

Assessing the Effects of the Conservation Reserve Program on Cavity-Nesting bees in Colorado

Agroecosystems

By

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ABSTRACT

The conversion of natural habitat for agricultural use continues to be a prevalent threat to wild pollinator populations, although the establishment of semi-natural habitats in agroecosystems can successfully bolster bee populations. Conservation Reserve Program (CRP) plantings provide a unique opportunity to understand the effects of conservation efforts on wild bee populations. To assess the conservation value of CRP plantings for native pollinators, I analyzed the contents of trap nests deployed in 29 field sites over the 2014 flowering season, including 18 CRP grassland plantings and 11 rangelands in northeastern Colorado. I compared the abundance and diversity of cavity-nesting bees between site types, and explored mechanisms driving reproduction and performance of the most common native cavity-nesting bees. Despite significantly higher plant species richness on rangeland sites, CRP plantings supported greater wild bee abundance and *Megachile* spp. fecundity, although there was no difference in genus richness between the two land types. Additionally, there was no significant difference in the body size of native female *Megachile brevis*. Only one introduced species was present in our samples (*Megachile rotundata*), and was not found in rangeland sites, suggesting that rangeland plant communities may play an important role in supporting native bee species. These results indicate that compared with rangelands, the Conservation Reserve Program provides significant benefits to wild bee populations. Further studies should investigate the persistence of these patterns over multiple flowering seasons and potential mechanisms driving cavity-nesting bee population dynamics within these agroecosystems.

Keywords: Conservation Reserve Program (CRP), rangeland, cavity-nesting bees, nests, cells, *Megachile*

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INTRODUCTION

The pollination services provided by native bees are essential to both agricultural and natural ecosystems (Michener 2007, Willmer 2011) especially given increasing pressures on food production by population growth and cropland acreage limitations. The abundance of pollinators directly relates to the seed set, fitness, and fruit quality of a plant (Calderone 2012), and economic estimates have valued crop pollination services provided by wild bees at more than \$3 billion in the United States alone (Hoffman Black 2011). It is estimated that in North America, a complete loss of native bee pollination services would result in the disappearance of seven out of the 60 agricultural crops that are essential to agriculture, like alfalfa, from the agricultural landscape (Ghazoul 2005). Additionally, wild bees pollinate approximately one third of the crops produced for livestock feed and grazing, which make up about 80% of the economic value of insect pollinators (Gallai et al. 2009). Despite the importance of pollinators in grazed landscapes, we still know relatively little about how land management in grazed systems impacts pollinator community dynamics, growth, or reproduction.

Given this reliance on pollination services, the popularly coined “global pollination crisis,” refers to the current paradox of greater demands for pollinator services in conjunction with decreased pollinator abundance and diversity. These declines are most commonly discussed in the context of managed honey bee populations. On a global scale it is true that the population of managed honeybees is increasing; however, this population is a) not increasing at a rate that matches the increase in pollinator-dependent crops and b) decreasing somewhat in Europe and drastically in the United States (Calderone 2012), declining 40% since the 1990’s (Cox-Foster et al. 2007). These declines have been attributed to introduced diseases, insecticide use, and habitat loss (Kremen et al. 2002, 2007, Aizen 2009). The susceptibility of honeybees to parasites and

pathogens most likely stems, at least in part, from the lack of genetic diversity within populations due to large honeybee breeding facilities (Winfree 2008). This decline has driven up honeybee rental costs, especially in the United States, from \$19.25 per colony in 1992 to \$89.90 per colony in 2009 (Bauer and Wing 2010). This increased cost to producers will continue to drive up crop prices and threaten global food security. Most importantly, despite global increases in honeybee hive abundance, pollinator-dependent crops are believed to have increased by more than 300% in the same period of time (Potts et al. 2010), which could lead to mass shortages in pollination services unless honeybee populations are complemented by wild bee pollinators.

While monoculture practices are considered to be the main driver of habitat loss for pollinators, the establishment of natural and semi-natural habitats in largely agricultural landscapes benefits wild bee populations through increasing floral diversity and thus diminishing the effects of habitat fragmentation caused by agricultural intensification (Rader et al. 2013). Monoculture cropland generally lacks the natural or semi-natural habitats that are conducive to diversified food resources and nesting sites for native bees (Ghazoul 2005). In addition, crop monocultures lack the diversity of sugars, amino acids, micro-vitamins, and minerals that bees are so highly reliant upon, not only for adult nutrition, but larval nutrition as well (Garibaldi et al. 2011). However, effective maintenance of areas that can serve as buffers for bees can provide pollination services in less-intensive agricultural systems where reduced pesticide use exists and the retention of nearby semi-natural habitat for nesting sites is maintained (Ghazoul 2005). Research shows that agricultural practices such as these are capable of maintaining diverse wild bee populations that maximize productivity and complement honeybee pollination services (Rader et al. 2014). Thus, cropland located near natural and semi-natural habitats seems to generally be more successful in maintaining wild bee abundance and diversity (Greenleaf and

Kremen 2006; Ricketts 2004; Klein et al 2003; Kremen et al 2004, 2002; Morandin and Kremen 2013). In addition, crops found in these systems generally have increased productivity, including enhanced yields (Ghazoul 2005). While hedgerows and roadside edges are well-researched habitat buffers for bees in agricultural ecosystems, non-traditional, semi-native habitats such as rotational fallows and rangelands have recently been targeted for their potential conservation value to wild bee populations (Stoate et al 2001, Le Feon et al 2010).

Given the role of wild pollinators as essential buffers for crop pollination and the importance of habitat conservation in maintaining their populations, the impacts of land management practices on wild pollinators are still relatively understudied in many agriculturally intensive areas. In regions dominated by industrial agriculture, marginally productive land may be set aside for conservation plantings, or be used as rangeland for livestock (Kothmann 1995). Conservation plantings have relatively low disturbance rates compared to surrounding land under production, and provide the potential for diversified vegetative ground cover (Dunn et al.1993). While research indicates the importance of intermittent natural or semi-natural habitats in supporting wild bee populations (Winfree 2010), the extent to which these conservation plantings are beneficial has yet to be determined. In order to move forward in creating efficient conservational strategies, it is imperative to understand how land-use affects bee communities and how current land-use and conservation practices may impact not only bee abundance and diversity, but also bee reproduction, performance, and population growth.

With increasingly intensive agricultural practices, rotational fallows such as those established by the Conservation Reserve Program (CRP) were recognized as essential to maintain land quality. Created in 1985 as part of the Food Security Act (Dunn et al. 1993), the Conservation Reserve Program was initially created to incentivize farmers to retire

environmentally sensitive and highly eroded land from production. To do so, the CRP provides an annual per acre rental payment to landowners, as well as half the cost of establishing vegetative land cover (e.g. trees, grasses, forbs) to aid in soil remediation (Ribaudo et al. 2001). While the original goal of the CRP was to minimize and reverse soil erosion on exploited cropland, the recognition of additional benefits to reestablishing semi-natural landscapes led to revisions of the CRP's objectives, including the creation of wildlife habitat under the State Acres for Wildlife (SAFE) practice (USDA 2008). Five years after implementation, the Food Agriculture, Conservation, and Reform Act (FACTA) created an environmental benefits index (EBI) to evaluate an expanded range of potential benefits of the CRP when considering prospective leases. The 1990 EBI thus included water quality and tree establishment considerations, in addition to soil quality estimations. In 1995 and again in 1996, the EBI was revised to explicitly incorporate the benefits to wildlife of establishing vegetative ground cover. These considerations comprised close to 30% of all evaluated benefits, indicating the extent of the CRP's potential to improve wildlife conditions (Ribaudo et al. 2001).

Prior to 2008, pollinator conservation efforts focused mostly on honeybee conservation and was limited to the Conservation Security Program and CRP SAFE practices (USDA 2008). In 2006, the National Research Council (NRC) released the *Status of Pollinators in North America*, followed closely by the 2008 Farm Bill, which extended pollinator considerations to all USDA conservational factions. Specifically, the Farm Bill authorized USDA incentive-based conservation programs to take practices supporting pollinator habitat under special consideration for payment (Xerces Society 2008). However, this bill was limited in its implementation to 'encouragement of pollinator habitat development and protection' (USDA 2008), leaving specific strategies for conservation generally ambiguous.

Despite this effort by the USDA to incorporate the creation of wildlife habitat into the objectives of the CRP, there currently is no strategy to evaluate the success of this initiative after leases expire. The CRP is the largest U.S. agricultural conservation program and has leased over 33.5 million acres, or 10% of cropland, in the U.S. (Ribaudó et al. 2001). Given the explicit objectives of the EBI in assessing potential sign-ups, CRP land provides an ideal study system for evaluating the effectiveness of varying conservation strategies. However, while the EBI was created to determine projected environmental benefits, there has yet to be a standardized measure of change in environmental quality (US GAO 1993) and realized benefits of the CRP upon contract completion. Additionally, critics of the CRP point to the lack of evidence-supported estimates of cost effectiveness, which are evaluated utilizing the predicted benefits of the program as outlined by the EBI, rather than incorporating data on long term changes in environmental quality over the course of a CRP lease (Ribaudó et al 2001). Not only would consistent follow-up to make these measurements helpful in the justification of the CRP, but could help develop a more effective EBI going forward. While there is minimal understanding of the environmental benefits of the CRP, there is even less regarding the effect of CRP plantings on bee communities. Recent efforts, as outlined below, have begun to broaden in conservational scope, but still currently lack follow through assessments of efficacy.

In 2012, the CP-42 Pollinator Habitat Program was established to specifically target bee conservation through the cultivation of pollinator habitat on rotational fallows leased under the CRP. This program provides a diverse seed mix of native flora, when available, with varying bloom periods to sustain bee populations throughout the flowering season. The USDA justifies this investment in pollinator health by highlighting the positive effects of increased bee pollination on crop production in surrounding agricultural land (USDA 2012). Upon inclusion of

the Pollinator Habitat Program, the Natural Resources Conservation Service (NRCS) expanded the EBI to include evaluations of potential pollinator habitat and current pollinator status in their administration of the CRP with new enrollments (Decourtye et al). The 2014 Farm Bill was revised to incorporate terms of ineligibility for contract termination if there is evidence that land leased by the CRP is pollinator habitat (Stubbs 2014). As of 2014, there was an estimated 5,499 acres of land enrolled in the CRP with pollinator habitat conservation initiatives (Stubbs 2014). The addition of CP-42 planting practices to the CRP was one of the first widespread initiatives for pollinator conservation in the United States, reflecting a growing awareness of the importance of maintaining not only honeybee but also native bee populations.

While much research has been devoted to the impacts of agriculture on wild bees, we know comparatively little about how grazing impacts bee communities. Rangeland comprises approximately 25% of global land area and provides 10% of the world's meat supply (Alkemade et al. 2012). Determining the value of rangeland for native bees is difficult, given the diversity of rangeland ecosystems, and potential responses of bee communities. Compared with cropland, rangelands typically have richer native floral resources (Kothmann 1995) and thus may provide better habitat for bees (Werling et al. 2014). This is especially applicable in the United States, where a majority of land used for livestock grazing has never been tilled for intensive crop production and was historically native prairie or grassland adapted to intermittent grazing by bison (Alkemade et al 2012; Milchunas et al. 1988; Knapp et al. 1999). Compared with rangeland, CRP land typically prohibits grazing (Dunn et al. 1993) and the type of planting assigned to a lease determines the initial plant community, typically not including a wide diversity of native forbs (Plantinga et al 2001; Stubbs 2014). Rangeland has been considered "semi-natural habitat", along with roadside edge and hedgerows, all providing necessary

foraging and nesting resources for pollinators within agroecosystems (Cingolani et al. 1005; Kothmann 1995; Biondini et al. 1998). However, rangeland composition varies regionally and throughout time, depending on seasonal variations in temperature and precipitation (Fuhlendorf and Engel 2001, Kothmann 1995) with potentially different implications for pollinators.

