

Nesting Preferences of *Osmia* Bees in the Rocky Mountains
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Abstract

Osmia is a genus of solitary, cavity-nesting bees which will readily nest in artificial nest-blocks. Nest-blocks (or “trap-nests”) have long been used to study cavity-nesting bees and have recently been popularized for conserving bees in urban and agricultural systems. What attributes of the nest-block, its placement, and its proximal environment are attractive to *Osmia*? At each of several existing sites with nest-blocks already set up in the Rocky Mountains, CO, I measured the orientation and height of each nest-block; the health, circumference, and color of each tree with a nest-block; and the internal temperature of each nest-block. *Osmia* preferred warmer nest-blocks and blocks on larger trees, but there was no observed effect of any other variable on the number of nesting *Osmia iridis*. Future research should focus on measuring internal nest temperature for longer periods of time and placing nest-blocks on a wider variety of tree circumferences.

Introduction

Pollination is essential for production of over half of the world's crops, and honey bees are extensively managed to provide this service. However, native bees are responsible for a considerable fraction of pollination in agriculture and could provide much more pollination, if their populations were supported by effective management or conservation techniques (Kremen, 2008). While less abundant in some agricultural environments, wild bees are often more efficient pollinators than honey bees and, in some cases, may enhance cross-pollination, increasing yield and quality of the crop (Garibaldi et al. 2014, 2017). In general, pollinator richness can contribute to pollination even when honey bees are present (Garibaldi et al. 2014). As risks to managed bees continue to escalate, the importance of having alternative methods of pollination increases. Managing or conserving native bees could provide a pollination buffer for crops requiring or benefitted by pollination (Kremen, 2008).

While native bees can contribute to crop pollination, few species actually perform this service (Senapathi et al. 2015). There are numerous arguments for conserving bees in general, however, including those that do not contribute to crop pollination. First, higher biodiversity means more diverse reactions to ecological disturbances. Therefore, if an ecological disturbance disrupts or eliminates a few species of a particular group, the ecosystem function of that group could be preserved by the presence of a few resilient species (Senapathi et al. 2015). However, bee species important to agriculture are usually resilient species while other species are more threatened. In addition, many species of bee do not contribute to pollination of any plant at all. For these reasons, Kleijn et al. (2015) argues that the cultural importance that humans place on biodiversity should be included in the justification for bee conservation. Supporting native bee populations is therefore in the best interests of stakeholders in agriculture and conservation as well as the general public.

One native bee group of interest is *Osmia*, a genus of solitary, cavity-nesting bees that is currently being managed in some agricultural systems to supplement or replace pollination by managed honeybees (James & Pitts-Singer, 2008). Not only are certain species, including *Osmia lignaria*, more efficient orchard pollinators than honeybees, but there are also many native species found in diverse environments throughout the world (Sedivy & Dorn, 2014; James & Pitts-Singer, 2008). These bees readily nest in artificial nest-blocks, also called “bee hotels” or trap-nests. Nest-blocks have long been used to study cavity-nesting bees and have recently been popularized for conserving bees in urban environments (MacIvor, 2017). Although the effectiveness of using nest-blocks alone to promote bee populations has been called into question (MacIvor & Packer 2015), the combination of extra nesting material with additional floral resources could be an excellent strategy in both urban and agricultural environments (Gaston et al. 2005, Sedivy & Dorn 2014, Blaauw & Isaacs 2014).

Despite the popularity of “bee hotels”, there is little published research on what attributes of nest-blocks are attractive to cavity-nesting bees. Some studies have found that bees may be attracted to blocks nearer to particular floral resources, previously occupied nests, nests occupied by conspecifics, and areas with more available nest material (Guisse & Miller, 2011; Everaars et al. 2011; Vorel, 2010; James & Pitts-Singer, 2008). Wood blocks with drilled holes seem to be more attractive to *Osmia* than other artificial nest materials (Sedivy and Dorn, 2014). Although the literature tends to show that *Osmia* prefer cooler, more shaded nests, these studies were primarily performed in low-elevation, warm climates (Martins, Ferreira & Carneiro 2012;

Hranitz et al. 2009). One exception, however, is a study performed in Leipzig, Germany, by Everaars et al. (2011), finding that *O. bicornis* was more likely to nest in blocks with more sun exposure at their low-elevation, cool climate site. All sites in the above studies have relatively narrow annual temperature ranges.

