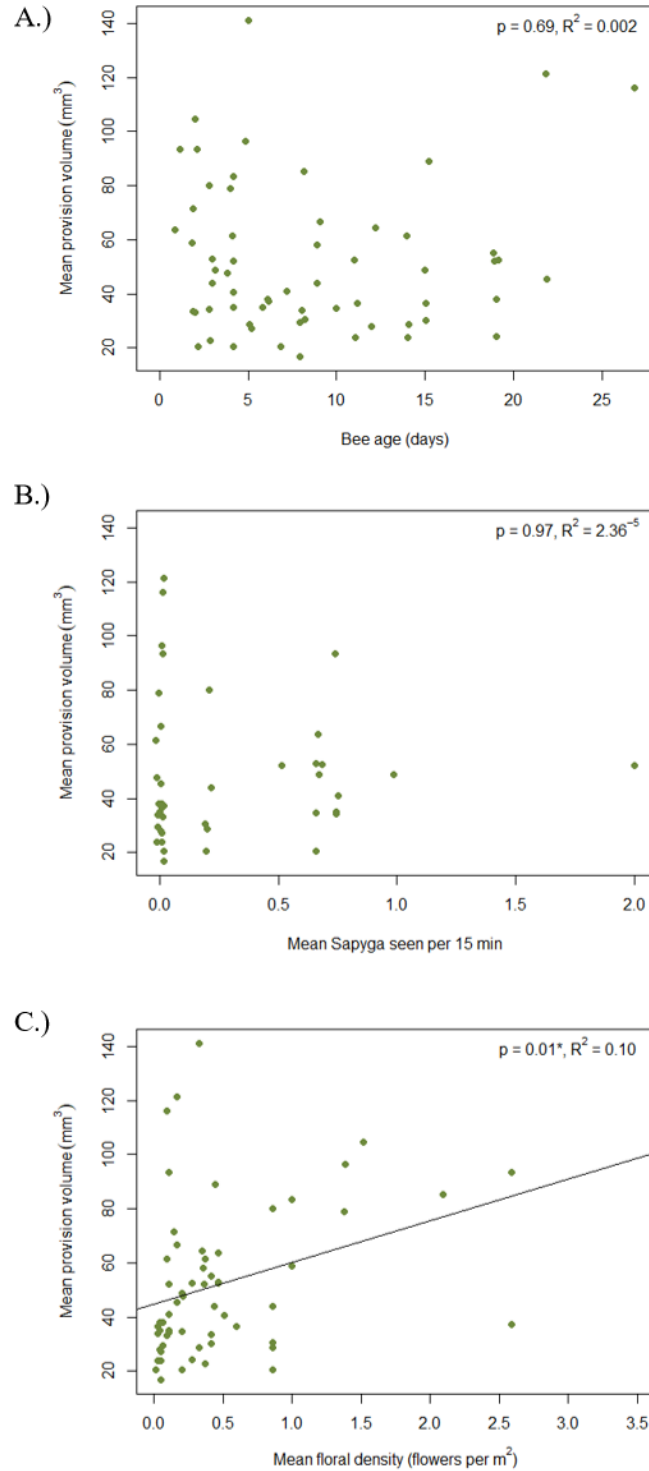
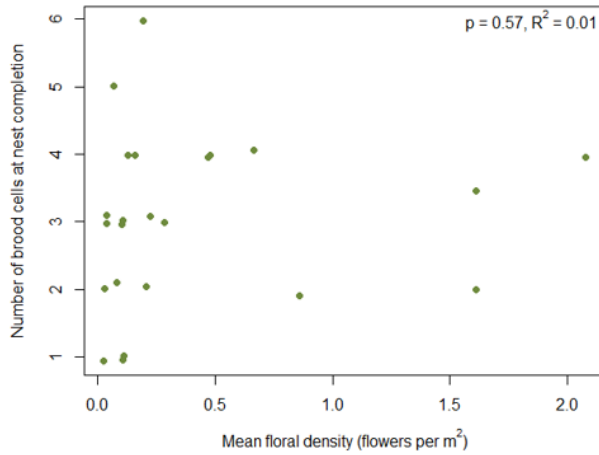


Figure 2. Univariate regressions showing the relationships of bee age (days), mean floral density (flowers per m^2), and mean *Sapyga* seen per 15 minutes of observation to mean brood cell provision volume. $N = 26$ *O. iridis* nests across 4 sites with 123 brood cells total.