The impact of rangeland use on bee communities depends on a number of factors. On the one hand, periodic and moderate grazing has been shown to increase the diversity of plant communities compared with non-grazed land (Biondini et al. 1998, Fuhlendorf and Engel 2001), which could benefit bee communities by supporting specialist bees or creating redundancy in floral resources over time (Winfree 2010). On the other hand, the intensity and timing of livestock grazing could have drastic negative effects on the timing or floral composition. For example, moderate grazing by livestock could result in competition with bees early or late in the flowering season, due to the seasonal limitation of pollen resources (Alkemade et al. 2012). On the Great Plains of the Western United States, drought seasons must be met with a drastic decrease in stocking rates or complete cessation of grazing to avoid the long-term damage that follows overgrazing during a drought (Kothmann 1995). The diversity of factors that influence grassland carrying capacity for wildlife and livestock grazing vary on broad spatial-temporal scales, which has historically presented a challenge for rangeland management policy makers (Kothmann 1995). Subsequently, very little is understood about pollinator populations in these habitats. This study aims to address this gap in knowledge by comparing bee communities on CRP plantings and rangelands. Both of these land types have experienced anthropogenic disturbance to differing extents, while still remaining essentially under human-use. The contrast in management strategies between CRP and rangelands allows us to compare pollinator

communities on these semi-natural habitats and potentially inform effective future approaches in conservation.

To determine the effects of land management practices on cavity-nesting bee communities within agroecosystems in Colorado, I used samples collected as part of a broader USDA-funded research project examining wild bee communities across 32 sites in the most agriculturally intensive region of northeastern Colorado. CRP enrollment in Colorado covered some 1,962,173 acres in 2014, although currently, only 962 acres of CRP land in Colorado are designated as pollinator habitat (USDA). The sites from which the cavity-nesting bees were sampled included both CRP-leased land and rangeland used for livestock grazing. These two landscapes differ greatly in disturbance regimes and plant community composition, providing an ideal study system to examine how these land-use factors affect pollinator populations. Using trap nested bees, I was able to ask both population and organismal-level questions regarding the current state of cavity-nesting bee communities in these grassland agroecosystems. Specifically, I asked: 1) How does land use affect the abundance and diversity of cavity-nesting bees? 2) Does bee reproduction and performance vary between land-use types? And, 3) what mechanisms drive cavity-nesting bee abundance, reproduction, and performance?

While, exploratory in nature, these questions followed a number of predictions based on initial observations and could have different implications, given competing hypotheses about bee response to both rangeland and CRP habitats. On the one hand, rangeland in our study area approximates ‘natural’ habitat, maintaining the landscape of native grassland steppe and potentially providing diverse native plant cover compared to the traditionally homogenous plant diversity of CRP plantings. Because native bees typically display preferential foraging on native flora (Moissett and Buchmann 2011), rangelands have the potential to attract a more diverse or

abundant community of wild bees. On the other hand, persistent disturbance regimes of livestock grazing on rangeland may shift resource abundance to be the primary limiting factor of bee populations, making undisturbed CRP plantings more attractive habitats for large populations of bees. Overall however, I did expect to find a greater abundance of cavity-nesting bees on CRP land due to my initial observations of high plant abundance compared to rangeland sites. Regardless of outcome, this study could contribute to our understanding of the benefits of conservation in agroecosystems, in addition to understanding potential changes in conservational strategies that could increase these benefits, especially in light of decreasing acreage enrollment in the CRP (USDA).

METHODS

Study Area

The solitary cavity-nesting bees used for this study were collected across five counties in Northeastern Colorado during the summer of 2014 (Figure 1a). Thirty sites were successfully surveyed for cavity-nesting bees, including 18 CRP plantings and 11 actively grazed rangelands (Table 1). The sites spanned three east-west corridors (hereafter, routes) roughly 100km long (designated North, Middle, and South, Figure 1b.). The region is agriculturally intensive, dominated by cereal crops such as corn and wheat, but also extensively used as rangeland for livestock (Schwantes 2015). Acreage enrollment per county within our study area ranged between approximately 39,000 to 144, 000, totaling 522,633 acres that were under CRP lease in 2014 (USDA 2014). The five counties in this study make up one quarter of total CRP enrollments in Colorado in 2014.

Short-grass steppe comprises 15 million acres of Colorado's eastern plains, making it the region's primary ecosystem (Mitchell 1993, Figure 1c). Grazing has long been an established practice on Colorado short-grass steppe, stemming back to wild bison populations prior to the establishment of commercial crop (Milchunas et al. 1988; Knapp et al. 1999; Fuhlendorf and Engle 2001) and livestock production. Ideally, dominant grass, forb, and wildlife species are well adapted to the kind of disturbance associated with moderate bison grazing. However, as livestock production has surpassed the levels of historical grazers (Kothmann 1995), there is an increasing threat for the decline of native species in rangeland habitats. Alternatively, large-scale agriculture eliminates entire natural habitats as a regular practice, setting the bar for rangelands classification as semi-natural agricultural landscape. Recently, the increasing use of rotational fallows to prevent the overproduction of crops and soil erosion puts temporarily retired cropland, termed

“set-asides” in the same category of agricultural semi-natural habitat. Thus our study compares two semi-natural habitats that have the potential to support wild bee communities while persisting under some degree of management to facilitate agricultural and livestock production.

Figure 1. Study sites were distributed through Phillips, Yuma, Washington, Logan, and Kit Carson Counties (a), and were split into three routes: (b) ‘North’ sites are in blue, ‘Middle’ in yellow, and ‘South’ in red. Much of the landscape, including the rangeland, was characterized by short-grass steppe (c).

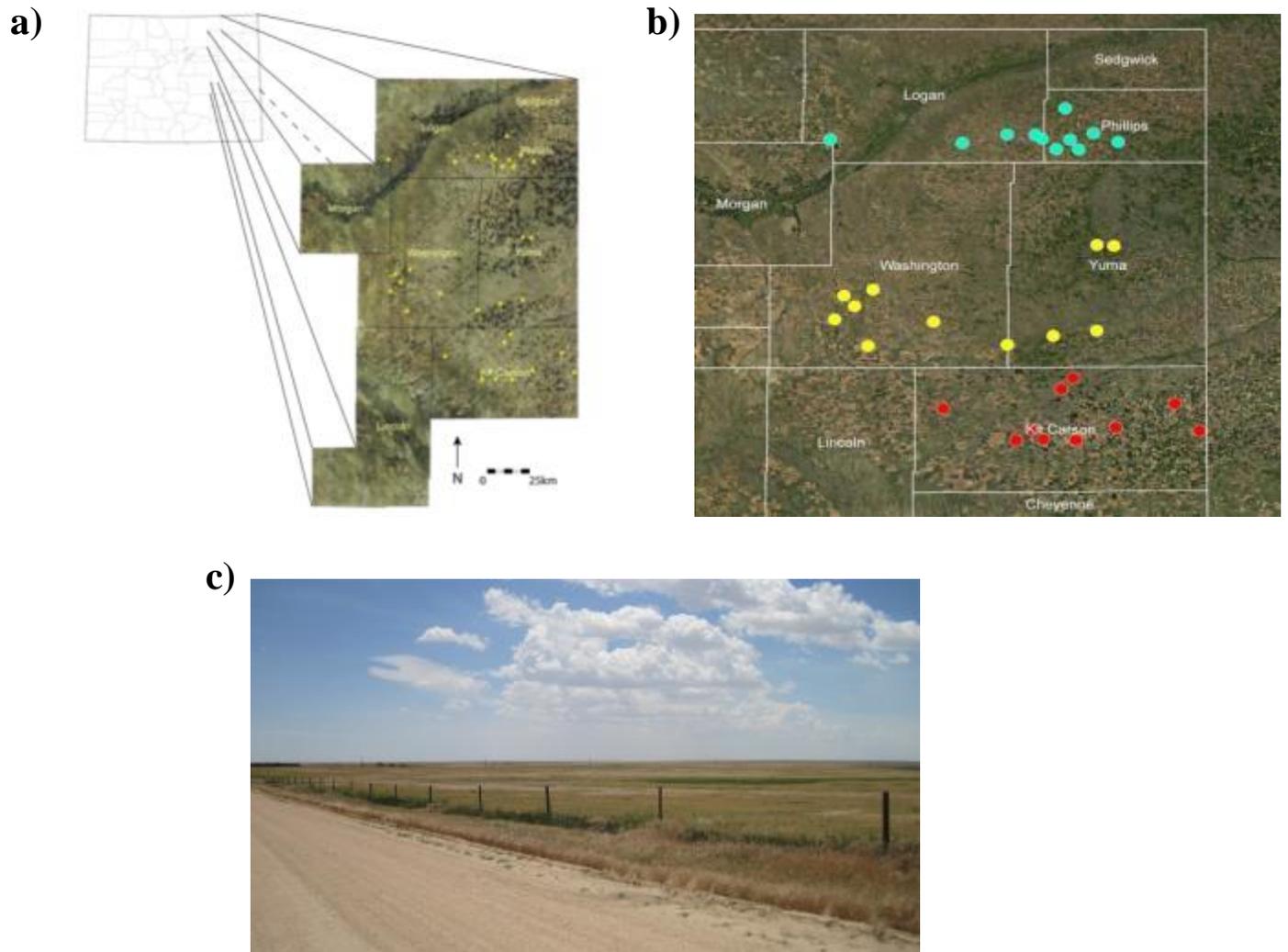


Table 1. Study sites are listed by site name, land-use type, and survey route.

Site	Type	Route	Latitude	Longitude	County
BA	CRP	Northern	40.52114N	102.97203W	Logan
BR	CRP	Middle	39.39426N	103.04741W	Kit Carson
CE	CRP	Middle	39.66435N	102.80456W	Washington
CR	CRP	Middle	39.77224N	103.45949W	Washington
ER	CRP	Middle	39.76278N	103.08356W	Washington
ET	CRP	Northern	40.55724N	102.80530W	Logan
H2	CRP	Northern	40.53902N	102.67283W	Logan
H3	CRP	Northern	40.49420N	102.53403W	Phillips
H4	CRP	Northern	40.56210N	102.47756W	Phillips
K1	CRP	Southern	39.31379N	102.39314W	Kit Carson
K2	CRP	Southern	39.41555N	102.16851W	Kit Carson
K3	CRP	Southern	39.29972N	102.07599W	Kit Carson
JW	CRP	Middle	39.89826N	103.31391W	Washington
LE	CRP	Northern	40.52546N	102.38425W	Phillips
MA	CRP	Middle	39.65989N	103.33290W	Washington
ME	CRP	Northern	40.53487N	103.47584W	Logan
N2	CRP	Middle	40.08410N	102.40041W	Yuma
PI	CRP	Southern	39.30438N	102.71327W	Kit Carson
C1	Range	Southern	39.2602N	102.77151W	Kit Carson
C2	Range	Southern	39.2604N	102.54388W	Kit Carson
DU	Range	Northern	40.66829N	102.58486W	Phillips
FR	Range	Middle	39.72453N	102.46528W	Yuma
KW	Range	Middle	39.82835N	103.38353W	Washington
LC	Range	Middle	39.87335N	103.42435W	Washington
L1	Range	Southern	40.52546N	102.38425W	Kit Carson
L2	Range	Southern	39.52397N	102.55627W	Kit Carson
SH	Range	Southern	39.26452N	102.66809W	Kit Carson
SC	Range	Northern	40.49528N	102.61854W	Phillips

Trap-nesting bees

To sample cavity-nesting bees, I (as part of a team of researchers) used two types of trap nests: bundles of bamboo internodes and pine nest blocks (Figure 2a-b), both of which are effective at capturing cavity-nesting bees and can provide unique insights into their biology and population dynamics. Bamboo trap nests were constructed of pieces of bamboo (3/8-7/16" in diameter) approximately 30 cm in length with the pith removed to create a hollow, cylindrical corridor ideal for cavity nesters. Each piece possessed a node at one end, ensuring that the bamboo was open at only one end for bees to enter and closed at the other. We bundled approximately 40 randomly selected pieces of the hollowed bamboo using duct tape, with each open end facing the same direction, ensuring easy access for the female cavity-nesting bees (Figure 2a). When installing the traps at each site, we secured the bundles with zip-ties to wooden stakes approximately 30 cm above the ground. We built and installed thirty bamboo nests in 2014, in addition to bee block trap nests, made from 1" x 6" pine common boards approximately 30 cm in length secured together with bolts (Figure 2b). Each block was drilled with 40 holes along the margins of the boards. Five holes of 8 different diameters (ranging from 1/16th to 1/2 inch) were drilled along each conjoining board to ensure the easy removal of nests at the end of the field season. We placed the bee blocks at each site using a stake inserted low to the ground, with the open holes facing away from the stake.