Still the question remains: what attributes of the nest-block, its placement, and its proximal environment are attractive to *Osmia*? To investigate this question, I studied *Osmia* at the Rocky Mountain Biological Laboratory (RMBL), Colorado, in an environment that is radically different from those described in the previously mentioned studies. This gave me the opportunity to test whether observed *Osmia* nesting preferences are context-dependent. I therefore measured the effect of several micro-habitat attributes on the number of nests per species and per nest-block at several established sites around Gothic, CO. As the sites around RMBL are at high elevation and bees experience short, cool summers, I expected that solitary bee development would be limited by the available heat, leading to more nesting per block in warmer locations and in blocks oriented in a direction that provides more sunlight per day. As some prior research has found that megachilid bees may be attracted to previously occupied nests, nests occupied by conspecifics, and areas with more available nest material (Vorel, 2010; James & Pitts-Singer, 2008; Forrest et al. 2015; Grundel et al. 2010), I expected to find more nests per species in blocks on dead or dark-colored trees. It is important to note, however, that mason bees appear to show no preference for nest-blocks occupied or unoccupied by conspecifics (Groulx & Forrest 2018), and there was a similar possibility that I would find a concentration effect, whereby there would be more nests per block on living or light colored trees (Everaars et al. 2011). There is some evidence that stingless bees prefer to nest in larger trees, and Samajima et al. (2001) found higher nest densities in areas with higher densities of large trees. Although stingless bees are social, I might expect a similar trend of more nests per block in blocks attached to large trees. Finally, there have been some observed effects of nest-block height, showing that bees prefer to nest at certain heights (Scott, 1995).

In summary, I will be testing two hypotheses: (1) nest-block temperature and visual characteristics of the proximal environment are predictors of *Osmia* nesting preference, and (2) nest-block orientation predicts nest-block temperature. To test these hypotheses, I measured the orientation and height of each nest-block; the health, circumference, and color of each tree with a nest-block; the internal temperature of each nest-block; and the number of nesting *Osmia* per block.

Methods

Sites and Study Organism

Nest-blocks at six existing sites in areas surrounding Gothic and Crested Butte, CO, were used for this study (fig.1); each site had 12 nest-blocks already in place for use in other studies. Nest-blocks were constructed of two pieces of wood glued together with holes 4-8mm in diameter drilled about 15cm all the way through the blocks (fig.2). Blocks were attached to trees at each site using a metal strap and screws. The sites were mostly on the edges of subalpine aspen or spruce-fir forests on hillsides near Brush Creek, Rosy Point, Forest Service trail 401, Elk Creek (Kebler Rd.), Kebler Pass, and Virginia Basin. Due to physical constraints of the primary researcher, nest temperature measurements were only taken at the Brush Creek, Rosy Point, and Kebler Pass sites, while all other variables were measured at all sites. In this area, several species of *Osmia* have been observed, including *O. coloradensis*, *O. iridis*, *O. montana*,

O. subaustralis, *O. tersula*, and *O. tristella* (Groulx & Forrest 2018). The group of study organisms, *Osmia*, is largely univoltine, but in our populations near RMBL it is typically semivoltine, meaning the larvae take two years to develop before emerging as adults (Torchio & Tepedino, 1982). Adult bees emerge May-July, live for 3-4 weeks, and nest from May-August. The larvae develop over the course of the summer, overwinter as pre-pupae or larvae, continue developing through the following summer, overwinter as cocooned adults, then emerge the next summer (Groulx & Forrest 2018). Some will take only one year to develop, but this appears to be rare, perhaps due to temperature constraints (Forrest, unpublished)

Measurement of Variables

We used a compass to measure orientation of the nest-block entrances and the orientation of the side of the block facing away from the tree (fig. 2). We measured the height of each nest-block relative to the ground. We recorded the species of tree that each nest-block is attached to, along with the circumference of the tree at the height of the nest-block. Tree health was assessed on a semi-quantitative scale where 0 was a completely dead tree, 1 was a completely healthy tree with no dead branches, and 0.5 was a tree with some live and some dead parts. As some trees were not dead but appeared dead at nest-block height, we also recorded a categorical estimate of the color of the tree at nest-block height such that trees with dark, rough bark were categorized as “dark” and those with light, smooth bark were categorized as “light” (fig. 2).

