

Effects of Climate Variation and Nesting Traits on Sex Ratios of Adult Solitary Bees

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ABSTRACT

In the face of environmental change, including climate change, many organisms' biological and ecological processes are being altered. Adult sex ratios are directly related to an organisms' reproductive success and long-term viability. Understanding what influences sex ratios in the face of these changes may help us to predict the success of an organism. Here we investigated how climate variation influences the adult sex ratios of twelve species of solitary bees present in what is now the Gothic, Colorado area. We looked at how climate variation, specifically snowmelt timing, may influence their adult sex ratios. We also explored how nesting traits may provide protection or susceptibility to climatic influence. Using long-term data collected by Dr. Rebecca Irwin's lab and Billy Barr, we ran generalized linear models to understand the relationship between snowmelt timing and the sex ratios of each of the twelve selected bee species. We also ran these same models, but categorized bee species based on nesting type (above-ground vs. below-ground nesters). Across all species, we found that in years with early snowmelt, the following year had a smaller proportion of females. The same trend was seen significantly with below-ground nesters. Above ground nesters exhibited the same pattern but the pattern lacked significance. We hypothesize that snowmelt does not have a direct effect on the sex ratios of solitary bees, but rather that it influences floral abundance which affects the ability of females to provision female vs. male offspring. Future studies should further explore this as it may have a direct influence on the long-term viability of solitary bees.

INTRODUCTION

Adult sex ratios are fundamental to understanding an organism's biological success and long-term viability (Szekely et al. 2014). Understanding what variables affect sex ratios could help us to predict how species will adapt in the face of climate variation and other types of environmental change. While other studies on sex ratios have been primarily focused on reptiles and amphibians like sea turtles (Schofield, Gail et al., 2017; Paukstis, Gary et al., 1993), there has been little research investigating what influences the sex ratios of arthropods, particularly the adult sex ratios of naturally occurring solitary bees. Solitary bees are important species, not only as pollinators for agriculture, but also within their own ecosystems (Danforth, B. et al., 2019). Multiple factors could influence the adult sex ratios of solitary bees, such as climate variation, floral abundance, and life history traits. The goal of this study is to assess how climate variation affects the adult sex ratios of solitary bees with varying nesting strategies.

Climate variation has a large influence on biological and ecological processes (Deutsch et al., 2008; Lambers et al., 2008). As climate variation becomes more common, understanding how it influences adult sex ratios of naturally occurring solitary bees is crucial to predict their success (Slominski & Burkle, 2019). Furthermore, understanding the relationship between the adult sex ratios of solitary bees and change in climate could make their adult sex ratios indicators of climate variation. For the purposes of this study, we specifically looked at how snowmelt timing influences the sex ratios of solitary bees. The previous year's snowmelt timing was used in our analysis because bees of the current year were laid in the previous year.

Along with climate variation, this study aimed to shed light on how different life history traits influence change or stability in adult sex ratios. The way an organism lives and fits into its

own niche can greatly influence the protection or susceptibility it has to change. In this study, we considered nesting as a life history trait. There are many different nesting traits exhibited across bees: some bees create their nests in soil, others use cavities, and other bees do not create nests but instead lay their eggs in another bee species' nest (brood parasitism) (Danforth et al., 2019). For this study we defined nesting traits into two categories, either above-ground or below-ground nesters. These trait values inherently provide different exposures to weather as well as different resource materials, and both weather and nesting resource availability could influence the adult sex ratios of solitary bees (Danforth et al., 2019).

In this study, we investigated 1) how climate variation, specifically snowmelt timing, influenced the adult sex ratios of solitary bees, and we asked 2) whether nesting traits lend protection or susceptibility to the effects of climatic variation on adult sex ratios of solitary bees. We hypothesized that H1) with the previous years' earlier snowmelt timing, there will be a lower proportion of females because females are typically more costly to provision because they require more floral resources, and that H2) the magnitude of impacts will be more noticeable in above-ground nesters. Answering these questions will help us to understand a fundamental aspect of solitary bee success and fitness, and direct us on the questions to explore next.

METHODS

Site Location and Layout:

Site locations were on what has historically been Ute Land and what was established as treaty land in the Treaty of 1868. What is now Rocky Mountain Biological Laboratory land, along with the other survey land that will be used in this study, was "ceded" by the Ute during Cession 515 (1868) and Cession 616 (1880) where they were removed to their current reservations in

southern Colorado and Utah. Surveying will take place in 16 different areas. These sites are located along an elevation gradient, with four sites at low elevation near Almont, nine sites located near Gothic, one additional mid-elevation site (Rustlers), and two sites at the highest elevation near Mexican Cut. The nine Gothic sites are in three different habitat types: dry meadow, veratrum dominated, and willow dominated. Two of the sites have weather stations nearby that have a similar meadow habitat. These locations have been used for a long-term bee phenology study since 2009. Surveying has historically taken place every two weeks, but this summer (2021) the surveying took place every three weeks to reduce the amount of lethal sampling. The sites used in this study are listed from low elevation to high elevation:

Almont Sites (low elevation): Almont (Figure 3d), Almont Curve (Figure 3d), Lypps (Figure 3c), CDOT (Figure 3c)

Gothic sites:

Block A (Figure 3e): David's, Tuttle, Willey

Block B (Figure 3e): Gothic, Sean's, Beaver

Block D (Figure 3e): Copper, Hill, Little

Additional mid-elevation sites: Rustlers (Figure 3b)

High Elevation sites: Mexican cut (Figure 3a), N. Elko (mapping unavailable for this site)

Each of the 16 sites is set up the same way with two 42-m transects running roughly perpendicular to each other. Some are not perpendicular due to obstacles (willows, rocks, and streams). Each transect has three 2 x 2-m plots where floral surveys are conducted. At each site, 30 bee bowls are placed on sampling days, 15 on each transect placed 3 meters from each other. Net surveying took place at each of the sites, within the area defined by the transects, on days that bowls were out.

Daily Sampling Routine

Field sampling had three different components: net surveying, bee bowl collecting, and flower phenology surveying. Each sampling had the same routine (some field days may vary depending on the number of sites that need to be sampled). Bee Bowls were set out first. After Bee Bowls were placed, netting surveys took place (AM and then PM). Once netting was completed, plant phenology data was collected. After both surveys were completed, the Bee Bowls were picked up and processed.

Netting

Netting was used, complimenting bee bowl collecting. At each site, hand netting was done for a total of two hours per day, one hour in the morning and one hour in the afternoon. Surveyors who were netting wandered through the site, netting any bees that were seen. When a bee was collected the survey time was stopped and the bee was processed. Solitary bees were lethally collected using a vial of 70% EtOH. Plant visitation, date, site name, time of day (AM/PM), and name of catcher was recorded and written on the vial. When bumble bees (*Bombus spp.*) were caught, their species, caste, and location of capture (plant, ground, flying..etc.) was recorded. Each bumble bee was marked using a paint pen to prevent recording recaptures. A “bee-squeezer” was used to carefully mark the bumble bee’s thorax. Once marked and recorded the bee was released.

Bee Bowls

Bee Bowls (LeBuhn et al. 2003) were used to document the abundance and community composition of bees. Each site had 30 bee bowls set out; 15 per transect. Bee Bowls were set out in the morning and collected in the afternoon, being left out for roughly six hours per sample day.

The “Bee Bowls” are 6 oz plastic weigh boats that are painted fluorescent yellow or blue, or are white (left unpainted). At each site there were 10 Bee Bowls of each color (10 yellow, 10 blue, 10 white). They were placed along the transect in an alternating order and were then filled with soapy water (Dawn blue dish soap). The time the Bee Bowls are placed and picked up were recorded.

At the end of the day, the Bee Bowls were collected. The bees that were collected in them were placed into pre-label vials filled with 70% EtOH. Each site had three vials, one for each color (yellow, blue, white).

Plant Phenology

Plant phenology data was collected on the same days bees were collected. At each site there were six 2x2m phenology plots. Within each plot every species of flowering plant, number of plants per species, and number of flowers per plant for 10 plants of each species was recorded.

Pinning and Processing Specimens

Once the specimens were collected and brought back to the lab they were processed and pinned. They were stored in the vials they were collected in, inside of a fridge until they were able to be processed.

To process the bees, they were first washed. We removed them from the 70% EtOH, placed them into a vial of water and shook them inside of the vial to remove any soap from the Bee Bowls or EtOH. We then removed them and dried them using a paper towel, or a hairdryer if necessary. During this process all label information was kept with the bees. Once the bees were washed and dried, they were then pinned and stored in BioQuip specimen boxes.

Data Entry

Data collected in the field was entered as frequently as possible. The data entered was also used to create labels for the bees using R. The data from this year was added to the bee phenology database that has been collected since 2009.

Data Selection

To understand how climate variation and nesting traits are impacting the sex ratios of solitary bees, we used the long-term data set that has been collected using the methods described above by the Irwin Lab and the long-term climatic dataset collected by billy barr. We selected twelve bee species within the database based on their abundance and frequency of occurrence. Each species was then examined to make sure there were males and females present every year (in the species selected there were a few cases in which females were only present, or there were no bees present in a given year). We selected eight below-ground nesters: *Agapostemon texanus* (Halictidae), *Andrena algida* (Andrenidae), *Dufourea harveyi* (Halictidae), *Halictus rubicundus* (Halictidae), *Panurginus cressoniellus* (Andrenidae), *Panurginus ineptus* (Andrenidae), *Pseudopanurgus bakeri* (Andrenidae), *Pseudopanurgus didirupa* (Andrenidae), and four above-ground nesters: *Ceratina neomexicana* (Halictidae), *Dianthidium heterulkei* (Megachilidae), *Hoplitis fulgida* (Megachilidae), *Hoplitis robusta* (Megachilidae), were selected.