Trap nests were left in every site from late May over the course of the flowering season. We collected traps in September after the majority of bees had nested. Upon collection, bamboo bundles and bee boxes were placed in plastic bags and left for several months to allow for potential developmental completion of provisioned eggs, larvae, and pupae. For some species, particularly in the genus *Megachile*, many late-summer broods overwinter in a pre-pupal,

diapause state. However, because early-summer broods often skip diapause to complete development and produce a second generation (Pitts-Singer and Cane 2011), all nests were left closed till late fall of each season to maximize developmental completion of live, early-stage specimens.

We dissected all bamboo and wood to extract nests and all provisioned cells from each trap nest. Each inhabited cavity was counted as one nest, given that each female typically constructs one site in her lifetime. Pine nest boxes had previously assigned letter/number combinations for each cavity, while bamboo nests were randomly assigned unique numbers within a site, which included the site name and the number, to create a unique “nest I.D.” for later analyses. Date and locality information were recorded for each nest extracted. I assumed that nests that contained closed cells upon extraction contained developing juveniles. All cells were separated into individual 1oz Solo® Clear Plastic Cups (Dart Container Corporation, Mason, MI). Each cell container was labeled with either a consecutively assigned Nest ID for bamboo traps or the respective letter/number nest I.D. for pine boxes (Figure 3). Cells were numbered from the back of the cavity to the front, in the order they were constructed and provisioned. Descriptive information was recorded regarding nest material, the condition of a cell (emerged/open, non-emerged/closed, or unclear), the life-stage of bees found in open cells, a description of open-cell provisions, and a description of open-cell bees (genus, sex, etc., Figure 4-5).

Non-emerged cells were left as such for approximately four months to allow non-diapausal bees to complete development. Cells that had not emerged after this time were then refrigerated for approximately 14 days to mimic winter temperatures, upon which they were removed and left at room temperature (68-74°F) for another 14 days. This manipulation of

temperature and photoperiod cues imitated the conditions necessary to break diapause in overwintering bees. After inducing emergence, I dissected any remaining unemerged cells to determine their contents. Dissected cells contained bees in all developmental stages, from eggs (assumed by the presence of a nectar/pollen provision), larva, pupa, and adults with pre-emergence, post-developmental completion mortality. These adults, as well as adults found throughout the collection process were pinned, labeled, and subsequently used to make species-level determinations. Any undeveloped bees, including larvae and pupa, were separated from the nest substrate, placed in gelatin capsules (Capsule Connection, LLC, Prescott, AZ), labeled, and then pinned in conjunction with their cell contents (Figure 3c). All specimens are being curated in the Entomology Section of the University of Colorado Museum of Natural History.

Figure 2. Two types of traps were used to sample cavity-nesting bees: (a) block nests and (b) bamboo bundles. A majority of nests collected were both from bamboo bundles and constructed by *Megachile* spp. bees (c).

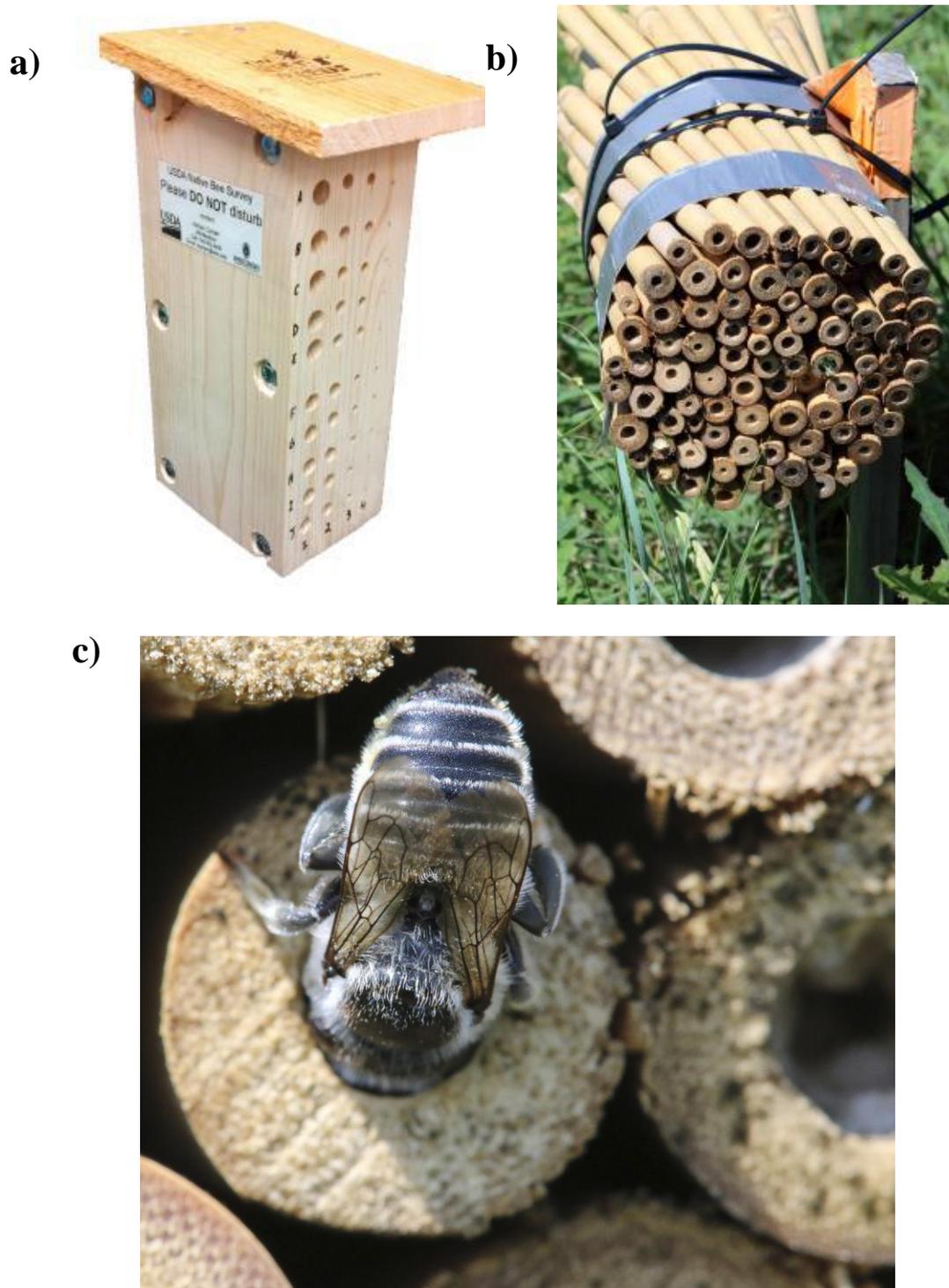


Figure 3. Each Solo® container holds one cell and if applicable, it's corresponding bee (a). Each container was labeled with site, date collected, nest, and cell number (b). The last step in curating our samples was to place all cell material into gelatin capsules, which were then pinned with a printed label (c).

a)



b)



c)



Richness and Abundance

Species I.D.'s were made for all adult bees by Virginia Scott, (University of Colorado, Boulder, Collection Manager of Entomology). Given limited literature regarding the systematics of *Osmia*, the bees we found in this genus were identified to morpho-species only. Additionally, for completed nests lacking adult bees, genus-level determinations were assigned according to nesting substrate. Each of the four genera identified have distinctive preferences in nest material (Appendix II, Figure 5, Figure 6, Figure 9), allowing for clear differentiation between genera based on the types of nests they constructed. On a foundational level, these assumptions were made using existing literature outlining nesting behaviors. Additionally, nests from which adult bees were extracted were used as reference for assigning genus-level determinations to empty nests (Figure 9). Given the multivoltine life-history of most *Megachile* spp (Appendix II) and my observations of noticeably more *Megachile* spp. nests (leaf and petal substrate, Figure 4a-b, 5b) than nests from other genera, I had reason to expect that nest abundance would be largely biased towards *Megachile*. I used total nest abundance and *Megachile* nest abundance as the response variables in our analyses of abundance (see *Statistical Analyses*).

To estimate richness, I totaled the number of unique species and genera observed from each of the sites. These counts were used as response parameters in my nest-level analyses. Nests that contained at least one cell with an identifiable adult bee were assigned a species-level I.D. under the assumption that empty cells within the same nest were constructed by the same female and were either emerged or had pre-development mortality, depending on placement within the nest. Because I had no way of knowing the exact fate of each cell, no assumptions about offspring success within individual cells were made. Within each genus, I calculated the total

number of nests per site. This count of nests-per-genus was my primary response variable for the diversity analyses (see *Statistical Analyses*).

Figure 4: Bees that completed development after traps were removed from their sites either emerged during processing, like this male *Megachile rotundata* (a), or did not successfully emerge, as in this case of a post-mortem, adult male *Megachile rotundata* (b).