An OctTemp 2000 (MadgeTech Inc., New Hampshire) thermocouple data-logger was used to measure temperature at each nest-block. The temperature of each nest-block was measured several times throughout July by first removing the top nest straw on the side of the nest-block away from the tree, then inserting a thermocouple wire. Each nest-hole measured was 6mm in diameter and the thermocouple wire was inserted to a depth of ~30-40mm. Temperature was sampled every 15 seconds for 5 minutes at each block until all 12 blocks per site were sampled. Ambient temperature was recorded by the same device at each nest-block. As ambient temperature increases throughout the day, a balanced design was used so that each block was measured at least twice per hour from 9:00am to 1:00pm MDT. Temperature was only measured in the early part of the day due to time constraints. Throughout the summer, the number of nests per insect species was recorded for each nest-block. Most bees were marked and observed throughout the season as part of another study, so any nests that were not a mother’s first nest were excluded.

Analysis

For each nest-block, temperature measurements were grouped into time-slots such that all measurements taken at 9:00 AM - 10:00 AM were averaged, then all measurements taken at 10:00 AM -11:00 AM were averaged, and so on for every hour from 9:00 AM to 1:00 PM. Then, all these values (4 means for each nest-block) were averaged to get a single average temperature for each nest-block. To calculate the average temperature difference between each nest-block and ambient temperature, this same process was used except the difference was calculated for every temperature measurement before taking any averages.

To get nest choice values, I took the sum of the number of unique mother insects per species per nest-block. As some mothers would create more than one nest in a single nest-block, only first nests were counted. Only nests that had at least one complete brood cell were included in analysis. Although there were nesting data on multiple hymenopteran species, wasps were not marked, and most bee species were continuing to nest while the data were analyzed. I focused on

Osmia iridis as much of the population was finished nesting before August, 2018, and there were more mothers of this species than others, therefore the data for this species were most likely to be complete. Furthermore, *O. iridis* nested earlier in the season than other species and their nesting choices were thus less likely to be affected by the nesting choices of other Hymenoptera.

R-Studio (R Core Team, 2018) was used to plot nest orientation versus temperature and was then used to test whether any of the other variables predicted the number of *O. iridis* mothers nesting in each block, treating each block as an independent replicate. Linear relationships were analyzed using a Poisson generalized linear model. Non-linear relationships are shown using a local regression (LOESS) but were not analyzed statistically.

Results

Data on tree color, health, and circumference, and on nest-block height and orientation, were obtained for a total of 72 nest-blocks at six sites. A total of 67 *O. iridis* nests were constructed in these blocks by 55 individual mother bees. Data on nest-block temperature were obtained for 36 blocks at three sites.

There was no significant association between tree color and health and the number of nesting *Osmia iridis* in each nest-block (fig. 3a-b; $df = 60$, both $z < 1.1$, both $p > 0.28$). The sample was biased as most trees sampled were dark and completely dead ($n_{\text{dark}} = 54$, $n_{\text{light}} = 18$; $n_{\text{alive}} = 14$, $n_{\text{half-dead}} = 11$, $n_{\text{dead}} = 47$). Many nest-blocks were placed at 0.6m - 0.8m high, and there was no clear relationship between nest-block height and number of nesting *O. iridis* in each nest-block (fig. 3c; $z = 1.1$, $df = 60$, $p = 0.27$). Many of the sampled trees were 0.5m - 1.0m in circumference; however, there was a significant positive relationship between tree circumference and the number of nesting *Osmia iridis* in each nest-block (fig. 3d; $z = 3.65$, $df = 60$, $p < 0.001$). This relationship was largely driven by two outlying points and zero values. When the tree circumference data were log-transformed, the positive relationship was still significant but was again influenced by one outlying point ($z = 2.95$, $df = 60$, $p < 0.01$), and when the outlying points were removed from the log-transformed or the non-transformed data data set, the relationships were no longer significant ($z = 1.5$, $df = 59$, $p = 0.14$; $z = 0.19$, $df = 58$, $p = 0.85$). The relationship between tree circumference and *Osmia* nesting preference was potentially affected by tree species and site. As shown in figure 3d, the site at Kebler Pass (KP) contained the only trees larger than 1.5m in circumference, and this site used all spruce trees whereas all other sites used aspen trees. However, as shown in figure 3d, the trendlines are still mostly positive when split by site (and thus, also split by tree species).