Analysis

Once the bee species were selected, sex ratios were calculated. This was done for each species by calculating the proportion of females to the total number of individuals of that species caught per year (female/total). Snowmelt timing was measured by the day of year the snowpack was completely melted (snowmelt (DOY)), using billy barr's data.

Data were analyzed using R Studio and the packages eemeans, ggplot2, effects, tidyr, and dplyr. Three generalized linear models were used to look at 1) how snowmelt influences the sex

ratios of all twelve bee species, 2) how snowmelt influences the sex ratios of the eight below ground nesters, and 3) how snowmelt influences the sex ratios of the four above ground nesters.

RESULTS

For the first analysis, we ran a generalized linear model with all twelve species to see the relationship between their sex ratios and the previous year's snowmelt timing, considering species as a factor. We found that while the different species had different female proportions (figure 1a) there was a significant trend between previous year's snowmelt timing and sex ratio ($p = 0.001634$). We found that years with earlier snowmelt timing resulted in a low proportion of females the following year (figure 1b).

In our second analysis, we ran two generalized linear models, looking again at the relationship between sex ratio and previous year's snowmelt timing. We separated the analysis into two, first with all eight below ground nesters and second with all four above ground nesters. Species were also used as a factor in these models. In the first model, we found that, like with all species, years with earlier snowmelt timing resulted in a lower proportion of female below ground nesters the following year ($p = 0.000916$) (figure 2a). For the second model, above ground nesters showed a similar trend but without significance ($p = 0.36596$) (figure 2b).

DISCUSSION

We found that across the twelve species, solitary bee sex ratios were significantly affected by the previous year's snowmelt timing. This is likely due to the impacts of snowmelt timing on floral abundance (Inouye 2008). Snowmelt may be indirectly impacting solitary bee sex ratios through its influence on floral abundance available in the surrounding area. For

solitary bees, floral abundance is directly related to nutrition availability. A change in resource and nutrition availability could result in a change in adult sex ratios. The development of female bees requires more nutrients than that of male bees, thus having a change in floral abundance could result in a bias towards one sex over the other (Danforth, B. et al., 2019).

When we ran our analysis to look at below ground and above ground nesters separately, we found that below ground nesters significantly showed the same trend as the analysis with all twelve bee species. We also found that above ground nesters showed the same trend but without significance. This difference in significance could be due to multiple issues. In particular, a lower number of above ground nesting species ($n = 4$) in our analysis compared to the below ground species analysis ($n = 8$). Further, each of these above ground nesting species were found at lower abundances overall compared to below ground nesting species, and there were a few years that completely lacked males. This smaller sample size in above ground nesters may be a result of a naturally lower abundance in the environment (Danforth, B. et al., 2019). The difference in data between the below ground and above ground nesters certainly informs our ability to make robust conclusions about how snowmelt timing is influencing them differently. As of now, there is no way for us to compare these two life history strategies in their ability to provide protection or susceptibility to climatic influence on their sex ratios. However, we can certainly say that below ground nesters are being significantly impacted by snowmelt timing.

Future Directions

We are very interested in further investigating how floral abundance is influencing the sex ratios of these twelve bee species. Floral abundance and phenology data have been collected for each year and could be used in a similar generalized linear model analysis. We would need to first review what floral species each individual bee species visits. This would allow us to

eliminate any floral data that is irrelevant to their life history. We are also interested in comparing how specialist and generalist lifestyles may provide protection or susceptibility to sex ratio changes influenced by floral abundance.

We are also curious about the natural differences in the abundances of above ground and below ground nesters. Gaining a further understanding of this could help us to elucidate their relative abundances and how declines in these communities may differ.

ACKNOWLEDGEMENTS

This study and the data collected from previous years has taken place on the traditional homelands of the Ute, particularly the Uncompahgre (Tabeguache) band. Most of the land this study takes place on, was established as treaty land in the Treaty of 1868 which promised the western third of Colorado to be Ute land, as long as the grass shall grow. What is now the Rocky Mountain Biological Laboratory, along with the other land this study takes place on, was “ceded” in Cession 515 (1868) and Cession 616 (1880) where the Ute were removed to their current reservations in Utah and southern Colorado. Since the arrival of Europeans and the onset of settler colonialism, dispossession of land has been a key part of the American conservation movement. Learning and discussing the place RMBL and our own research has in this act of settler colonialism is an essential part of understanding the power of PLACE.

