

How season length and diet breadth limit mason bee success across an elevational gradient

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Abstract:

Climate change is having a major effect on species all over the world, possibly opening new ranges in which these organisms can live. Animals pollinate most plants around the world and bees account for most of the pollinator activity. Bees are often categorized into two types based on diet breadth: generalists and specialists, depending on the number of different flower species they harvest pollen from. The survival of offspring is associated with the amount of time available for development, as offspring born late in the season are less likely to survive the winter. We tested whether shorter and colder seasons at high elevation impact offspring development in mason bees, while also testing whether a bee's diet breadth affects its offspring's success. Hollow paper tubes were placed in wood boxes at sites of low and high elevations for bees to construct nests. Nests were monitored until we observed them being completed, and then we applied one of three treatments: (1) transplanted to a high-elevation site ("up"), (2) transplanted to another low-elevation site ("sham"), or (3) left in place (control). Larval development data was also collected and helped to provide a timeline for the development of different mason bee species throughout the season. We found that cocoon success was not associated with the day of transplant (i.e the day an egg was laid); however, up and sham treatments had lower offspring success than control nests. We also found that generalists and specialists differ slightly depending on when a nest was transplanted. All of this helps us to better understand the true range limits of these mason bee species, and to see if climate change is expanding these living distributions. Our results give some indication that upper range limits in these bee species could be influenced by the short season lengths we see in Rocky Mountain ecosystems.

Intro:

Climate change is impacting species across the world, causing shifts in living distributions of these organisms (Cozier, 2004; McCabe et. al, 2021). Range-shifts usually occur across an altitudinal range, in which organisms are tracking some aspect of their abiotic or biotic environment (Tomuolo & Ward, 2018). It is believed that nearly 84% of species have moved up in elevation since the 1970's due to changes in climate (Thomas, 2010). It is expected that organisms are shifting upward such that they are maintaining their climatic niche. There is some concern that changes in climate and the geographic distribution of species could increase extinction risk and affect biotic interactions (Gomez & Lacher, 2019; Blois et al., 2013). Therefore, it is important to try and understand the types of organisms that are being affected by these changes in climate.

Animals pollinate more than 85% of plants worldwide, and bees are the most important pollinators due to the fact they spend most of their lives harvesting pollen from flowering plants to feed their offspring, also known as nest provisioning (Parreño et al., 2022). Bees are categorized into two groups based on their diet breadth: specialist and generalist pollinators. Specialists limit themselves to gathering pollen from a few related plants, while generalists are seen to visit various plant species over time, with preference changing in response to past experience and perceived rewards (Wcislo et al., 1996).

The survival of offspring is associated with the amount of time available for their development, as offspring born late in the season are less likely to survive the winter (Cahill et al., under review; J. Forrest., unpubl. data). It is critical that bees emerge early enough in the season after overwintering as female bees need to begin building their nest and to provide enough food for their offspring. The major issue is at higher elevations, floral resources do not become as abundant until late in the season, likely due to snow preventing any flowers from developing. At lower elevations, abiotic factors may allow for flowers to become more abundant earlier in the season (Inouye & Wielgolaski, 2003). Flowers being abundant later in the season at high elevations can delay the female's ability for nest provisioning due to not having a sufficient amount of floral resources at these high elevations until late in the season, which can impact the survival of their offspring (Wong & Forrest, 2021).

I am interested in testing whether shorter season lengths at high-elevation sites impact offspring development in mason bees. My overall question is: do shorter and colder seasons at

high elevation limit offspring development, while considering the "floral landscape" of high-elevation sites (specifically, the fact that flowers come into bloom much later in the season at high elevations than at low elevations)? I predict that offspring born earlier in the season will have higher offspring success (i.e chance for survival) than the offspring born later in the season. I am also interested in testing whether generalist or specialist bees have a greater success rate during overwintering. I predict that generalist bees will have a greater survival rate during overwintering due to being able to start nest provisioning earlier than specialists who might have to wait for their floral host-plants to bloom.

Methods:

Study Organisms:

A number of bee species (ground- and cavity-nesting) occur at both high-elevation sites (Virginia Basin and Mount Baldy); however, trap-nest records from past years have shown that certain species, which are common at lower-elevation sites rarely or never occur at high elevations. I was working at what seemed to be the upper range limits of the bee species I studied; therefore, I conducted transplants using nests constructed by these species to understand if climate change is removing constraints on the range limits of these species. They include: *Osmia iridis*, *O. montana*, *O. coloradensis*, *O. lignaria*, *O. pikei*, and *Hoplitis fulgida*. These mason bee species are solitary bees who build nests in pre-existing cavities in woody substrate (Wilson et al., 2010). After mating, females will locate an acceptable area to start constructing their nests, which consist of a linear series of cells made of mud and pulp from leaf fibers. Each cell will usually contain a mass of pollen, nectar, and a single egg. Eggs are laid in the late spring - early summer, spend the first winter as larvae in cocoons, pupate the following summer, spend the next winter as adults in cocoons, and emerge the following spring.