Most nest-blocks were oriented at 90 – 180° from North (or, between East and South), and therefore the sides of the nest-blocks tended to be facing $\pm 90^\circ$ from East or South (fig. 4a,c). There was no clear relationship between orientation of the nest-blocks and the number of nesting *O. iridis*; however, there seemed to be slight peaks near 90° and 180° (fig. 4b,d). There was a slight negative relationship between orientation of nest face and internal nest temperature such that nests facing East (90° from North) tended to be warmer (in the morning) ($z = -1.55$, $df = 34$, $p = 0.12$; fig. 4e).

There were significantly more *O. iridis* nesting in warmer blocks (fig. 5b; $z = 2.51$, $df = 34$, $p = 0.01$). Again, however, this relationship was driven by one outlying point, and when this point was removed, the relationship was no longer significant ($z = 1.16$, $df = 33$, $p = 0.25$).

Discussion

Both hypotheses were partially confirmed. *Osmia iridis* did appear to prefer warmer nest-blocks and blocks on larger trees but my results did not suggest any preference based on tree health or color or nest-block height. As expected, nest-block orientation seemed to predict nest-block temperature, although this relationship was not significant. Many variables had values that were close together because the study measured variation in nest-blocks that were already in place. For example, most nest-blocks had been placed, according to existing recommendations, on dead trees and facing approximately East or South (Bosch & Kemp, 2001). For some relationships, this meant that significance disappeared after removing outliers. However, as this was an exploratory study, any trends are interesting for follow-up in future studies. For example, although the trends of *O.iris* preference for larger trees and warmer nest-blocks are not robust, these relationships could be verified by more data across years. I expected a normal or clumped relationship between nest-block height and the number of *O.iris* nesting per block, where there would be more *O.iris* nesting in blocks of a particular height but fewer nesting in blocks lower or higher. There were several *O.iris* nesting in blocks of around 0.75m in height (fig.3c); however, the peak in occupancy at this height was not strong. Future studies could place nest-blocks at a wider range of heights in order to test this relationship further.

Nest-Block Temperature and Orientation

It is possible that temperature preference is context-dependent considering seemingly conflicting evidence that *Osmia* prefer shaded nests over those in direct sun in one study, yet more *Osmia* nested in blocks in sunnier spots in another (Martins, Ferreira & Carneiro, 2012; Everaars et al., 2011). My study was conducted in a cooler, montane environment, and I found that *Osmia* preferred warmer nests, in agreement with Everaars, et al. (2011). As with many other insect taxa, *Osmia* require sufficient heat-accumulation to develop into adults, and therefore, nest-block temperature should be an important determinant of offspring survival (Forrest & Thomson, 2011; J. Forrest, unpublished). It is possible that *Osmia* nest choice might thus depend on nest-block temperature and that this choice would be dependent upon both heat-accumulation required for full development and the available heat in the environment. There could be a temperature limit where developing young or their food supply would be harmed by high temperatures, and this might explain why some studies have found a preference for more shaded nests (Hranitz et al., 2009). Figure 5a somewhat supports this possibility, as slightly fewer *O.iris* nested in blocks at high mean temperatures. I found that temperature and orientation are related: nest-blocks facing East tended to be warmer in the morning than nest-blocks facing in other directions (figure 4e). *O.iris* had a slight preference for blocks facing approximately East and South, however this relationship was not strong and there was a bias in nest-block orientation. Still, there was wide variation in the internal temperature of the nest-blocks, perhaps owing to variation in the amount of shade each block experienced regardless of orientation. One difficulty in testing the relationship between nest-block orientation and number of *O.iris* nesting is that the relationship is not predicted to be linear. Internal nest temperature could be a better predictor of *O.iris* nest-block choice than nest-block orientation, and this would be an interesting direction for future research.

Future Directions

Future studies might usefully focus on the relationship between nest-block orientation and internal temperature and testing the relationship between nest temperature and *Osmia* nest choice. Based on limitations of my study, future researchers should measure internal nest-block temperatures over more hours of the day to get a more complete representation of the temperatures that bees experience. I attempted to calculate the mean difference between external (or ambient) and internal nest-block temperature; however, because the OctTemp 2000 data logger was kept in an enclosed plastic container, it accumulated heat throughout the day, and my ambient temperature measurements were biased. If using a similar method to my study, future researchers should keep the temperature data logger shaded and un-enclosed in order to get accurate temperature readings. Any future studies on the effects of tree qualities on the number of nesting *Osmia* should have nest-blocks placed on trees of different species and circumferences, in order to test the relationship I found. They should also place nest-blocks facing in all directions, to get more evenly distributed orientation data.