I would like to thank the Rocky Mountain Biological Laboratory for hosting me this summer and facilitating my research, as well as the National Science Foundation for funding me. Thank you to my mentor Becky, for guiding me through my research experience here. Thank you to billy barr for collecting the climate data I used in this study. I am forever grateful for the team I got to spend this summer with, Jade McLaughlin and Michael Troutman. I would like to

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POSITIONALITY STATEMENT

Here I acknowledge that my experiences, background, and position have informed the way I investigate and interpret my data. I acknowledge that this is simply one way to interpret and collect data, and that other traditional ways of knowing that have been continually discredited are as valuable as what has been described here. My undergraduate education has taken place at a landgrab university and is only possible because of violent colonization, removal, and genocide of Indigenous peoples. Similarly, my ability to conduct research at the Rocky Mountain Biological Laboratory is a direct result of settler colonialism and the removal of Indigenous peoples. I acknowledge that I am continually learning and an imperfect human, but I seek to actively educate myself and make the field of ecology more conscious of its created landscape and history.

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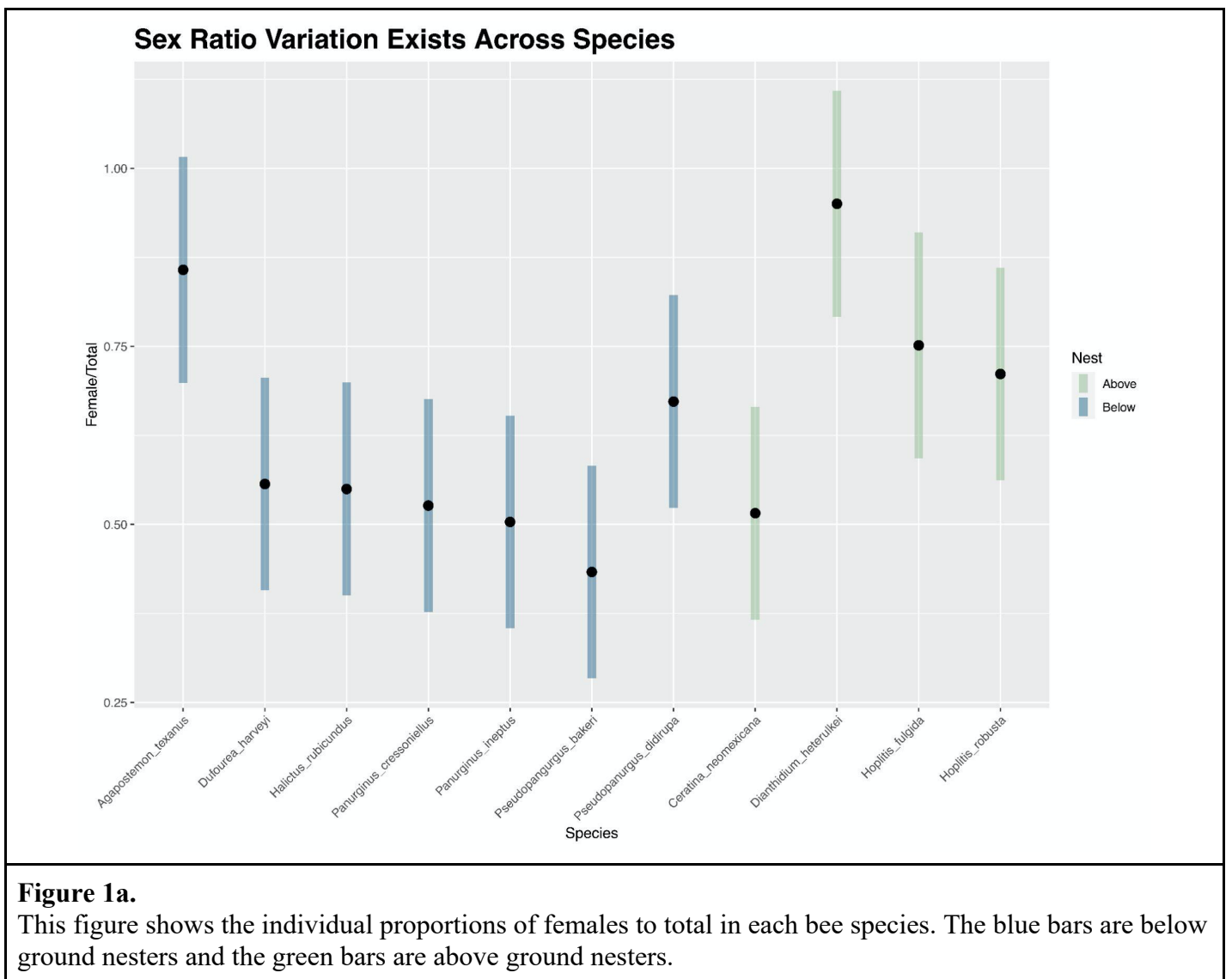
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TABLES AND FIGURES