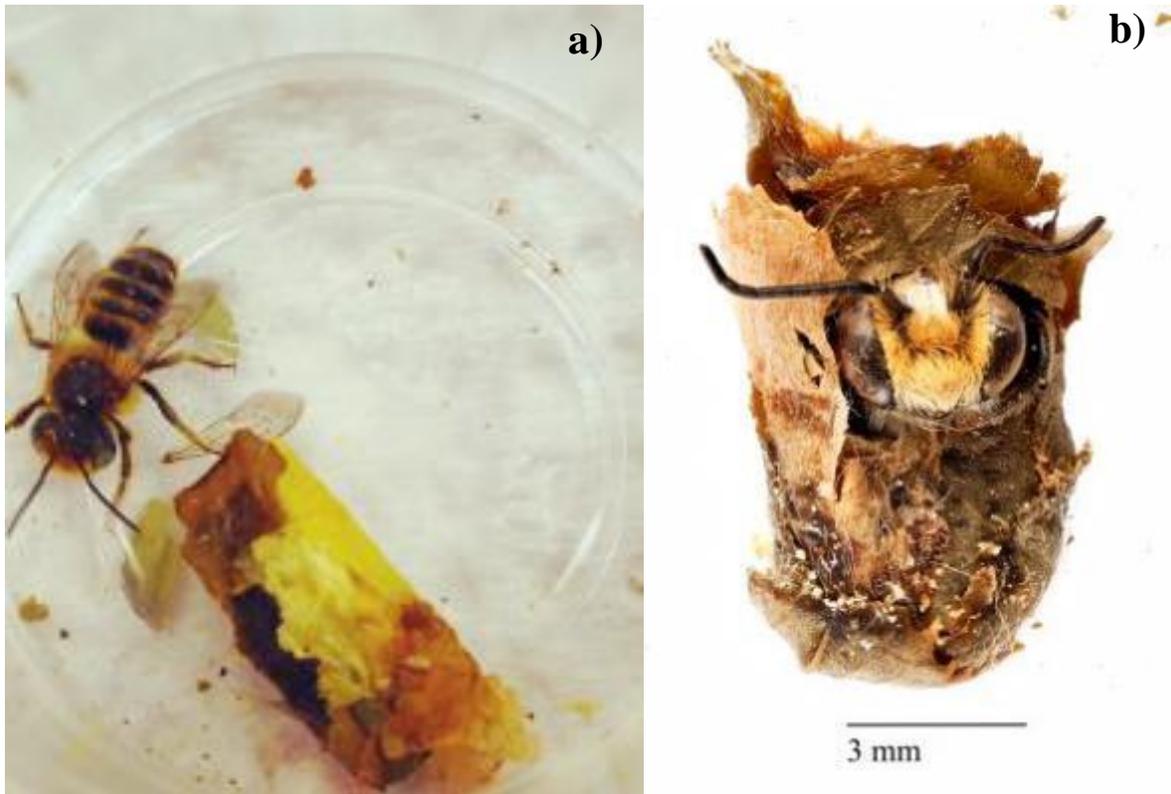
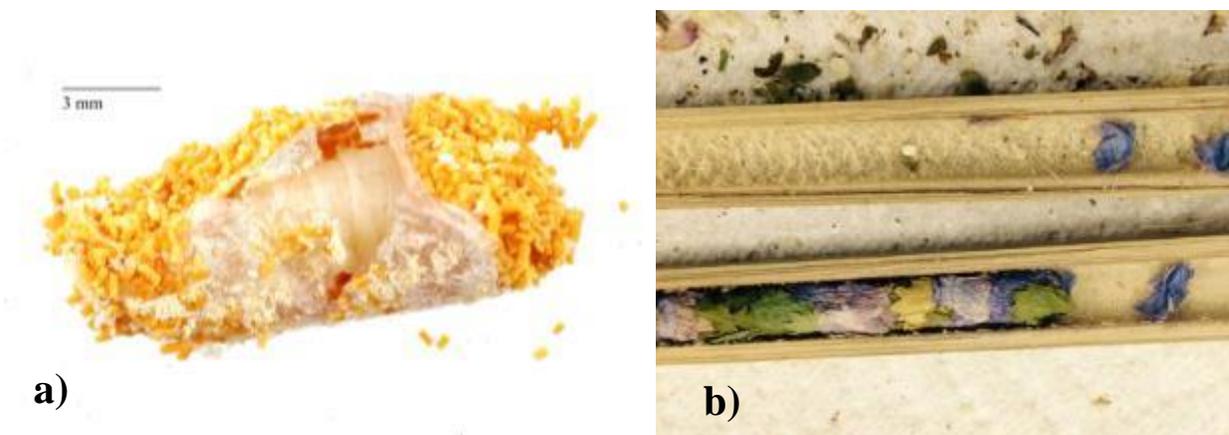


Figure 5: *Lithurgopsis apicalis* cells were easily identified by large *Opuntia* spp. pollen grains (a), while leaf and petal pieces were characteristic of *Megachile* nests (b).



Reproduction and Performance

Given that the provisioning of cells within a nest directly reflects a female bee's reproductive effort, the number of cells within a nest can be used as an estimate of reproductive effort and fecundity. Fecundity generally refers to an individual's reproductive capacity and can be measured by the number of eggs produced, offspring survival, offspring body size, and a number of other factors (e.g. Honěk 1993, Allaby 1994, Paini et al 2004). While a female cavity-nesting bee can oviposit up to two eggs in one cell, no more than one individual typically completes development within a cell (Rozen and Hall 2014). Additionally, it is unlikely that a female bee would expend energy to build and provision a cell without depositing an egg. Thus, in this study I utilized the number of cells constructed per nest as an index of female egg-laying success and as estimate of her fecundity.

Individual cells were easily identifiable by either their distinctive partitions or by the presence of a larval provision. *Megachile* use leaf or petal caps to close completed cells (Pitt-Singer and Cane 2011; Michener 1953). *Lithurgopsis* compose their cells almost entirely of *Opuntia* pollen (Rozen and Hall 2014), and *Osmia* and *Ashmeadiella* use mud and dirt to line the inner walls of a cavity with larval provisions serving as cell dividers (Cane et al. 2007; Sheffield et al 2011; Michener 1939). Additionally, *Osmia* cells were consistently larger than *Ashmeadiella* cells, facilitating differentiation between the two mud cells (Appendix I, Figure A3). Cell numbers were assigned by relative position within a nest (Figure 6). Thus Cell #1 corresponded to the first cell constructed by a female, found either at the nodal end of a bamboo segment or at the back of a pine block. The next cell was counted as Cell #2, then Cell #3 etc., continuing through the last cell of the nest, located nearest the cell entrance. The total number of cells was recorded for each nest. Again, due to the bias of nest and cell abundance towards

multivoltine bees (*Megachile* in our study), I chose to only use *Megachile* cells per nest in analyses (see *Statistical Analyses*) (Figure 6a-b).

Body size in female bees is often used as a measure of performance and fitness, given a strong positive relationship between mother size, egg production, and offspring size, which is positively correlated with larval over-wintering success (Tepedino and Torchio 1989; Seidelmann 2014; Bosch and Kemp 2004). While opportunistic polygyny in solitary male bees results in a non-significant relationship between male body size and mating success (Seidelmann et al 1999), larger females have greater capacity for transporting floral resources to a nest and can provision more cells in a shorter amount of time (Tepedino et al. 1984, O'Neill et al. 2010). Larger bees also tend to have proportionally larger wings that allow for greater foraging distances (Cane 1987). J. H. Cane established the use of intertegular span as an accurate measurement of non-eusocial bee body size in 1987. Tegulae are sclerites covering the base of the forewings and are a maintained morphological characteristic across all bees and many insects (Figure 7a-b). Cane's research shows that there exists a significant relationship between the dry weight of a bee and the distance between the inner margins of each tegula (Figure 7c) (Cane 1987).

For emerged bees, I used a dissecting stereomicroscope fitted with an ocular micrometer (Figure 8a) to measure the intertegular distance (hereafter, ITD) of each bee as the shortest distance between the bases of an individual's tegulae. I used a stage micrometer to convert ocular micrometer measurements to millimeters (0.8065 mm per ocular unit, Figure 8b). For each bee, the initial ITD measurement was recorded and analyzed in arbitrary ocular units then converted to millimeters post-analysis to contextualize comparisons of body size. Given that *Osmia spp.*, *Ashmeadiella bucconis*, *Megachile rotundata*, and *Megachile onobrychidis* adults were absent

from our rangeland nests, the small sample size of *Lithurgopsis apicalis* individuals (11 CRP and 6 rangeland adults), and the high relative abundance of *M. brevis* individuals found on both CRP and rangeland, I compared only *M. brevis* ITD in my measurements of performance. Because they are native to the region, have multivoltine life histories, and are polylectic (Michener 1953), *M. brevis* provides an excellent model for understanding how land management practices may support native, solitary bee populations. I used female ITD as an indicator of body size and as the primary response for performance in my analyses (see *Statistical Analyses*).

Figure 6: Each individual unit shown in (a) and (b) is one *Megachile spp.* cell with a larval provision and an expected one egg or developing bee. The cell farthest from the cavity entrance (right) in (a) would be “Cell 1”. *Lithurgopsis apicalis* cells are provisioned primarily with *Opuntia* pollen (b).

a)



b)



c)



Figure 7: The tegula of a female *Megachile brevis* is basal to the left wing in the lateral view (a) and then zoomed in (b). Taken from Cane 1987, the intertegular distance is the span between two tegulae and is given by “y” (c).

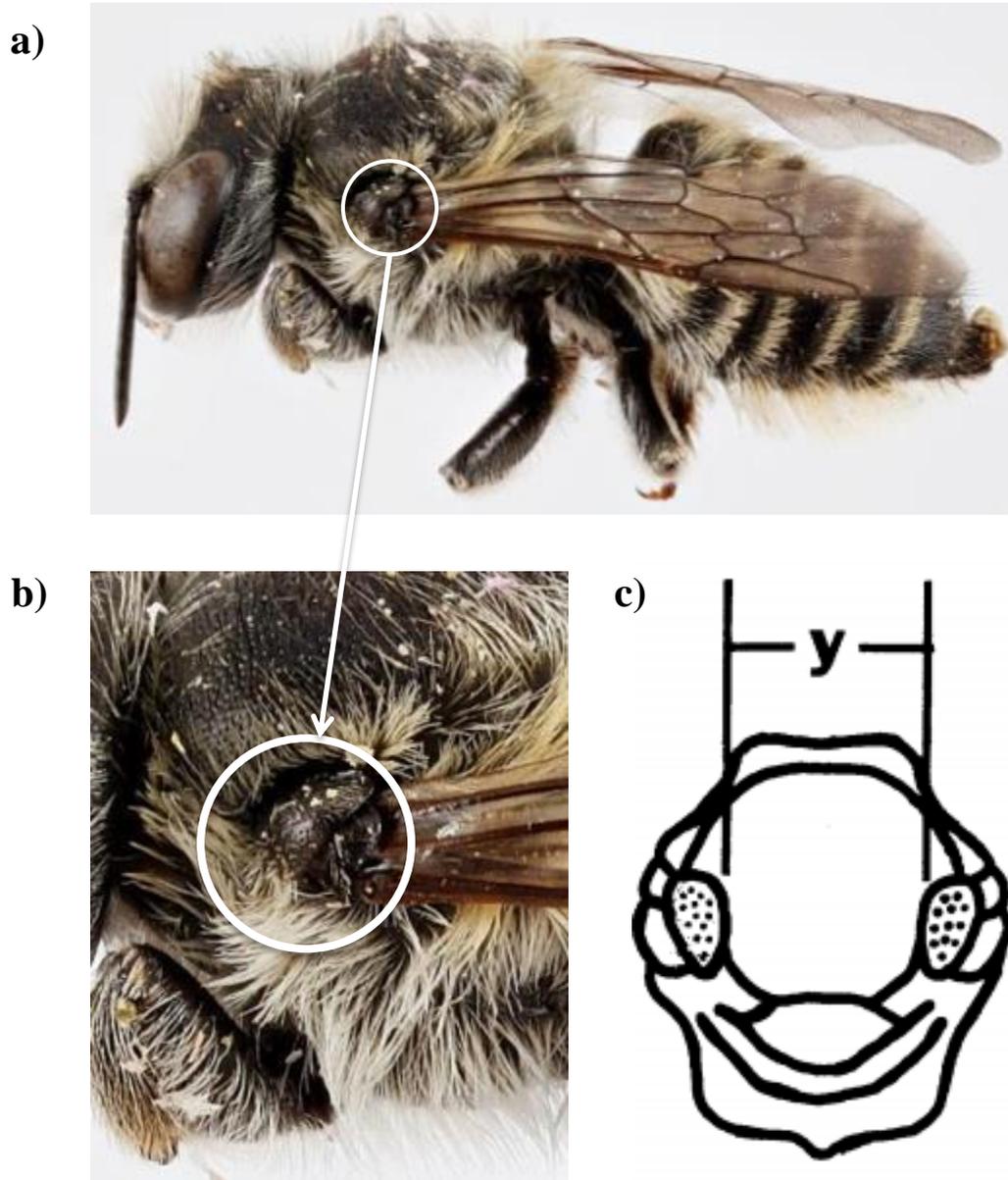
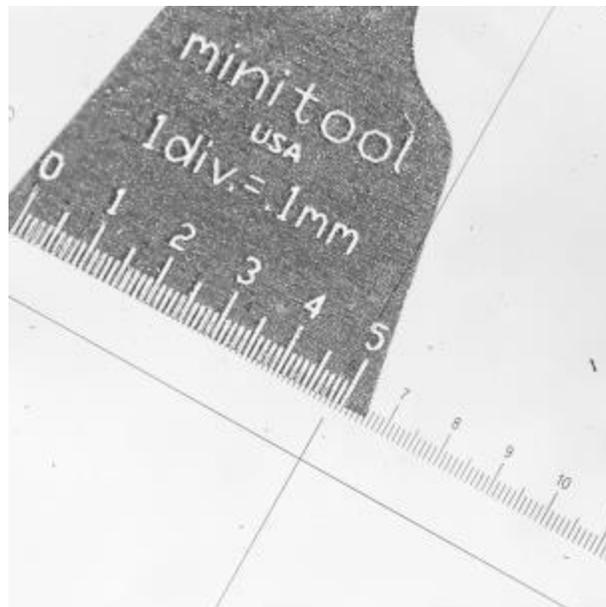