Conclusion

Understanding nesting preferences is useful to any future studies on *Osmia*, and potentially on any cavity-nesting Hymenoptera. It can also be useful to conservation and management efforts, as nest-blocks could go unused if not placed in attractive environments. Although not robust, my findings suggest that, in a cold, montane environment, *Osmia* prefer warmer nest-blocks placed on larger trees.

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Tables and Figures

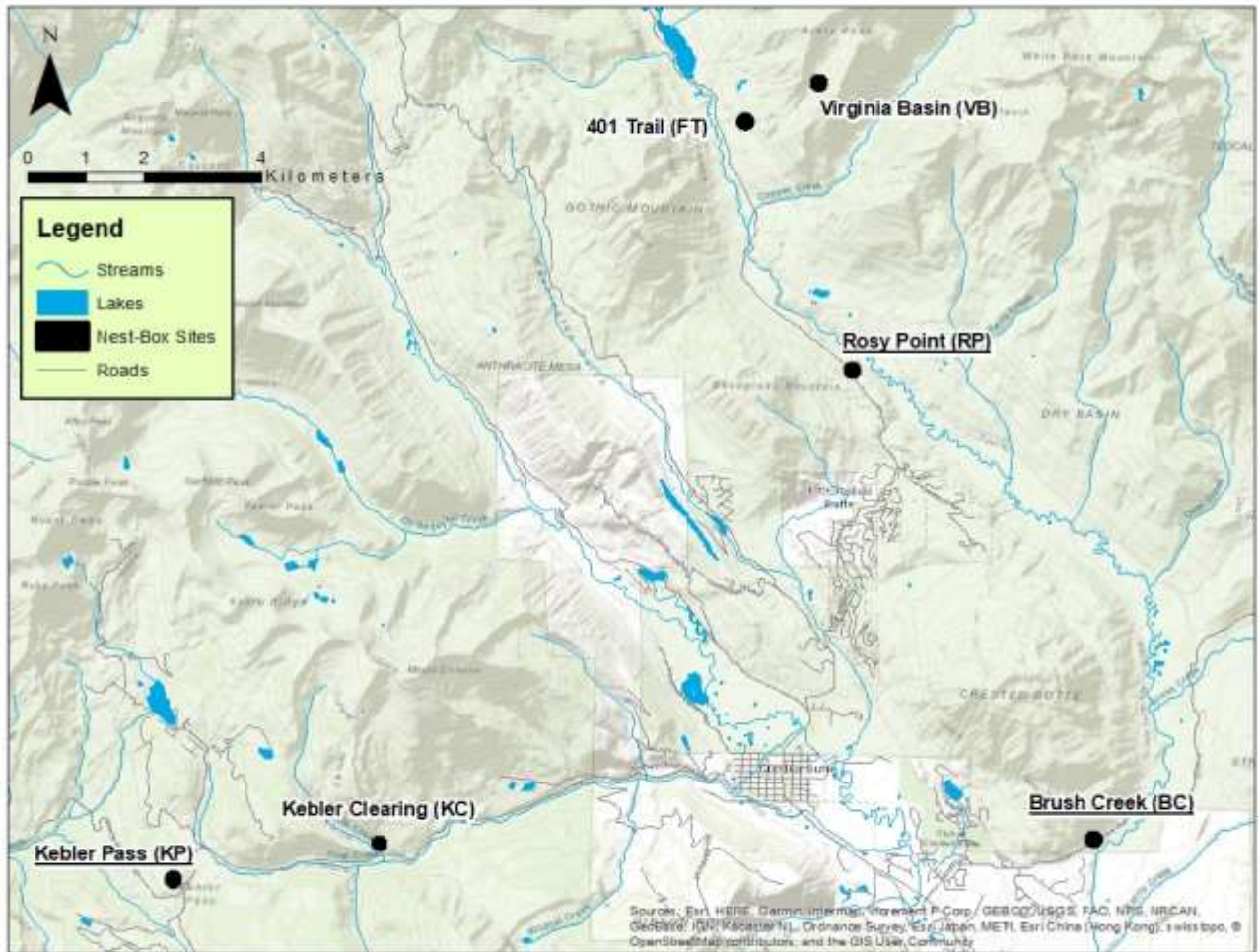


Figure 1. Research sites around the Rocky Mountain Biological Laboratory, CO (located near FT site). Site code is in parentheses, underlined sites show where temperature measurements were taken.