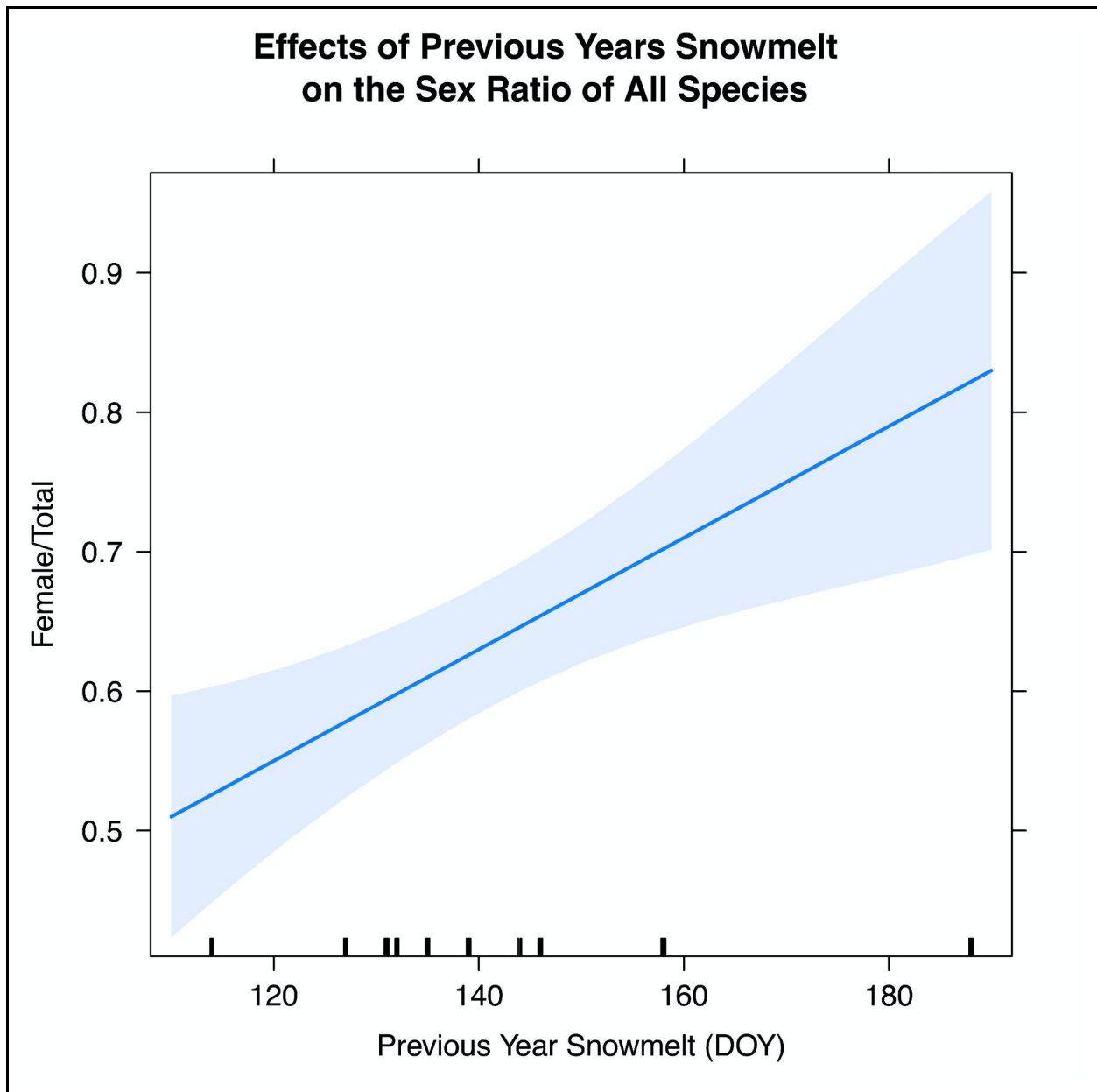


Figure 1b.

This graph shows the relationship between the proportion of female bees to the total number of bees and the previous year's snowmelt. We see that in years with early snowmelt, there is a lower proportion of female bees in the following year.

