Project Report – January 2015

Understanding the effects of nutrition and Juvenile hormone on reproductive output in alkali bees (*Nomia melanderi*)

Principal Investigator

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Justification

Reproduction is a critical aspect of native pollinator biology, because the number of pollinators available each year is limited by the number of offspring produced the previous year. Furthermore, pollination by bees is mostly the result of female bees collecting nectar and pollen to provision their developing offspring. Understanding the factors that influence reproductive output, as well as the dietary needs of female bees during different stages of their reproductive cycle is thus a crucial element of maintaining pollinator populations.

Alkali bees are important native pollinators of alfalfa (*Medicago sativa*), and thus are a valuable resource for alfalfa seed growers. I have proposed experiments to investigate the reproductive biology of female alkali bees (*Nomia melanderi*). I will conduct experiments to learn how variation in nutrition and hormones affect reproductive development. I will also investigate how dietary needs and preferences change throughout the reproductive cycle and throughout the growing season. In conducting these experiments, I will also develop methods for rearing alkali bees in the laboratory. The funding for my proposed research was received after the last field season ended, so I have not yet had the opportunity to conduct the proposed research. This report is therefore a summary of the experiments I plan to conduct in summer 2015. I have also included preliminary data that I collected during a brief visit to Touchet Valley, WA in June 2014. These data are very preliminary, and the results should thus be considered as merely suggestive until additional, more rigorous experiments are performed.

Objectives

1. Identify nutritional and hormonal factors that influence variation in reproductive potential in female alkali bees

Variation in nutrition and hormone cycling during development and in the early stages of adulthood influence reproductive activity in other bees, but this relationship is untested for alkali bees. I plan to test these factors by treating females with Juvenile hormone (JH), which is well known for its role in insect gonad development (Nijhout 1994), and by manipulating the protein content of food resources provided to bees. I will measure ovary development in females subject to hormone treatments on low or high protein diets, as compared to females on similar diets that were 'sham' or 'solvent' treated. In the sham treatment, bees will be handled the same way they would have been if they received a hormone treatment, but they will not receive any hormone. Under the solvent treatment, bees will be treated with the solvent used to administer the JH, to test for non-specific effects of the treatment. I will use newly emerged females for the first round of these experiments for two reasons. First, this is an especially sensitive time for reproductive development among bees. Females of a bee species related to alkali bees emerge with undeveloped ovaries, and protein consumption is required for their eggs to develop (Kapheim et al. 2012). Second, this is a practical way to standardize age among experimental bees. It is also a phase of the life cycle that can be easily recognized by seed growers, should practical applications arise from this research. I will repeat the experiment in larvae, as hormone and diet fluctuations during the larval stage are known to influence the ovary development and overall condition (e.g., protein and fat stores) at the time of emergence in other bees (Bloch et al. 2002, Wheeler 1996).

Preliminary data indicate that there is a great deal of variation in egg development among free-flying alkali bee females collected from the same bee bed at the same time of year. I dissected the ovaries of bees I collected from Mike Ingham's bee bed and alfalfa field, and measured the longest oocyte as an indicator of ovary development (i.e., reproductive potential). I also measured head width as a proxy for body size. In general, larger females had more developed ovaries (linear regression: F = 7.65, R^2 = 0.14, n = 49, p = 0.008, Figure 1).