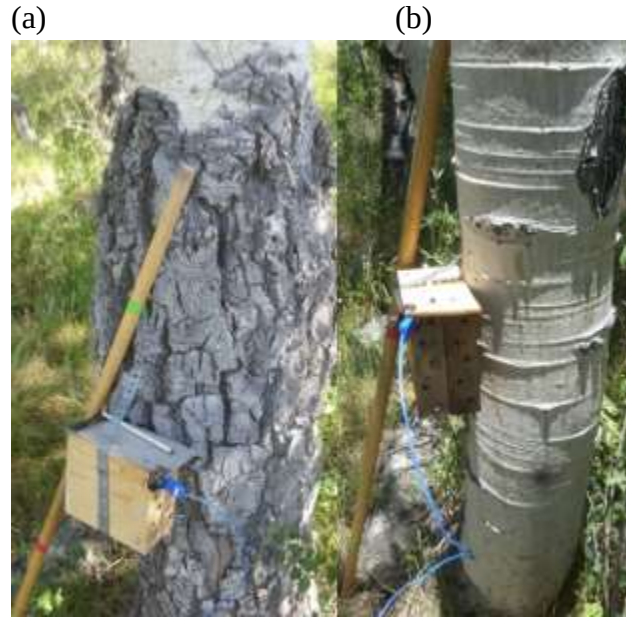


Figure 2: A thermocouple wire sampling internal nest temperature at two nest-blocks at Brush Creek. Although both trees were fully alive, tree (a) was classified as “dark” due to the bark around the nest-block which appears dead while tree (b) was classified as “light” due to the mostly light-colored, living bark.