The Effect of Previous Year Snowmelt on the Sex Ratios of Below Ground Nesters

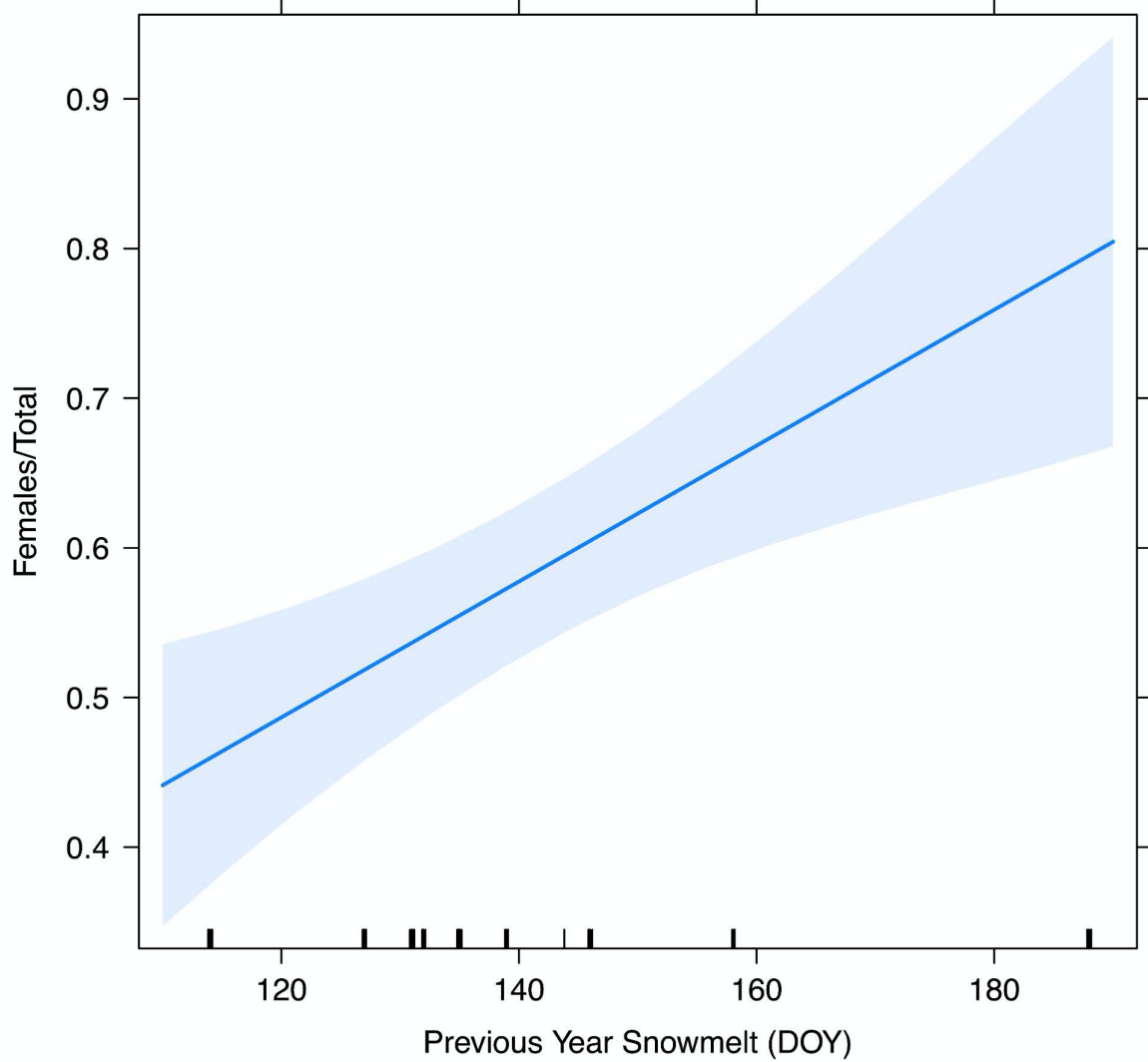


Figure 2a.

This graph shows the relationship between the proportion of female below ground nesting bees to the total number of bees and the previous year's snowmelt. We see that in years with early snowmelt, there is a lower proportion of female bees in the following year.