Study Sites:

Nests that were transplanted were sourced from four low-elevation sites at Brush Creek, Gothic Townsite, South Gothic Meadow, Judd Falls trail (2784 m, 2909 m, 2937 m, 2978 m elevation respectively). Each source site contains between 5 and 7 trap-nests. Nests from these sites were either transplanted to another source site (sham), to one of two high-elevation sites

(Virginia Basin or Mount Baldy; 3440 m and 3433 m elevation respectively), or left in place (control). Transplant sites contained five to seven empty trap-nests attached to a tree or stump approximately 1 meter above the ground which served to receive transplanted nests. A trap nest is a wooden block of 2"x6", approximately 14 cm in length with deep holes of 3 diameters, 4.5, 6.1, 8.2mm driven into them, and are attached to a tree. Each of the holes contains a hollow paper tube where the individual bees construct their nests.

Transplant Experiment

Trap-nests at source sites were monitored every 3-7 days beginning in late-May or early June for *Osmia iridis*, *O. montana*, *O. coloradensis*, *O. lignaria*, *O. pikei*, and *Hoplitis fulgida* nesting. Once a nest was observed being completed and sealed at a source site, I removed the nest (i.e. extracted the paper straw containing its nest contents from its original trap-nest), and applied one of three treatments: nests are either (1) transplanted to a high elevation site, (2) transplanted to another low-elevation site (sham), or (3) left in place (control). Transplanted nests are inserted into empty trap-nests located at the transplant site where they remain for the rest of the field season. Treatment (2) was included to control for any effects of transporting nests on offspring development. For each of the target species, pairs of nests that are completed on approximately the same date are first identified. One nest (straw) from each pair remains in situ, while the second is transplanted to a different source site.

For both high-elevation transplants, shams, and controls, the realized number of transplanted nests ultimately depends on how many nests are available at lower-elevation sites (i.e one species may not build enough nests, so we cannot perform transplants for that particular species).

Scoring Larval Development

Offspring success was scored at the end of the field season (late July to early-August). To do this, I first cut small openings into the paper straws adjacent to each brood cell within the nest. During the assessment, nests were given a score from 0-6 with 0 meaning there was a larva present but the pollen was unfinished, and 6 meaning a full cocoon was spun. Nesting straws are then repaired with tape upon completion of the assessment. Asteraceae specialists (*O.*

coloradensis and *O. montana*) were not scored because of difficulty seeing a larva in the cell. Scoring was done by a single observer.

Table 1. Larval development score classification

Larval Development Score	Larval Development Classification
0	Pollen unfinished with larva present
1	Frass present but pollen unfinished
2	Frass present and pollen finished
3	Low cocoon silk visible (some indication that silk production started but larva still clearly visible)
4	Medium cocoon silk (silk cover ~50% of larva, though sometimes this is hard to tell)
5	High cocoon silk (lots of silk present, but larva still visible)
6	Full cocoon (larva totally concealed)

Floral density observations

30m x 1m transects were also set up at the high elevation sites, and then all entomophilous flowers were counted throughout the season. Flowers were counted differently depending on the taxon. Individual flowers were counted for some species while flower heads were counted for others if individual flowers were too small and numerous to count. Floral surveys were completed during the 2022 and 2023 season. In 2022, surveys were completed at one of our low elevation sites (Brush Creek) from June 3 - August 5 every 7-12 days. Floral surveys were also conducted at two high elevation sites (Mt. Avery and Mt. Baldy) from June - July 27 every 5-16 days. In 2023, surveys were conducted from July 1 - July 31 every 6-11 days. Transects were not set up for low elevation sites in 2023, as there would be too many flowers to count. Instead, I used the 2022 flower data collected by the Forrest lab for low elevation flower density. These flower surveys were used to provide context for how floral conditions might differ

between elevations, and when naturally occurring bees would be able to start nesting. Offspring success data was used from the 2021 and 2022 seasons, while also combining it with larval development data from this season (2023). Floral density data was used from both the 2022 and 2023 seasons.

Data Analysis

No statistical tests were performed on floral survey data as it was meant for descriptive purposes only. A binary success model was used for cocoon success from 2021 and 2022, where the response variable was either a 0 (no cocoon) or a 1 (cocoon). For this data we ran a generalized linear model where we modeled success against date of transplant, treatment, diet breadth, and year. We also performed an ordinal regression model on the larval development data collected from 2022 and 2023. The ordinal regression modeled the different stages of larval development against treatment and species.

Results:

Over the course of three years, we observed and transplanted 302 nests containing a total of 1300 brood cells. 104 of the nests (540 cells) were from the 2021 and 2022 seasons, while 198 nests (760 cells) were from 2023. Out of the nests transplanted in 2021 and 2022, 78 nests (382 cells) were transplanted to higher elevation sites, while the remainder were left for control and sham treatments. For 2023, 160 nests (565 cells) were transplanted to higher elevation sites with the rest being left for control and sham treatments. The 2021 and 2022 seasons saw four generalist nests and 100 specialist nests transplanted, while 2023 saw 89 generalist and 69 specialist nests transplanted. The lack of generalists in 2021 and 2022 was because previous transplant experiments did not take into account generalist nests, as they were mostly focused on *Osmia iridis*.