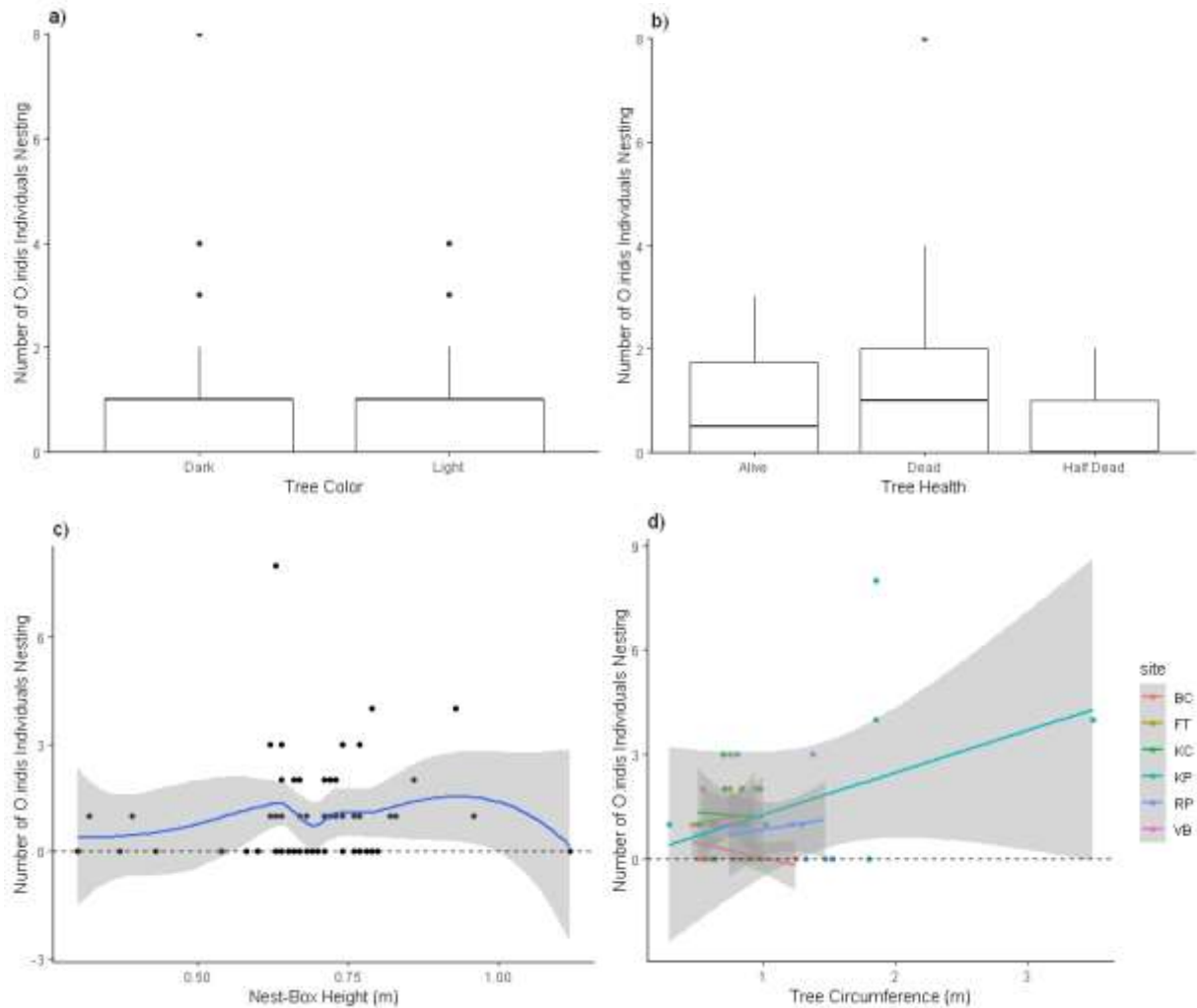


Figure 3. (a) The relationship between tree color and number of nesting choices by *Osmia iridis* per block ($n_{\text{dark}} = 54$, $n_{\text{light}} = 18$); (b) the relationship between tree health and the number of nesting choices by all *Osmia* bees ($n_0 = 14$, $n_{0.5} = 11$, $n_1 = 47$); (c) the relationship between nest-block placement height in meters and the number of nesting choices by *O. iridis* (smooth model shown; Poisson glm regression: $z=1.098$, $df=60$, $p=0.27$), (d) the relationship between tree circumference in meters and the number of nesting choices by *O. iridis*, points and trends shown per site (Poisson glm regression: $p<0.001$, $z=3.647$, $df=60$; log-transformed Poisson glm regression: $p<0.01$, $z=2.948$, $df=60$).