Figure 8. *Megachile brevis* intertegular distance, used as an indicator of body size, was measured using an ocular micrometer (a) and a stage micrometer to make practical conversions to millimeters (b)

a)



b)



Mechanisms driving bee community dynamics, reproduction, and performance

Of the potential mechanisms that influence patterns of bee abundance, diversity, reproduction, and performance, resource availability plays a significant role in facilitating pollinator populations. For oligo- and mesolectic genera, the availability of a specialized host plant is highly, if not directly correlated to a species' ability to survive and effectively reproduce. For polylectic species, foraging resource requirements are less of a limiting factor. However, for polylectic, multivoltine species like *Megachile*, floral resources must be diverse in seasonality to ensure that early, late, and mid-season bees have access to larval provisions and nest materials throughout the flowering season. Given the diversity of oligolectic, mesolectic, polylectic, and multivoltine bees found in our samples, floral resource diversity was hypothesized as the largest potential factor driving the patterns we observed in the bee communities in our samples. From May to August of 2014, we recorded the number of different plant species found on each site. Flowering forbs and grasses were counted and identified four times throughout the season, then totaled at the end of sampling upon collection of our trap nests. I used this count as a measure of plant species richness for each site, including flowering species and grasses due to evidence that a positive relationship exists between non-competitive grass abundance and pollinator species richness on set-asides and semi-natural landscape (Kuusaari et al. 2011). We did not collect information regarding bloom time and duration. I subsequently compared plant species richness to land-use type, total nest abundance, *Megachile* nest abundance, *Megachile* cell abundance, and *M. brevis* ITD using model comparisons.

Statistical Analyses

In my final analyses, I eliminated three sites from my sample. Disturbance early in the season rendered traps from N1 (CRP) and SH (Range) irrecoverable. Only the bamboo trap was recovered from SB (CRP), which contained one nest (Appendix I, Figure A1) with 12 cells and six *Megachile brevis* individuals (three males, three females). I chose to only use sites with a minimum of two nests to facilitate my tests of proportions and subsequently removed SB site from all analyses. Additionally, only nests from the genus *Megachile* were ubiquitous across all sites and comprised $87.43 \pm 4.0\%$ of total samples. Subsequently I chose to focus only on *Megachile* nests, cells, and individuals after my initial analyses of total nest abundance and diversity. Prior to all of my analyses, I determined the distribution of my response variables using JMP® Pro version 13.0.0 (Appendix I).

Richness and Abundance

I employed a Kruskal Wallis Rank Sum test to non-parametrically analyze genera richness per site. Because only fully-developed, non-emerged bees were able to be identified to species, the analysis of species level diversity between the two site types was not justifiable. Within species richness, I chose to compare the proportion of nests produced by species native to the region to non-native, introduced nests. Traps from four sites, all CRP-leased, did not contain any adult bees upon dissection (KW, ME, K2, and H3) and were thus eliminated from this diversity analysis, as no species-level determinations were able to be made. I used a chi-squared analysis of equal proportions to analyze the proportion of nests containing native bees to non-native bees by land-use type. Finally, I used two-sample, unpaired t-tests with a log transformation to compare total and *Megachile* nests per site between CRP and rangeland.

Reproduction and performance

To account for variations in the life histories and discontinuity of abundance across sites between all collected genera, I eliminated all non-*Megachile* nests in my analyses of reproductive output and performance. I used a Pearson's chi-squared analysis of goodness-of-fit to determine the distribution of *Megachile* cells per nest. To compare reproductive output between CRP and range land sites, I fit a negative binomial generalized linear mixed effects model to the response variable, cells per nest, against the fixed effect of land-use type, with site as a random effect. Factors affecting reproductive success were likely uniform for all *Megachile* spp. bees within a site. Given this, *Megachile* nests within a plot were not independent and site was included as a random effect in my analyses to prevent pseudoreplication.

Of the nine species of bees identified across all sites, only one, *Megachile brevis*, occurred in high enough abundance in traps from both land-use types to use for comparisons of bee performance. Given my a priori expectation for females to be larger than males (Moisset and Buchmann 2011), I eliminated male adult *M. brevis* from my analyses of performance. This difference by sex was upheld in my analyses (Figure 2). I determined the distribution of my measurements of female *M. brevis* ITD with a Shapiro-Wilk test of normality. I compared *M. brevis* body size between land-use type with a generalized linear mixed effects model. Nest and site were included as random effects to account for pseudoreplication given the organismal-level of the response variable.

Mechanisms driving bee community dynamics, reproduction, and performance

Prior to testing my response measures against my hypothesized mechanism, I first determined if plant species richness did in fact vary between the two land-use types. To do this I

performed a two sample t-test with plant richness as the response and land-use type as the determining factor. Consequently, I used generalized linear modeling (Bates et al. 2015) to compare plant species richness, land-use, and their interaction as fixed effects against my four response measures: total and *Megachile* nest abundance, *Megachile* cells produced per nest, and female *M. brevis* ITD (Table 2). For models with body size as a cell-level response I incorporated nest as a random effect to account for pseudoreplication. Neither body size nor *Megachile* cell abundance model comparisons included site as a random effect, given that plant species richness was counted on a site-level basis, and was thus used as a quantitative metric for site.

To select the best fit model for each response, I utilized Akaike's an Information Criterion (AIC) to compare the AIC score of each model (AICc), the change in AIC between the model with the lowest score and the remaining models ($\Delta AICc$), the weight of the score (AICcWt), and the relative likelihood of a model given the data (ModelLik) (Appendix I). Models with the lowest AIC score and highest relative likelihood were chosen as the best-fit model for the effect of fixed effects on the response variables. All analyses were performed using R version 3.3.2 GUI 1.68 Mavericks build (7288).

Table 2. Fixed effect parameters for mechanism model comparison (excluding nest as a random factor for ITD span analyses).

Response		Nest abundance, <i>Megachile</i> nest abundance, Megachile Cell Abundance, Female <i>Megachile brevis</i> inter-tegular distance (ITD)
Model	K	Parameters
1	4	Land Type + Plant Species Richness + Land Type*Plant Species Richness
2	3	Land Type + Plant Species Richness
3	2	Land Type
4	2	Plant Species Richness

RESULTS

Richness and Abundance

In total, we collected 356 nests from northeastern Colorado in 2014. Three quarters of these nests came from Conservation Reserve sites (267 nests), with the remaining 89 nests were extracted from rangeland traps. I was able to identify nine species of bees from nests containing fully developed adults. This included four *Megachile* species (*M. rotundata*, *M. brevis*, *M. onobrychidis*, and *M. montivaga*), three *Osmia* sp., *Lithurgopsis apicalis*, and *Ashmeadiella bucconis*. Of these, *M. rotundata* is the only species not native to the region (Pitts-Singer 2011). Additionally I found three species of parasitic *Coelyoxis* in native *Megachile* nests and one parasitic wasp in the Crysidae family. About 85% of all nests identified were *Megachile* and were found on every site. *Osmia* nests were the least abundant overall.

For comparative purposes I combined captures from the four genera of non-parasitic, cavity-nesting bees, including *Megachile*, *Lithurgopsis*, *Osmia*, and *Ashmeadiella*. *Megachile* nests exceeded all other nests almost sixfold, and comprised a majority of the total nests ($87.43 \pm 4.0\%$), as well as on both land types (CRP: 89.09%, Range: 74.16%). Of the species identified, no *M. rotundata* or *Ashmeadiella* nests were found on rangeland sites. *Osmia* nests made up a higher proportion of overall nests on range sites (20.22% versus 3.75% in CRP). Five *Lithurgopsis apicalis* nests were found on each land type, with 80% of nests on CRP and rangeland being concentrated on one site. Bees of every identified species were found on CRP land, but only adult *Megachile brevis*, *Megachile montivaga*, and *Lithurgopsis apicalis* were found from rangeland nests.

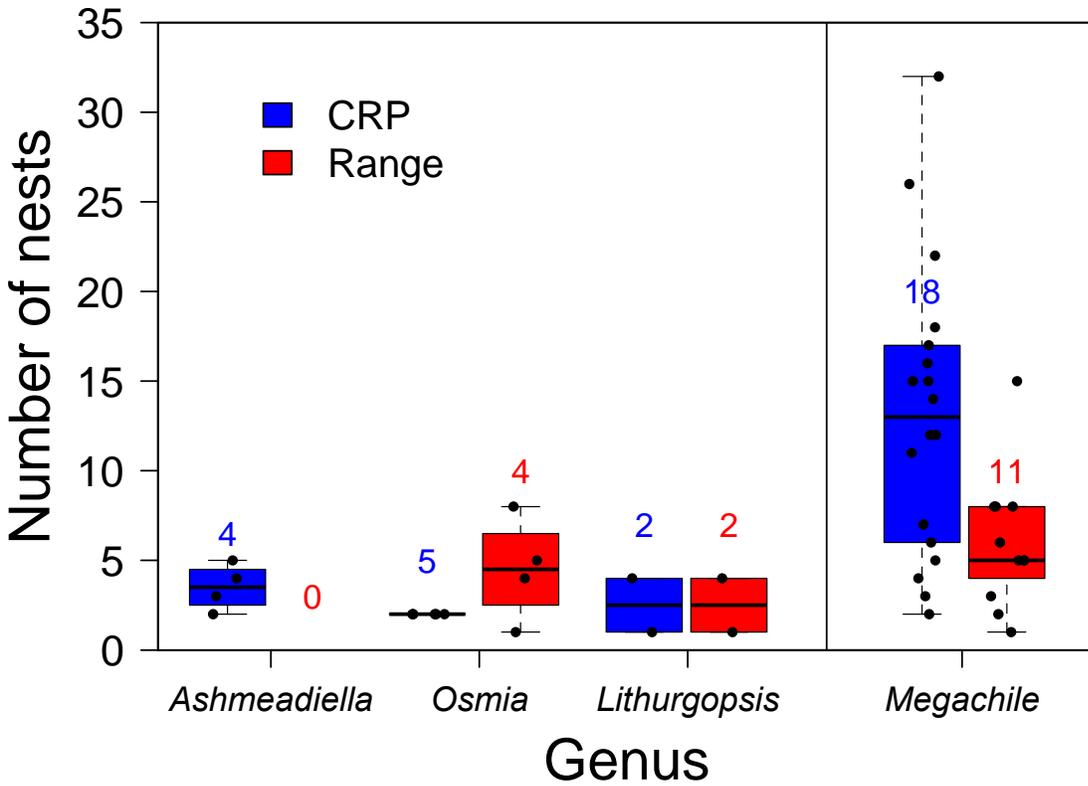
Site genus richness did not significantly differ between CRP and range lands ($X^2_1=0.0158$, $p=0.9001$), and had a mean of 1.59 ± 0.14 genera per site. Rangeland nests also

contained 100% native adult bee species, which was a significantly greater proportion of native nests than was found on CRP sites ($X^2_{24} = 76.35$, $p < 0.001$).