Floral density at different elevations

The highest floral density at Brush Creek was around 35.8 flowers/m² on July 26. Highest flower density at our Avery site was 19.38 flowers/m² on July 18, while the highest flower density measured at our Baldy site was 56.6 flowers/m² on July 25. In 2023, similar surveys

were conducted; however, they were only conducted at high elevation sites. The highest floral density recorded at Avery was 19.3 flowers/ m² on July 1, while the highest floral density at Baldy was 51.8 flowers/m² on July 28. In 2022, there were abundant flowers at Brush Creek earlier in the season than earlier in the season than at Avery.

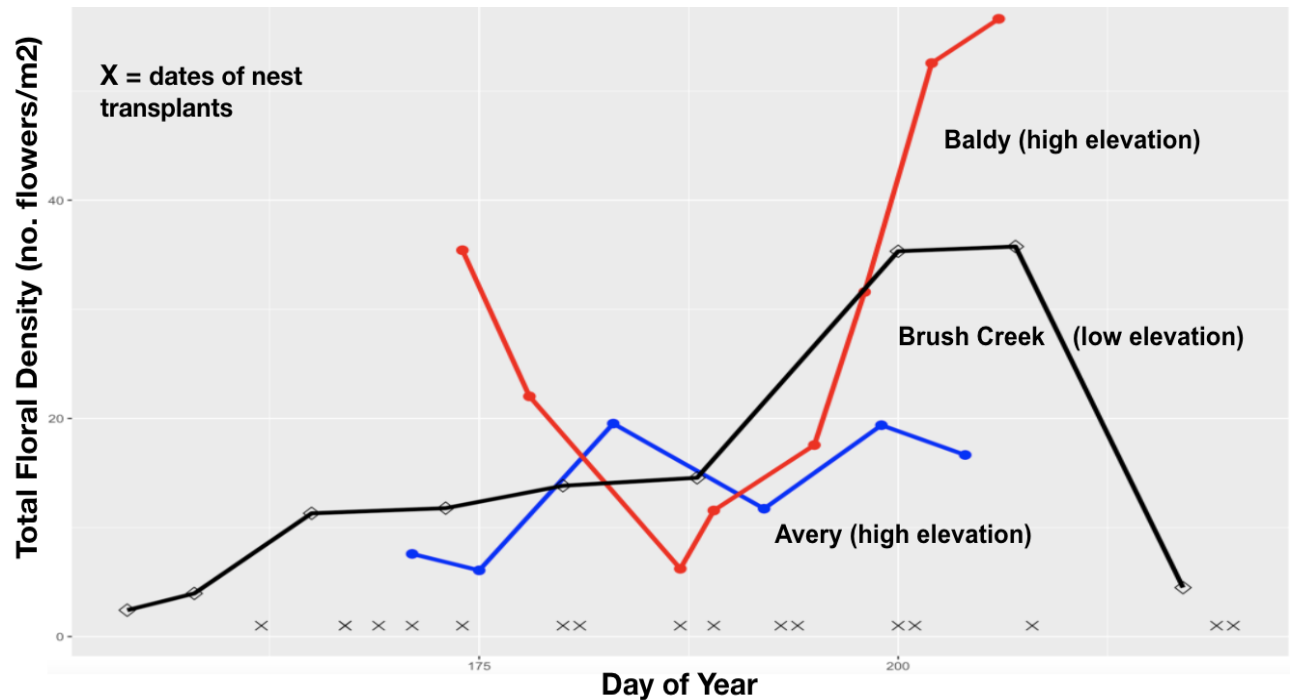


Figure 1. Comparison of total floral density at low elevation and high elevation sites in 2022. X's on the x-axis represent days we transplanted nests