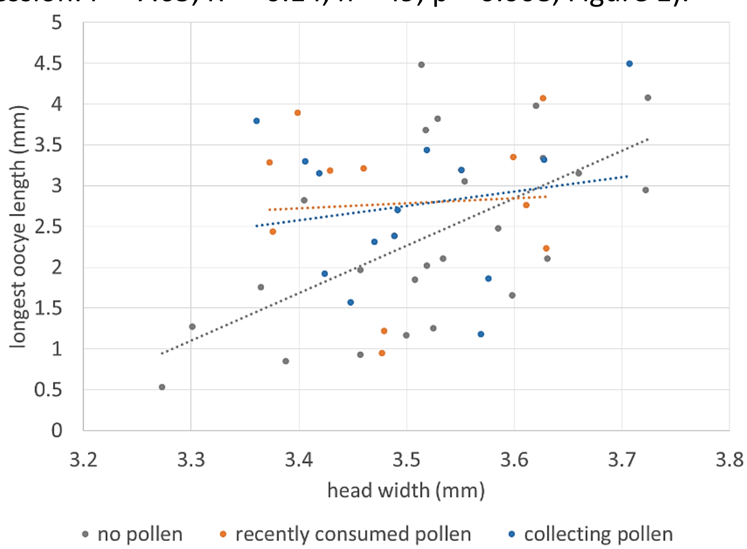

Figure 1 Body size is a good predictor of ovary size in bees that have not associated with pollen (grey), but not for bees that have been collecting (blue) or consuming pollen (orange).

Further inspection of the behavior of these bees at the time of collection suggest that this relationship between body size and ovary development may not be consistent across the entire reproductive cycle. Bees consume pollen to acquire protein for biological processes such as oocyte development. Bees collect pollen on their legs to provision the cells in which they will lay these eggs. Thus, bees associate with pollen to different extents throughout the reproductive cycle. The subset of these bees that had no pollen anywhere on their bodies at the time of collection showed an even stronger relationship between body size and ovary development (linear regression: F = 12.61, R^2 = 0.36, n = 24, p = 0.002, grey line in Figure 1). However, body size was not a significant predictor of ovary development among the bees that were either consuming or collecting pollen prior to collection (as evidenced by finding pollen in the gut during dissection or pollen on their legs at the time of collection), (linear regression: consuming pollen – F = 0.05, R^2 = 0.004, n = 13, p = 0.84, orange line in Figure 1; collecting pollen – F = 0.40, R^2 = 0.03, n = 14, p = 0.54, blue line in Figure 1). I divided oocyte length by head width to create an index of ovary development that is corrected for body size. This index was, an average, higher among bees either collecting or consuming pollen prior to collection than bees not associated with pollen, but this difference was not statistically significant ($p = 0.16$). Additional experiments are needed before conclusions can be drawn, but these data suggest that females consuming and collecting pollen *may* have more reproductive potential than females not associating with pollen. These bees were not standardized by age or nesting activity, so differences in the life cycle of these bees may also have contributed to this variation. The controlled laboratory experiments proposed for summer 2015 will test the hypothesis that protein from pollen leads to more reproductive potential.

2. Identify how reproductive potential corresponds to dietary preferences

Bees use floral nectar to provision their cells for egg-laying and to as energy to fuel flight during foraging. There is a maximum load that bees can carry, however, so they must tradeoff nectar and pollen collection during foraging. In honey bees, sensitivity to the sugar concentration of nectar changes with experience and reflects nutritional needs (Pankiw and Page Jr 1999, Pankiw and Page 2003). I have proposed experiments to determine whether similar variation in dietary needs occurs among alkali bees, and how this corresponds to reproductive activity. Preliminary data collected in June 2014 suggests that alkali bees do vary in their sensitivity to sugar concentration in nectar. I used the proboscis extension response (PER) test to determine the gustatory response score (GRS) for each bee. This is a measure of sucrose sensitivity determined by counting the responses of each bee to increasing sucrose concentrations. Bees that are the most sensitive to sucrose have a high GRS (e.g., 7). Bees that are the least sensitive to sucrose do not respond at all, and their GRS is 0. In a pilot experiment, I found that GRS scores ranged from 0-5 among 29 female bees tested at all seven sucrose concentrations (Figure 2). This suggests that the dietary needs, and thus floral visitation rates, of bees may vary with variation in irrigation practices, which can alter the concentration of nectar concentration.