After eliminating SB, I had a total sample size of 355 nests across 29 sites (18 CRP and 11 rangelands). The mean number of nests per site was 12.24 ± 1.54 . The abundance of nests collected from CRP traps (14.78 ± 2.10) significantly exceeded that of range lands (8.09 ± 1.53) by almost 200% ($t_{24.2} = 1.98$, $p = 0.030$, Figure 9a). *Megachile* nest abundance was also significantly higher on CRP sites than rangeland by a magnitude of more than 250% ($t_{21.7} = 2.67$, $p = 0.007$, Figure 9b).

Figure 9. Nests in my sample were divided between four genera over 29 sites, with the distribution highly biased towards *Megachile* by both abundance and site. The number of sites from which each genus was collected is superimposed (a). I only found native species in rangeland traps (b)

a)



b)

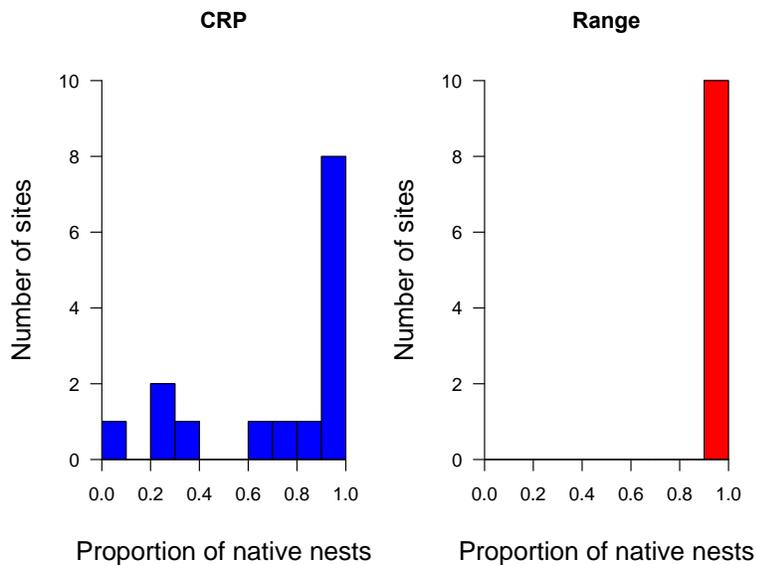


Figure 10. Bee species captured in trap nests included a) *Megachile rotundata*, b) *M. brevis*, c) *M. montivaga*, d) *Osmia* sp.1, e) *Osmia* sp.2, f) *Ashmeadiella bucconis*, g) *Lithurgopsis apicalis*, and h) a cleptoparasitic *Coeliyoxis* sp. from a *Megachile* nest.

a)



b)



c)



d)



e)



f)



g)



h)



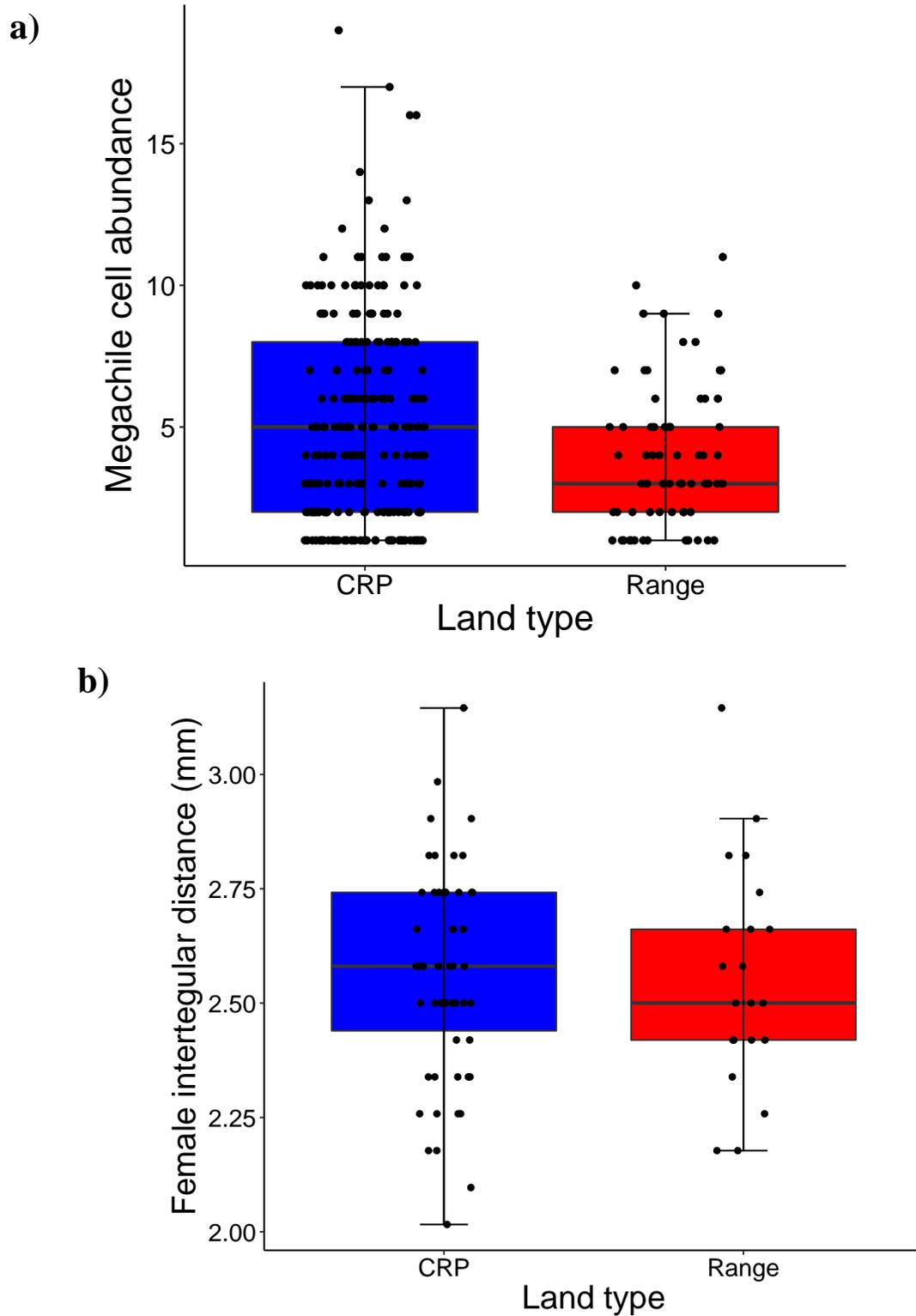
Reproduction and Performance

I dissected a total of 1,710 cells from nests, of which I analyzed 1,698 (eliminating 12 cells from SB). *Megachile* cells comprised 87% of all cells, with 1,479 total cells. CRP nests had 1,367 cells, 89% of which were *Megachile* (1,217). Rangeland nests had 262 *Megachile* cells (79% of total) out of 331 cells overall. Across the entire sample, the mean number of cells per nest was 4.78 ± 0.18 . Over 70% of cells were extracted from bamboo traps (1,212 cells). On CRP land, 256 identifiable *M. rotundata* cells were extracted, although likely a low estimate given our inability to identify nests to species-level. Thus introduced *M. rotundata* accounted for at least 15% of *Megachile* reproductive output on CRP sites. Again, *M. rotundata* was not observed on rangelands. Subsequently, *Megachile* on CRP sites produced significantly more cells per nest than those on rangelands ($z = -2.15$, $p = 0.031$, Figure 10).

My measurements of emerged bee ITD initially came from 433 adult bees found in nests and included head width, radial cell length, and ITD, resulting in a total of 1,299 measurements. I chose to use only ITD in my performance estimates, given overwhelming evidence of the high correlation between ITD and dry body weight (Cane 1987). Upon making species-level determinations, I decided to only analyze *Megachile brevis* adults, with a total of 153 bees taken from 47 nests. (Interestingly, I observed the same number of *M. brevis* nests as *M. rotundata* on CRP land). The mean male ITD of *M. brevis* was 2.99 ± 0.03 mm, while females had a mean span of 3.18 ± 0.03 mm. Approximately 48% (58 bees) of *M. brevis* were female in CRP traps, with 66% (21 bees) in rangeland traps. Female body size did not differ significantly between land-use types ($t = -0.06$, $p = 0.950$, Figure 11).

Figure 11. CRP sites supported significantly more *Megachile* cells per nest than rangeland sites

(a). However, female *M. brevis* did not differ significantly in body size by land use type (b).



Mechanism of plant species richness driving abundance, reproduction, and performance

Our counts of plant species richness per site ranged from five to 32 species, with a mean of 16.24 ± 1.09 . A broad range of species were observed across both land-use types, including three species of *Opuntia* (host of *Lithurgopsis apicalis*), alfalfa (*Medicago sativa*), two species of *Plantago*, Solanaceae, and over 130 more species. My analyses did not include specific vegetation cover-type due to the qualitative nature of the floral data at this stage. Rangelands had significantly higher plant richness than CRP plantings ($t_{24,18} = -2.153$, $p = 0.021$, Figure 12), with 14.78 ± 1.60 species on average per site compared to 18.64 ± 0.81 species per site on rangelands, thus supporting my hypothesis that plant species richness would differ between the two land-use types.

The best-fit model for nest abundance included only the fixed effect of land type (AICc = 21.983), but showed only a marginally significant difference in nest abundance between CRP and rangelands ($F_{1,27} = 3.590$, $p = 0.069$), and did not include plant species richness (Figure 13a). This divergence from the originally significant effect of land-use on nest abundance is likely due to the initial use of a one-sided t-test, per my initial hypothesis that CRP sites would support more abundant populations of bees than rangeland sites. Plant species richness as an effect was not applicable, as it was excluded from the best-fit model.

Model comparisons for *Megachile* nest abundance produced three models varying only marginally in their AICc parameters of goodness-of-fit. The model including only the fixed effect of land-use type had an AICc of 21.85 and suggested a significant effect of land-use type on *Megachile* nest abundance ($F_{1,27} = 7.025$, $p = 0.013$). Including the effects of land-use and plant species richness (AICc = 21.92) maintained the significant relationship between CRP land and greater *Megachile* nest abundance ($F_{1,26} = 7.229$, $p = 0.012$), but showed no significant effect

of plant species richness on *Megachile* nests ($F_{1,26}=1.784$, $p = 0.193$, Figure 13b). Finally, including the interaction effect of plant richness and land-use ($AICc = 22.64$) resulted in no significant relationship between *Megachile* nests and the richness response ($F_{1,25}=1.793$, $p=0.193$) or the interaction term ($F_{1,25} = 1.1253$, $p = 0.299$). The effect of land type was still significant ($F_{1,25} = 7.264$, $p = 0.013$).

The best-fit models for my analyses of *Megachile* reproductive output included the fixed effects of land-use and plant species richness ($AICc = 1,526.10$), and land-use only ($AICc = 1,526.60$). Both models suggested a significant relationship between cells per nest and site type ($F_{1,301} = 309.79$, $p = 0.010$ and $F_{1,302} = 307.32$, $p = 0.010$, respectively), and CRP nests produced more cells than rangeland nests. There was no significant effect of plant species richness on *Megachile* reproduction ($F_{1,301} = 307.29$, $p = 0.114$, Figure 14a).