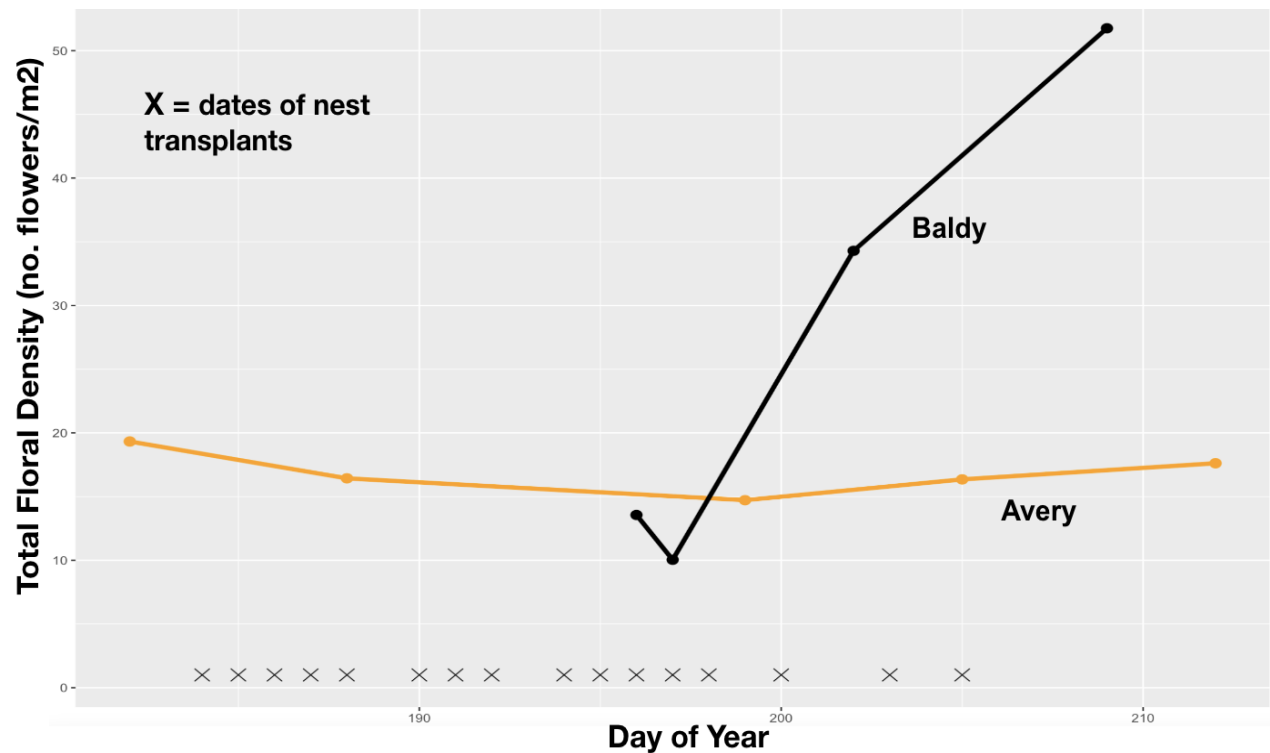


Figure 2. Comparison of total floral density at different high elevation sites in 2023. X's on the x-axis represent days we transplanted nests

Cocoon success compared to day nest transplanted

There was no significant relationship between cocoon success and the day nests were transplanted (estimate = 0.005, $p = 0.77$) during the 2021 and 2022 seasons; however, there was a significant relationship between cocoon success and treatment: Nests that were transplanted up in elevation (estimate = -3.43, $p < 0.0001$) or to a different low elevation site (sham; estimate = -3.81, $p < 0.0001$) had significantly lower cocoon success compared to nests that were left in place (control).

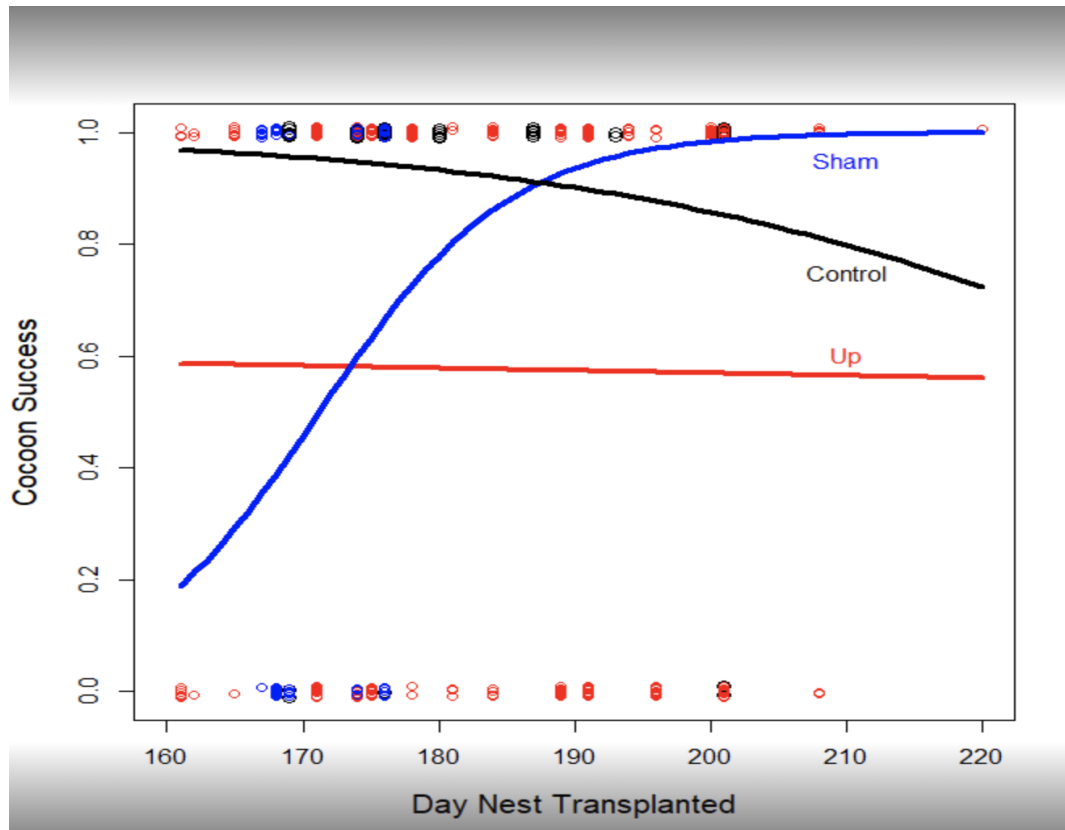


Figure 3. Cocoon success of nests compared to the day they were transplanted for each treatment type from 2021 to 2022. Day 160 is equivalent to June 9th, while day 220 is equivalent to August 8th; $n = 382$ larvae (up), $n = 73$ larvae (sham), $n = 85$ larvae (control). A score of 1 indicates the larva successfully spun a cocoon. Data points were jittered vertically to improve visualization.