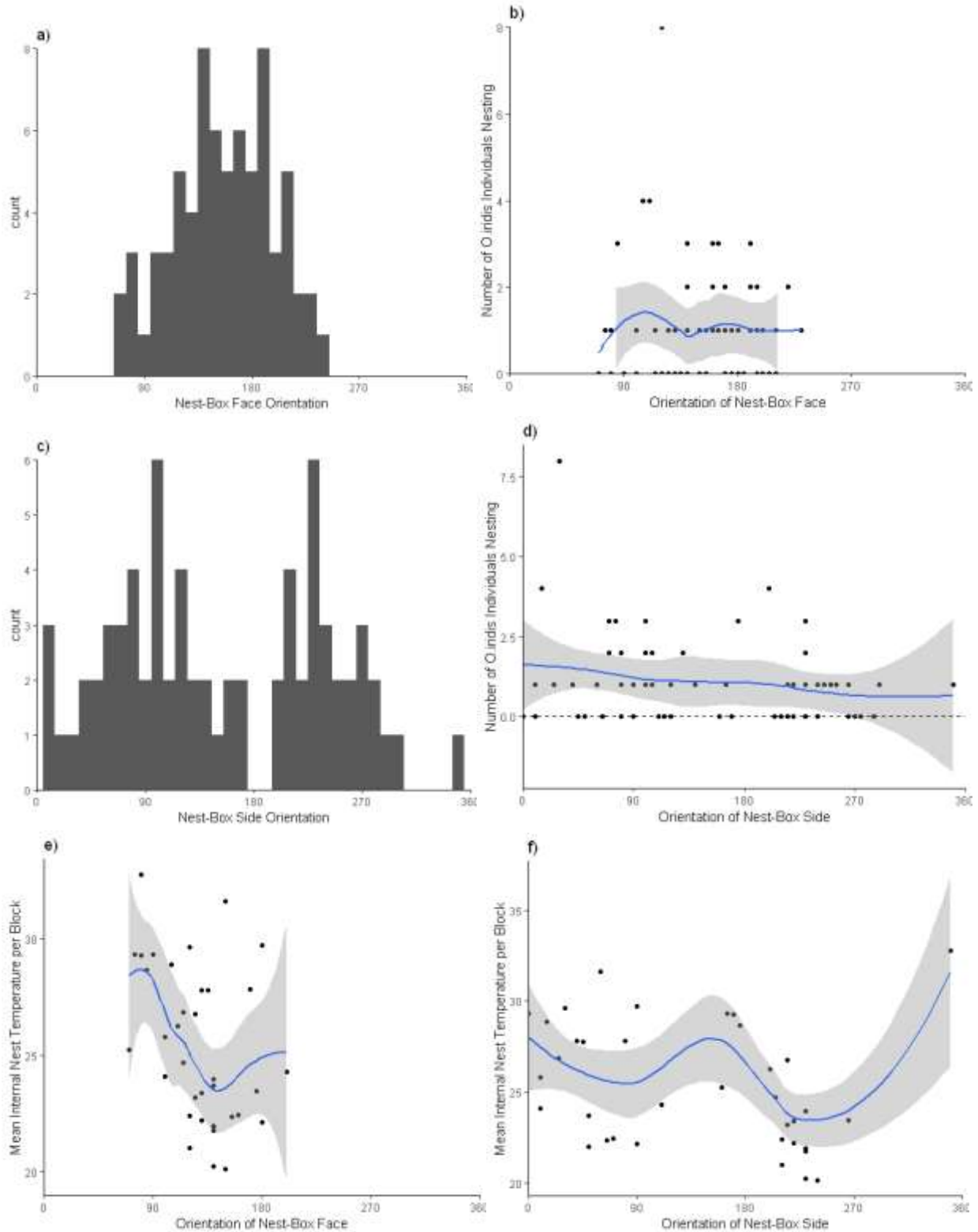


Figure 4. (a) The distribution of nest-block faces, measured as degrees from North. (b) The relationship between nest-block face orientation and number of *O. iridis* nesting per block. (c) The distribution of nest-block sides, measured as degrees from North. (d) the relationship

between the orientation of the nest-block sides and number of *O.iris* nesting per block. (e) the relationship between orientation of nest-block face and mean internal nest-block temperature (smooth model shown; Poisson glm regression: $z=-1.55$, $df=34$, $p=0.12$). (f) the relationship between orientation of nest-block side and mean internal nest-block temperature

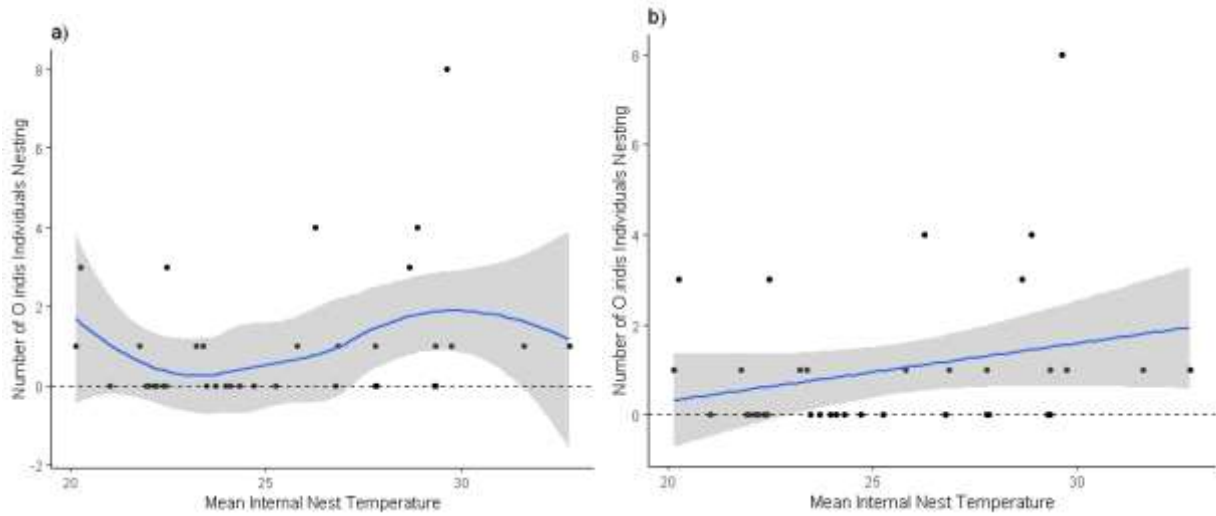


Figure 5. (a) The relationship between mean internal nest-block temperature and nest-block choice by *O.iris* with a smoothed trend line. (b) Same as (a) except showing the generalized linear model ($z=2.51$, $df=34$, $p=0.012$).