Finally, the best fit model for *M. brevis* body size included only the fixed effect of plant species richness ($AICc = 15.90$) and showed no significant effect on female *M. brevis* ITD ($F_{1,301} = 309.79$, $p = 0.010$ and $F_{1,302} = 307.32$, $p = 0.010$, Figure 14b). Best-fit model coefficients are included as statistical support in Appendix I.

Figure 12. Rangeland overall had significantly higher plant species richness than CRP land.

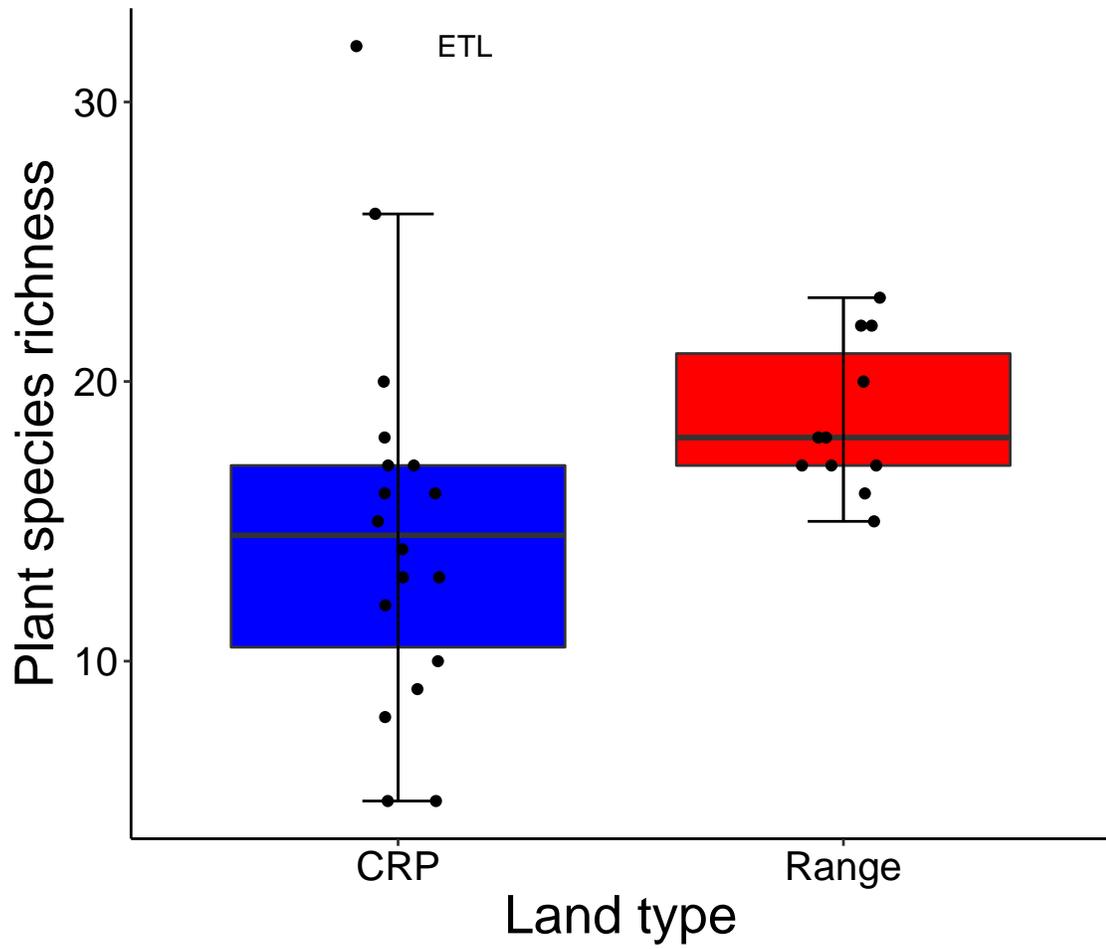


Figure 13. While CRP and rangeland varied significantly in nests, there was no significant effect of plant species richness on either total nest abundance (a) or *Megachile* nest abundance (b)

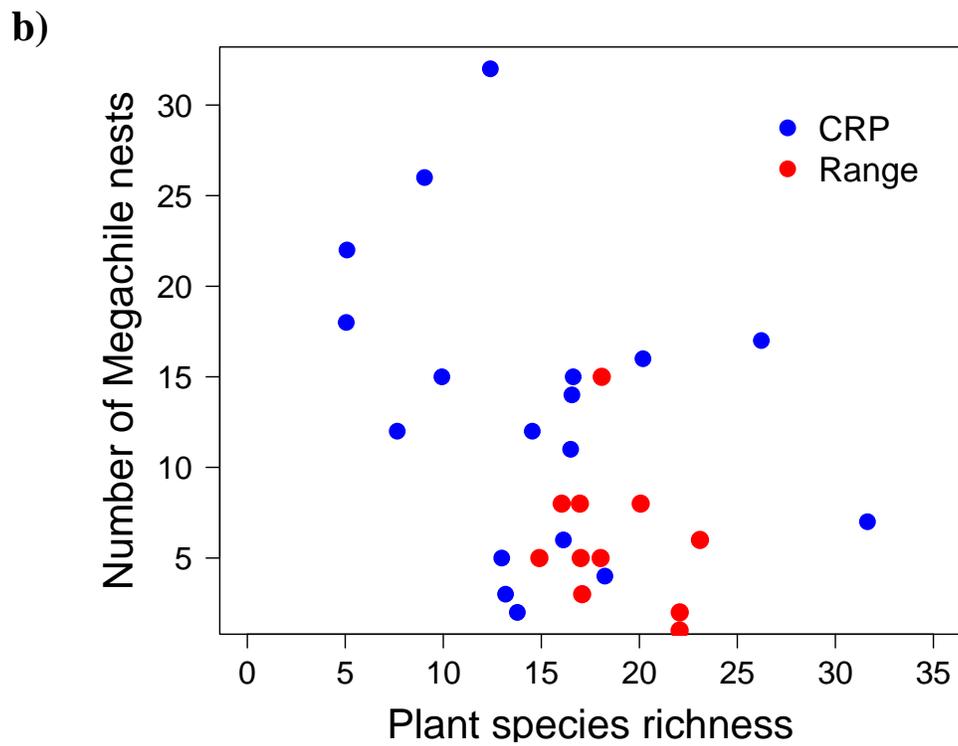
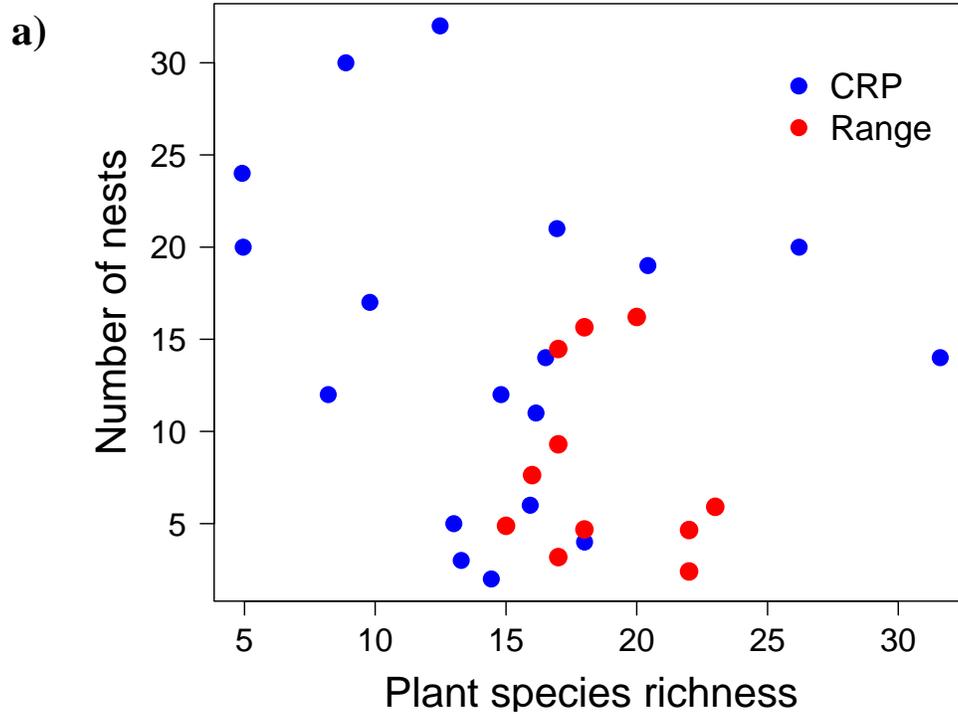
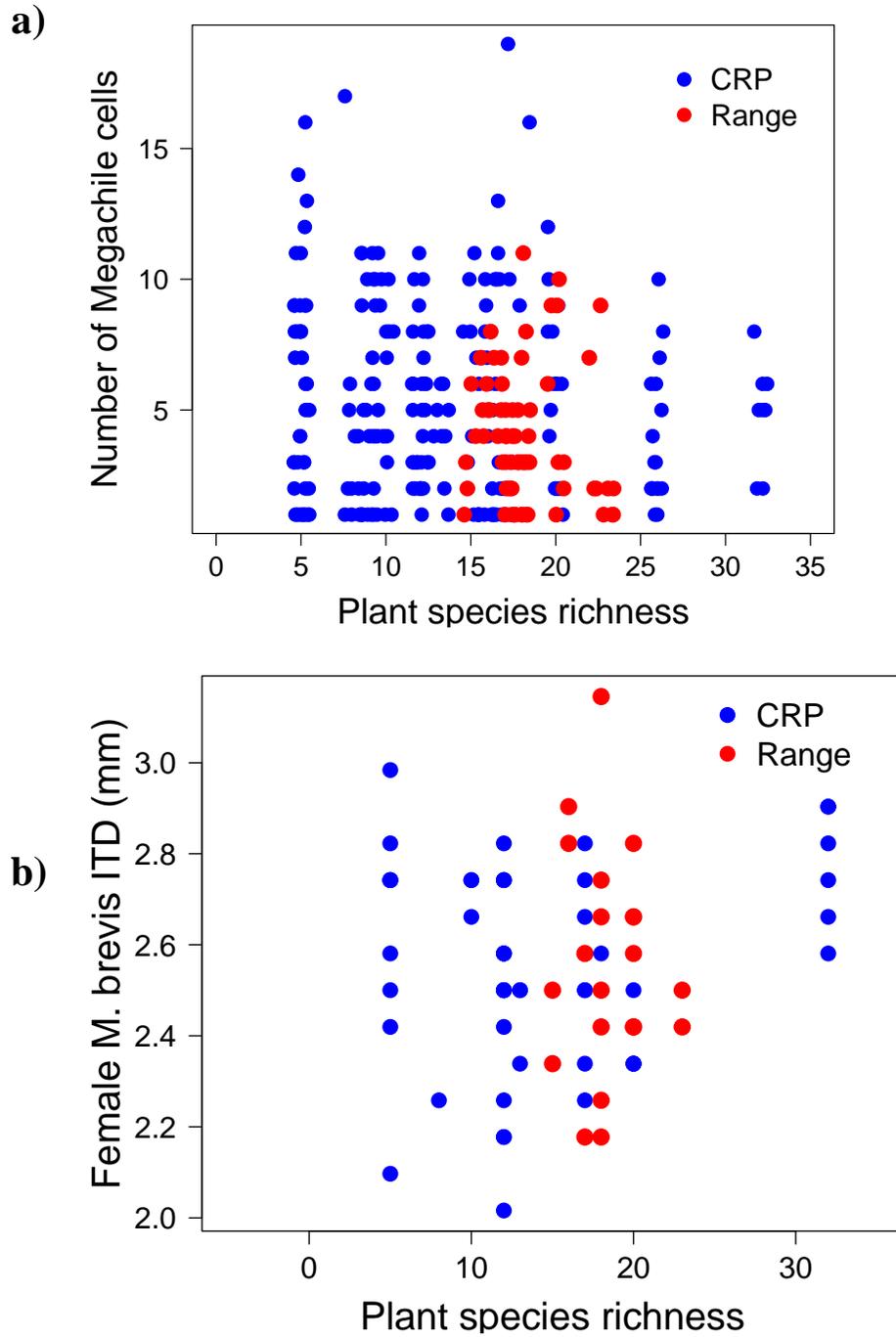


Figure 14. Reproductive output (measured by cells per nest) was greater on CRP sites than on rangeland sites but was not significantly affected by plant species richness (a). Female *M. brevis* body size remained uniform throughout the samples, regardless of land-use or plant species richness (b).