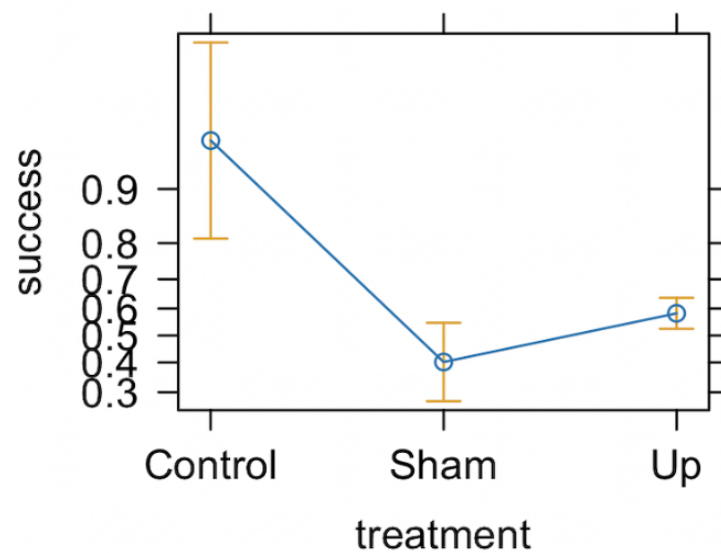


Figure 4. Cocoon success of nests vs. treatment type in 2021 and 2022.

Generalists vs. specialists

Diet breadth did not significantly affect the relationship between date of transplant and cocoon success (estimate = 0.309833, $p = 0.4150$). Generalists had slightly poorer success when transplanted later compared to specialists who stay fairly constant no matter when they are transplanted.

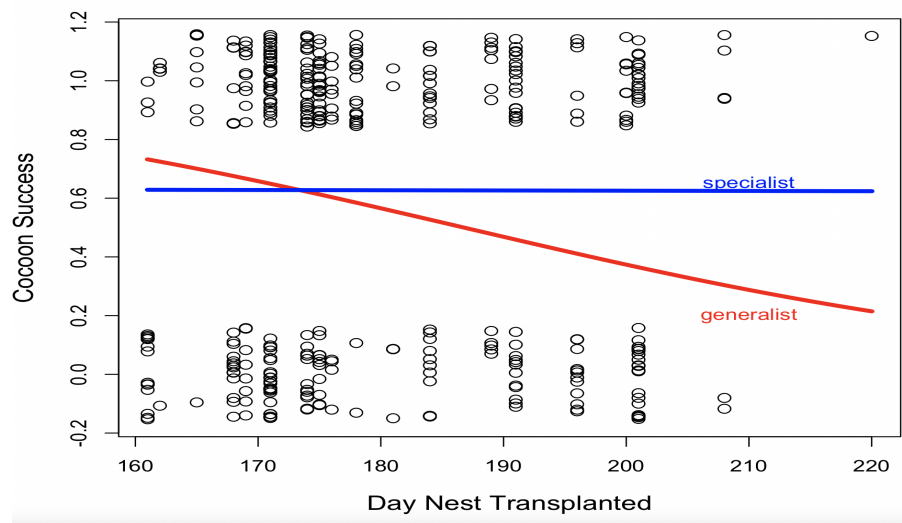


Figure 5. Cocoon success of nests vs. the day they were transplanted for each pollinator type. Data from 2021 to 2022; $n = 35$ generalists and 505 specialists. Points are jittered vertically to improve visualization.

Larval development stages

Nests transplanted earlier were further along in their development when checked. Nests transplanted up saw slower development compared to the other two treatments (Fig. 5 and 6). The Fabeae-specialist, *Osmia iridis*, had more cocoons spun than the other species in the study.