The Effect of Previous Year Snowmelt on the Sex Ratios of Above Ground Nesters

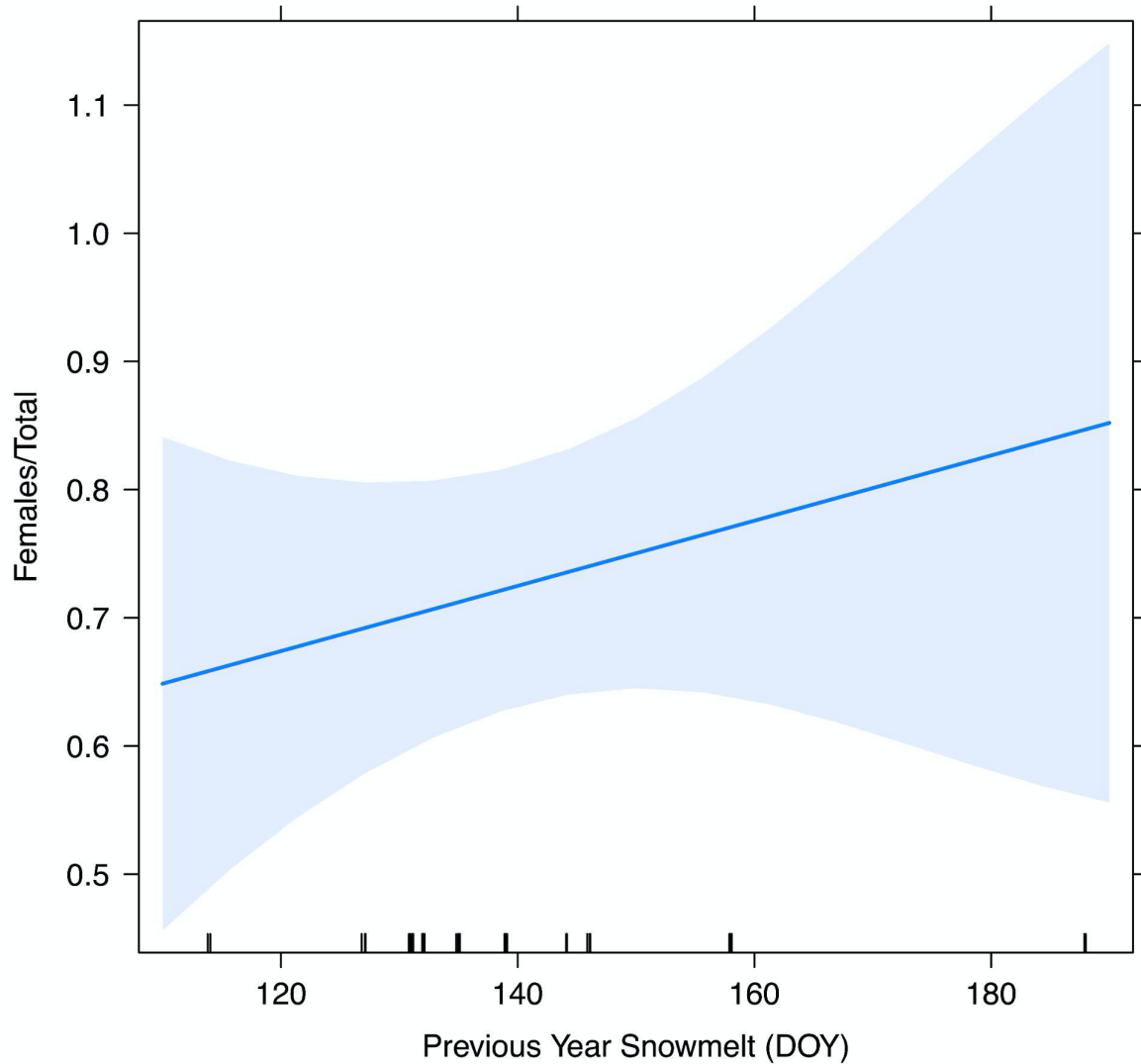


Figure 2b.

This graph shows the relationship between the proportion of female above ground nesting bees to the total number of bees and the previous year's snowmelt. We see an insignificant relationship between the proportion of female bees and the previous year's snowmelt, but we see a trend similar to those seen in figure 1b and 2a.