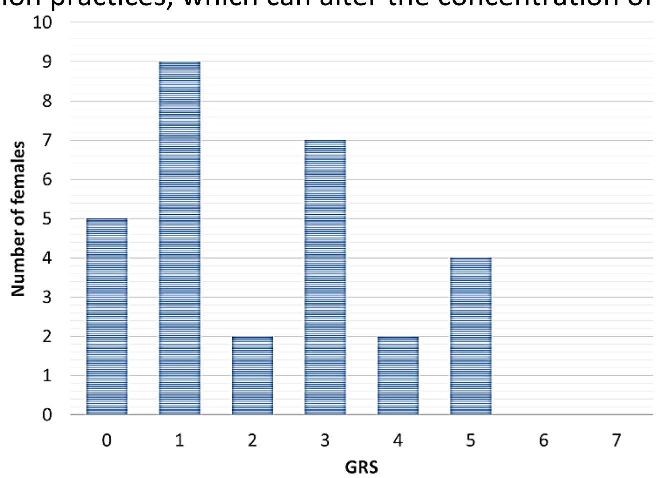

Figure 2 Female alkali bees vary in their sensitivity to sucrose concentration of nectar, as indicated by variation in the gustatory response scores (GRS) among 29 females.

Variation in how attracted bees are to different floral nectars may also depend on the stage of their reproductive cycle. Bees collect pollen to provision cells in which they lay eggs, but consume pollen for biological processes, including oocyte development. Preliminary results indicate that bees that were collecting pollen to provision their cells may be less sensitive to sugar than other females (Figure 3 left). However, there do not appear to be differences in sugar preferences among females consuming pollen and other females (Figure 3 right). Experiments planned for summer 2015 will follow up on these results, and will control for hunger state at the time of collection by feeding all bees to satiation prior to testing, which may also influence the gustatory response. I will also use bees described under objective 1 in PER assays to directly test the role of nutritional needs on dietary preferences.

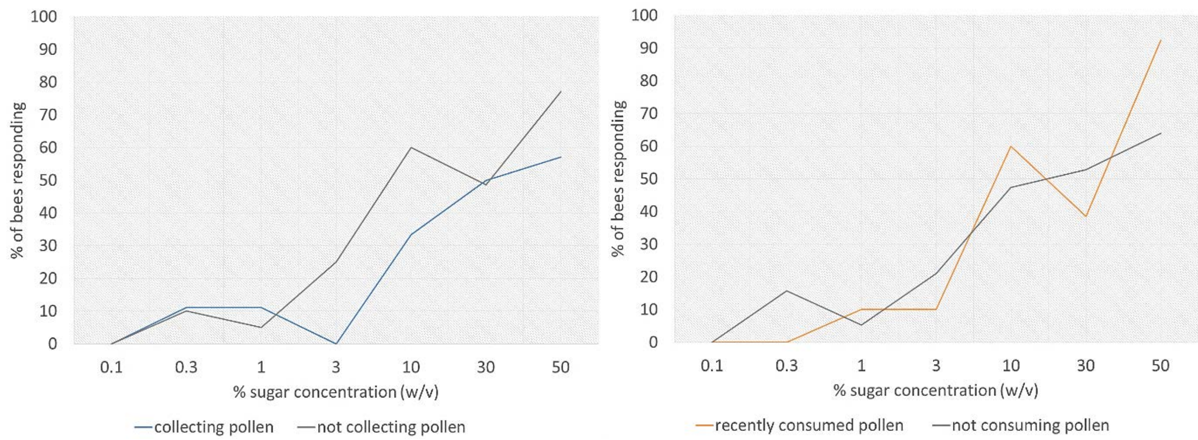

Figure 3 Response to sugar water may increase with increasing sucrose concentration slower among females in the pollen collection phase of their reproductive cycle (blue line on left) than among other bees (all other lines).

3. Develop rearing methods for alkali bees in the laboratory

An important component of a successful pollinator management strategy is the ability to rear bees under controlled conditions. This provides the opportunity for monitoring and treatment at early life stages, as well as the ability to sync emergence with crop cycles. I will develop lab-based rearing methods for alkali bees during summer 2015 and continue refining these methods in 2016. I expect to try a variety of artificial cell materials, temperature, and humidity regimes.

References

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