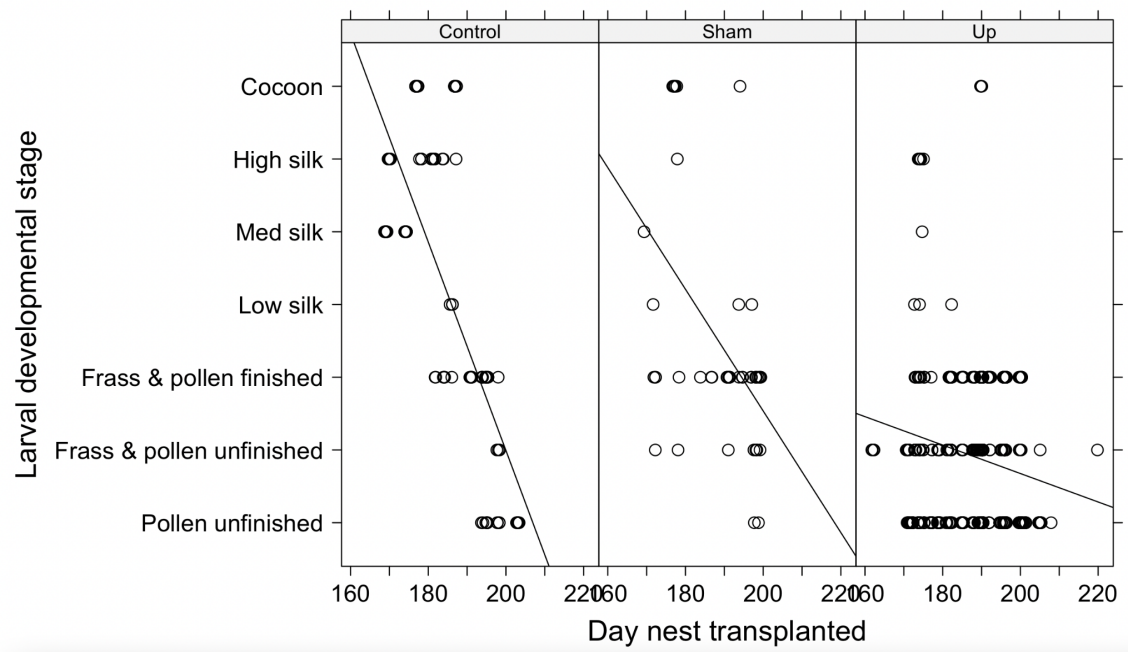


Figure 6. Stage of larval development as a function of the day nests were transplanted for each treatment type. Data from 2022 and 2023.

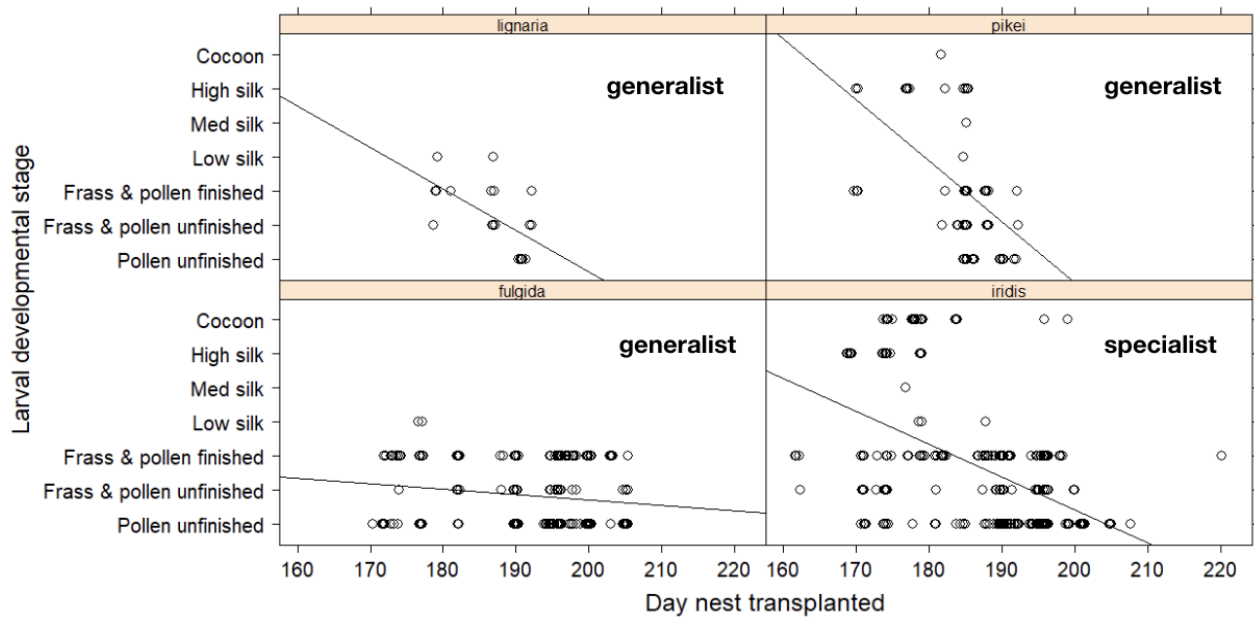


Figure 7. Stage of larval development as a function of the day nests were transplanted for each species/pollinator type from 2022 to 2023.

Discussion:

Results from these experiments show us that shorter seasons do have a slight impact on the development of mason bee offspring. This informs us on the range limits of these species, as at higher elevations, we see seasons are even shorter than normal due to factors such as late snow melts preventing floral resources from emerging (Inouye & Wielgolaski, 2003). The data collected helps us gain a better understanding of where these bee species can live as climate change is warming their current environments and opening up new areas for these bees to occupy.