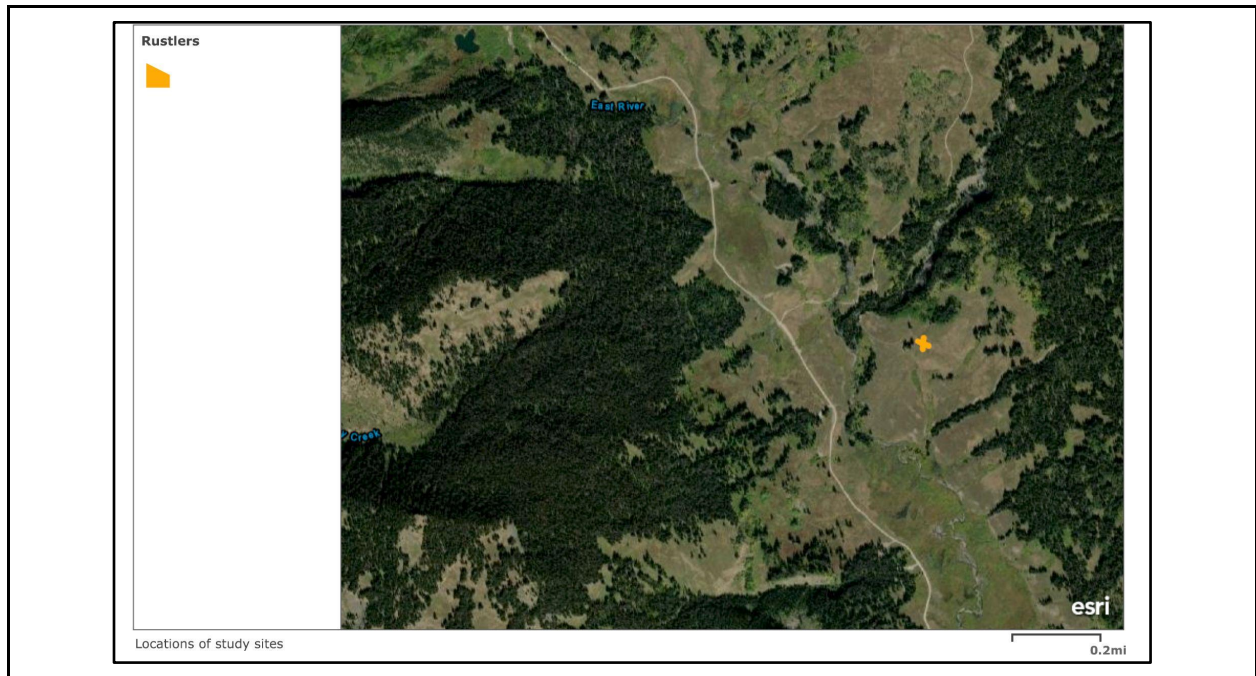


Figure 3a.

This figure shows the site known as Rustlers. Maps were made using ArcGIS Online.

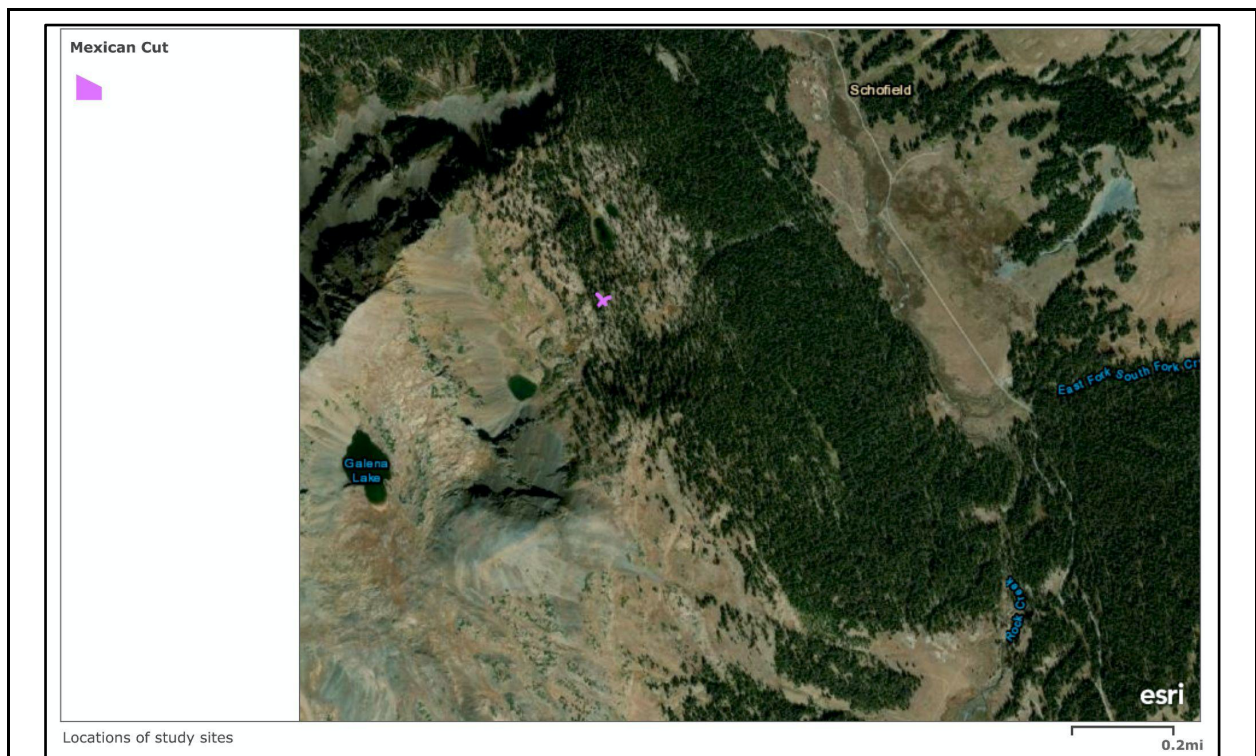


Figure 3b.

This figure shows the site known as Mexican Cut. Maps were made using ArcGIS Online.



Figure 3c.

This figure shows the site known as Lypps and CDOT. Maps were made using ArcGIS Online.



Figure 3d.

This figure shows the site known as Almont and Almont Curve. Maps were made using ArcGIS Online.