A.)



B.)

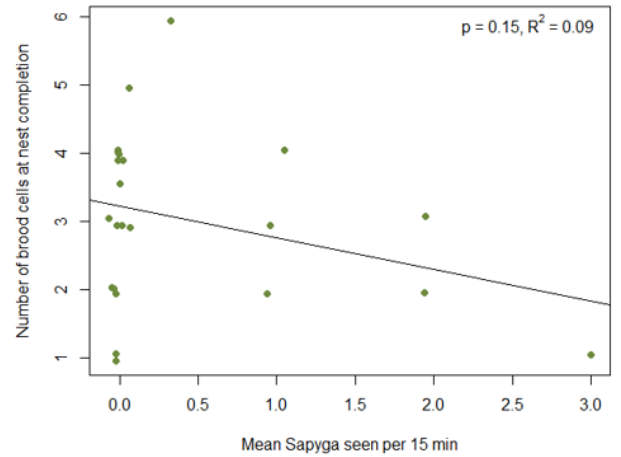
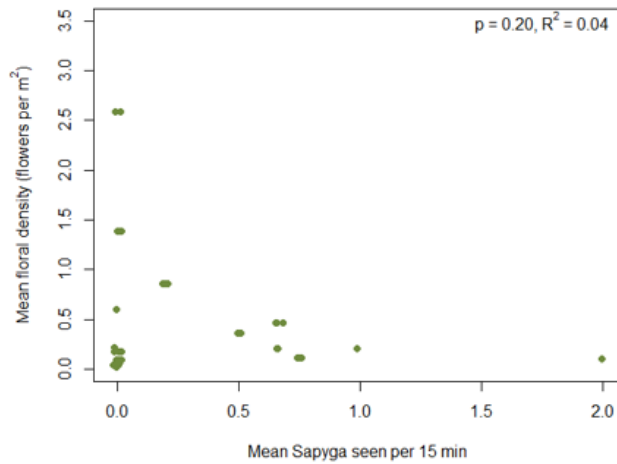


Figure 3. Univariate regressions showing the relationships of mean floral density (flowers per m²) and mean *Sapyga* seen per 15 minutes of observation to the number of brood cells in a nest at completion. N = 26 *O. iridis* nests across 4 sites with 123 brood cells total.

A.)



B.)

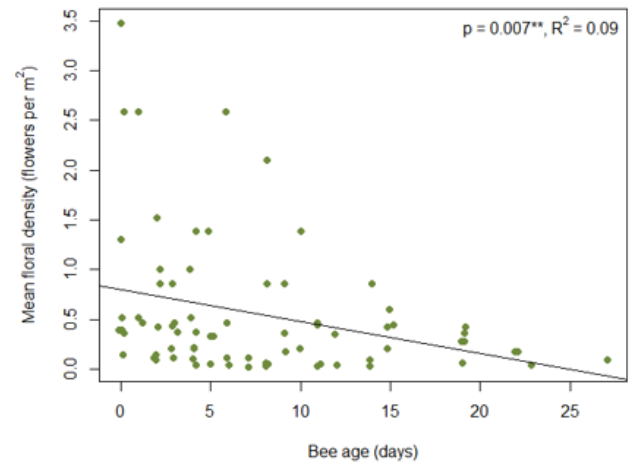


Figure 4. Univariate regressions showing the relationships between mean *Sapyga* seen per 15 minutes and bee age (days) to mean floral density (flowers per m²). N = 26 *O. iridis* nests across 4 sites with 123 brood cells total.