Floral density at different elevations

Floral environments at our Baldy and Brush Creek sites can change rapidly throughout the season, while at Avery, the floral environment seems to stay fairly consistent (Fig. 1 and 2). Floral surveys give us context for what the floral environments are like for pollinators at these sites during a certain time of season. These surveys also emphasize the fact that we were transplanting nests well before peaks in flower abundance at the high elevation sites. The timing of flower availability limits how early bees can start nesting at a given site, so information on flower density tells us when naturally occurring bees would be able to start nesting. At high elevations, bees could be constrained by local flower phenology, which could delay nest provisioning by female bees and increase the chance that her offspring will not survive the winter. While this is the pattern we would expect to see, the floral surveys conducted show not to be the case, as Brush Creek had high floral density later in the season compared to Avery and Baldy that saw some of the highest floral densities earlier in the season. This could be due to factors like timing of snow melt, as previous studies have shown that timing of snowmelt can alter floral environments and change traits in floral expression (Powers et. al 2021). There is also lots of floral variation between sites, so one site might have a flower that is super abundant later in the season while the other sites do not have that flower. In an ideal model, we could have sites that have the same species of flower, and the sites would only contain flowers these bee species use.

We do not know how representative the flower abundance data is of resource availability/phenology for the bees. For example, *Osmia iridis* only uses *Lathyrus* and *Vicia* and if these flowers aren't part of the counts early in the season, females can't start provisioning. In

other words, while Figures 1 and 2 seem to indicate that bees can start nesting around 170-180, this might not be the case for all species. What we need to do is to examine the relationship between transplant times and the specific floral hosts bees might be using.

Cocoon success and larval development in relation to day nest was transplanted

Cocoon success was slightly (not significantly) associated with the day of transplant. The up and control treatment lines follow the pattern we expected, that nests completed and transplanted later would have lower cocoon success than ones transplanted earlier in the season (Fig. 3). The sham treatment line is not the best for modeling what the treatment should actually look like. We would expect it to look similar to that of the up treatment, but we had few replicates in the sham treatment later in the season, which is likely why we see a positive correlation between cocoon success and the day the nest was transplanted. While we did not see a significant relationship between cocoon success and the day nests were transplanted, we did see a significant relationship between cocoon success and the type of treatment performed (Fig.4). Sham and up treatments did significantly worse than the control treatment. Low success in the “up” treatment could be because these bee species cannot tolerate the cool conditions that we see at high elevations; however, the fact that the bees in the sham treatment did worse than those in the up treatment leads us to believe there could be some effects on offspring development just from moving the nests around.

The larval development data gives us insight into the development time of different species in the different treatments. We expected that nests that were transplanted earlier in the season would be at a later stage in their development than nests that were transplanted later (as we observed; Figs. 6-7). When nests were observed being completed, they were transplanted as soon as possible, so that day nest transplanted was as close to the day an egg was laid as possible. Therefore, nests that were transplanted earlier contained eggs that were laid earlier in the season. This means that these eggs had more time to develop because they were laid earlier in the season, which is why we see a negative trendline on Figures 6 and 7. The Fabae-specialist *Osmia iridis* seemed to have more cocoons spun than the other three species, which happen to be generalists. This is likely because this species has earlier phenology than the generalists due to its host-plants flowering earlier in the season.

Generalists vs. specialists

Generalists and specialists differed (non-significantly) in response to day of year. Generalists did slightly worse when transplanted later compared to specialists whose cocoon success stayed fairly constant no matter when they were transplanted. Our model for comparing diet breadth in response to day of year was not the best because we did not have an even distribution of generalist and specialist (4 generalist to 100 specialist) pollinators from the 2021 and 2022 seasons. Ideally, this analysis would have been done with 2023 cocoon success data where we had a more even distribution of generalist and specialist nests (89 generalist to 69 specialist); however, we can see how development differs between diet breadths (Fig. 7). While this does not tell us whether generalists or specialists have greater success during overwintering, it can tell us that specialists (specifically *O. iridis*) were further along in their development when checked. This is again likely because this species has earlier phenology than the generalists due to its host-plants flowering earlier in the season. The fact that *O. iridis* was further along in their development than the other species leads me to believe some specialists will have greater offspring success than some generalists because their floral resources are abundant earlier in the season. Another idea to consider is that certain flower species might flower earlier in the season, which can impact when bees can begin nest provisioning. We observed a lot of Asteraceae-specialists like *Osmia coloradensis* and *Osmia montana* earlier in the season, likely due to the fact that *Taraxacum* flowers were more abundant in the month of June. We also observed that many *Hoplitis fulgida* (generalist) individuals were more abundant later in the season compared to some other species we saw earlier. Whether the phenology of when certain species emerge had an impact on the results from this analysis would have to be investigated further in another study.

One thing to mention is that we really should have included Nest ID as a random effect in our models. We did not do this because we were having problems running the model in R because of data convergence problems. We were pretending that each larva (i.e. each data point) is independent (i.e. we were pretending that each larva had a different mom and came from a different nest and are not related at all). In reality, many of the larvae are siblings and come from the same mom which could affect how well they perform (i.e. maybe one mom gives birth to offspring that develop a lot faster because the mom did a good job provisioning). Having Nest ID as a random effect helps account for this.

Conclusion:

When looking at the floral density data, we seemed to perform transplants before peak flowering (especially when looking at 2023 high elevation floral data). We found that cocoon success was not associated with the day of transplant; however, we noticed certain species building nests earlier in the season and some building nests later. Transplant up and sham treatments did much worse than control nests, so moving nests could have an impact on success, which is not great for this experimental design. Generalists and specialists also differed in response to day of year (though not significantly). Larval development data shows that nests transplanted earlier in the season were more likely to further along in their development than nests transplanted later. This is because when nests were observed being completed, they were transplanted as soon as possible, so that day nest transplanted was as close to the day an egg was laid as possible. This means that these eggs had more time to develop because they were laid earlier in the season.

Future Directions

We would want to check nests again from this season (2023) and score them, as we could not score cocoon success due to it being too early in their development when we checked them. Doing this could help us better compare offspring success between generalist and specialist pollinators, as there was a more even number of nests transplanted compared to the 2021 and 2022 seasons. Another interesting idea could be to look and see if the bees emerge successfully as adults after overwintering at the transplant site. Spinning a cocoon is not the best proxy for offspring success, as individuals can still die in their cocoons (L. Wong, personal observation). However, it is the best we can do at this moment because we are not supposed to release transplanted organisms into new environments at RMBL. Another idea could be to establish new high elevation transplant sites to be sure we can generalize the effects of elevation, as it is difficult to do with only two high elevation sites. This would help us to better understand the true range limits of these mason bee species, and allow for us to investigate if changes in the climate are expanding the range in which these bee species can live.

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Literature Cited:

- Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and the past, present, and future of Biotic Interactions. *Science*, 341(6145), 499–504.
<https://doi.org/10.1126/science.1237184>
- Crozier, L. G. (2004). Field transplants reveal summer constraints on a butterfly range expansion. *Oecologia*, 141(1), 148–157. <https://doi.org/10.1007/s00442-004-1634-z>
- Gómez-Ruiz, E. P., & Lacher, T. E. (2019). Climate change, range shifts, and the disruption of a pollinator-plant complex. *Scientific Reports*, 9(1).
<https://doi.org/10.1038/s41598-019-50059-6>
- Inouye, D.W., Wielgolaski, F.E. (2003). High Altitude Climates. In: Schwartz, M.D. (eds) Phenology: An Integrative Environmental Science. Tasks for Vegetation Science, vol 39. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-0632-3_13
- McCabe, L. M., Aslan, C. E., & Cobb, N. S. (2021). Decreased bee emergence along an elevation gradient: Implications for climate change revealed by a transplant experiment. *Ecology*, 103(2). <https://doi.org/10.1002/ecy.3598>
- McCabe, L. M., Cobb, N. S., & Butterfield, B. J. (2019). Environmental filtering of body size and darker coloration in pollinator communities indicate thermal restrictions on bees, but not flies, at high elevations. *PeerJ*, 7. <https://doi.org/10.7717/peerj.7867>
- Parreño, M. A., Alaux, C., Brunet, J.-L., Buydens, L., Filipiak, M., Henry, M., Keller, A., Klein, A.-M., Kuhlmann, M., Leroy, C., Meeus, I., Palmer-Young, E., Piot, N., Requier, F., Ruedenauer, F., Smagghe, G., Stevenson, P. C., & Leonhardt, S. D. (2022). Critical links between biodiversity and Health in Wild Bee Conservation. *Trends in Ecology & Evolution*, 37(4), 309–321. <https://doi.org/10.1016/j.tree.2021.11.013>
- Powers, J. M., Briggs, H. M., Dickson, R. G., Li, X., & Campbell, D. R. (2021). Earlier snowmelt and reduced summer precipitation alter floral traits important to pollination. *Global Change Biology*, 28(1), 323–339. <https://doi.org/10.1111/gcb.15908>

- Rahbek, C. (1995). The elevational gradient of species richness: A uniform pattern? *Ecography*, 18(2), 200–205. <https://doi.org/10.1111/j.1600-0587.1995.tb00341.x>
- Tomolo, S., & Ward, D. (2018). Species migrations and range shifts: A synthesis of causes and consequences. *Perspectives in Plant Ecology, Evolution and Systematics*, 33, 62–77. <https://doi.org/10.1016/j.ppees.2018.06.001>
- Thomas, C. D. (2010). Climate, climate change and range boundaries. *Diversity and Distributions*, 16(3), 488–495. <https://doi.org/10.1111/j.1472-4642.2010.00642.x>
- Wcislo, W. T., & Cane, J. H. (1996). Floral Resource Utilization by solitary bees (hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology*, 41(1), 257–286. <https://doi.org/10.1146/annurev.en.41.010196.001353>
- Wilson, J. S., Wilson, L. E., Loftis, L. D., & Griswold, T. (2010). The montane bee fauna of north central Washington, USA, with Floral Associations. *Western North American Naturalist*, 70(2), 198–207. <https://doi.org/10.3398/064.070.0206>
- Wong, L. H., & Forrest, J. R. (2021). The earlier the better? nesting timing and reproductive success in subalpine cavity-nesting bees. *Journal of Animal Ecology*, 90(5), 1353–1366. <https://doi.org/10.1111/1365-2656.13460>