DISCUSSION

Overall, I found that CRP plantings in agricultural landscapes support greater nest abundance and *Megachile* reproductive output than rangelands. While CRP plantings had more nesting bees, diversity on both a genus and species level was extremely low across all of the sites and did not significantly differ between the two land-use types. Additionally, land-use did not appear to affect individual bee performance, as native *M. brevis* female body size did not differ significantly between the two land types. While rangeland sites did have greater plant species richness than CRP land, plant species richness was not a significant factor driving any of the response variables, suggesting that other mechanisms are involved in cavity-nesting bee diversity and reproduction. Regardless, these results suggest that CRP plantings in northeastern Colorado have positive effects on bee communities, supporting more abundant and reproductively successful populations of cavity-nesting bees than rangeland.

The high nest abundance I observed in traps collected on CRP plantings follows closely with the findings of multiple studies. A 2004 meta-analysis compiled 127 publications reporting the effects of conventional agricultural land and rotational fallows, or “set-asides”, on focal taxon biodiversity and population density (either birds, insects, spiders, and plants); Conservation Reserve plantings fall under the umbrella of set-asides in that intensively managed land is removed from production during at least one growing season and experiences minimal disturbance, as is outlined in the requirements for enrollment in the CRP (Van Buskirk and Willi 2004; U.S. Food Security Act 1985). Additionally, conventional agriculture in these analyses covers not only traditional crop production but also intensively managed meadows, which characterizes any given rangeland exposed to high stocking rates and grazing intensities. Across the 127 studies evaluated, population densities (i.e. abundance) were consistently and

significantly greater on set-aside plots than conventional agriculture. Interestingly, the spatial, temporal, and methodological variation between the studies had no effect, suggesting that rotational fallows overall uniformly increases population densities (Van Buskirk and Willi 2004). Because the large ‘sample’ size should negate stochasticity, and population densities for all four taxa were greater on set-asides, it is not unreasonable to assert that our findings were consistent with this evidence for the benefits of set-aside cropland.

Interestingly, despite compelling evidence from the same meta-analysis of a parallel increase in biodiversity and population densities on rotational fallows, I did not observe a higher genus or species-level richness in CRP bees than rangeland bees. However, there was a noticeable lack of diversity throughout the entire sample of bees (mean genus richness = 1.5862 ± 0.1361). Unfortunately, there exists no baseline, observed level of diversity against which I can compare these findings. In 2013, the plains of northeastern Colorado were known to be home to only 19 recorded species of bee. Through just one USDA-funded research spanning two years, over 203 species of bee have reported. Thus attempts to explain the diversity, or lack thereof, within our sites and across the entire study are limited by previous biodiversity knowledge.

While I do not know if cavity-nesting bee species and genus-level richness was comparatively low in 2014 or persists at a low state diversity that includes the nine species observed in this study, I can make a priori assumptions about the cause for this homogeneity. At a basic level, all bees historically have two primary limiting factors: nesting substrate and floral resources (Danforth 2007, Westrich 1996). Short grass steppe, the dominant landscape of our study area well-adapted to moderate bison grazing, is historically comprised of low-level forbs adapted to grazing by early-American bison (Kothmann 1995, Alkemade et al. 2012). For cavity nesting bees especially, this contains limited nesting sites (e.g. dead wood, trees, long pithy-

stemmed plants, etc.). Additionally, intensively managed agricultural lands tend to have homogenous landscapes with short flowering seasons dominated by a single central crop bloom and low extraneous plant diversity (Steffan-Dewenter and Tschardtke 1999). In conjunction with the inherent limitations of the landscape, a series of additional restrictive factors arise: a) bee population robustness is positively related to floral richness and abundance (Hopwood 2008), b) cavity-nesting bee populations are highly sensitive to disturbance by nest limitation (Williams et al. 2010), c) cavity-nesting bees tend to be smaller than most taxa and thus have smaller foraging ranges (Greenleaf et al. 2007, Biesmeijer et al. 2006), and d) foraging distance determines the extent and breadth to which wild bees can provide pollination services to crops (Greenleaf et al. 2007). Combined, these factors indicate that cavity-nesting bee community composition may be homogenous on intensively cultivated land.

Despite low diversity, my results still support the overall value of CRP plantings for wild bees, compared with rangeland. While these results are largely supported by the literature, certain limitations of this study make it difficult to determine the mechanisms driving these patterns. Knowledge of our study sites is limited to land-use type and qualitative observations from the field season. While I had reason to a priori expect clear distinctions between CRP plantings and rangelands due to differences in past management strategies and current disturbance regimes, I did not have access to individual CRP planting details (age, seed mix planted, etc.), given confidentiality of parties participating through the NRCS. My results did indicate a significant difference in overall plant species richness between CRP and rangelands; however, overall total plant species richness is not an ideal estimate to account for inter-site differences in floral resources (i.e., nectar and pollen host plants). Thus I am constrained in my ability to speak to the potential impacts this variation may have on cavity-nesting bee

communities. For organisms within rotational fallow ecosystems there exists substantial evidence that biodiversity and population density is positively related to the age of the fallow, the extent of natural vegetative succession following cessation of crop production, and the size of a plot. Additionally, there exists a negative relationship with surrounding land-use intensity on density and diversity (Van Buskirk and Willi 2014). Given the limiting factors to cavity-nesting bees listed above, exploring bee response to flowering species richness or abundance, as well as proximity of these resources to sufficient nesting sites, could help elucidate more distinct patterns.

As previously alluded to, the past and present management of CRP plantings in this study could have impacts on the relationships I expected to find. Studies supporting the value of rotational fallows to pollinator communities point to a number of factors influencing the magnitude of these benefits (Dunn et al. 1993, Van Buskirk and Willi 2004). Specific to the Conservation Reserve Program, these factors include time since enrollment, the assigned planting type (based on an estimated EBI score), and subsequent management decisions and disturbance since initiation, such as emergency grazing. At least two CRP sites were allowed to be grazed in 2013 under such an emergency, although none were grazed in 2014. It is therefore likely that management factors such as these contributed to site-by-site variation we observed in our surveys. For example, plant species richness on CRP land spanned the entire range of richness counts across *all* sites, including rangeland. Thus CRP plantings were comprised of anywhere from five to 32 species of plants. The same pattern was true for nest abundance, with CRP traps producing between one to 32 nests (SB and H3, respectively). While CRP land *overall* supported more abundant cavity-nesting populations than rangeland despite these disparities, my ability to determine the mechanisms driving this result is restricted by the availability of

information pertaining to the CRP sites from which we sampled. A. Carper has been in contact with the USDA about access to data on our CRP plantings in hope that additional metrics could help determine how management on CRP fields impacts cavity-nesting bees.

In addition to the limited data availability on the CRP land used in our study were not available for my analyses, rangeland ecosystems as a whole are poorly understood on a global, historical scale. Rangeland has high variation on both a spatial and temporal level, with grazing disturbance persisting as the only historically unifying factor (Kothmann 1995; Alkemade et al. 2012). In recent assessments of rangeland systems however, attempts to standardize the quantification of grazing intensity are continuously confounded by disparities in rangeland management strategies, stocking rates, and fluctuating ecosystem carrying capacities (Alkemade et al. 2012). Given the challenges repeatedly faced by prior rangeland studies, the inclusion of site-specific livestock management and grazing intensities over the summer of 2014 was beyond the scope of this study. However, addressing this gap on a theoretical level could help to inform future studies investigating land-use factors that drive pollinator communities. Before discussing the value of assessing pollinator communities in conjunction with patterns of grazing intensity on rangeland, we must first address the potential impacts of varied-intensity grazing on rangeland plant communities and subsequent pollinator populations.

My a priori expectation of high plant species richness on range sites was informed by established land management practices outlined in this paper's introduction, as well as prior studies both comparing plant species diversity on cattle-grazed prairie to CRP-leased fallows. While plant species diversity may not be the best metric of habitat value, it provides an interesting comparison between the capacity for range and CRP lands to support high biodiversity, and how management may impact it. For example, Biondini et al. (1998)

recommend decreased grazing practices after finding that grazing intensity could not exceed 50% of aboveground net primary productivity (ANPP) without causing a vegetative community shift into a potentially irreversible transitional phase. Fuhlendorf and Engle (2001) on the other hand, asserted that only rangelands that are *managed* to be homogenous through anthropogenic interference with floral composition or patch-biased feeding would be heavily affected by intensive grazing. However, in terms of this study, the lack of abundance and overall plant community composition data leaves only basic species counts with which to postulate. While the effect of grazing intensity of rangeland species richness is unknown in the context of this study, we do know that in general, CRP lands were largely homogenous in plant composition, as predicted by the literature (Ribaudo et al. 2001, Dunn et al. 1993). Counts of plant species richness within our study system were significantly higher on rangeland than CRP plantings, thus upholding these previous findings. While plant species diversity has repeatedly been linked to bee species diversity, a number of additional factors must be considered in assessing pollinator community dynamics. By not including plant abundance or community composition assays in my analyses, I face the limitations of plant species richness as a minimally informative factor in assessing plant-pollinator dynamics. Additional factors potentially exceeding plant species richness as a driving mechanism in my study are addressed below.

Bee population dynamics are determined largely by the availability of nesting habitat and the abundance and quality of adjacent sources of pollen and nectar (Westrich 1996). Through the installation of trap nests, we essentially removed nesting sites as a limiting factor for cavity-nesting bees in our study. Therefore, floral resource availability was the likely the most limiting factor to bee abundance, diversity, reproductive output, and performance. The extent of this limitation varies between species of bees, determined by foraging range and preferences

(Steffan-Dewenter et al 2002; Beismejer et al 2006). Additionally, multiple prior studies have shown that native bees prefer to forage on native plant species (Morandin and Kremen 2012) and that native plant diversity is positively related to bee diversity (Williams et al 2010). However, native bees will forage on exotic plant species in the absence of native flora (Morandin and Kremen 2012) Given the trade-offs between quality, quantity, and proximity of floral resources to suitable nesting sites, it is possible that the native species observed on CRP land either a) chose the non-native, abundant resources of CRP plantings over limited, native flora on rangelands, or b) emerged on or near a CRP planting and were primed to stay given abundance of resource and unwillingness to disperse, neither of which are mutually exclusive hypotheses.

Specifically, the abundance of *Megachile* bees on CRP sites could easily be explained by both of these mechanisms potentially driving bee abundance on conservation plantings in this study. *M. rotundata* and *brevis* were the most abundant *Megachile* in our sample. Additionally, both are polylectic and have multivoltine life histories (Pitts-Singer 2011, Michener 1953). While being a generalist forager somewhat allows for flexibility in choosing a nesting site, a multivoltine life-cycle requires access to resource patches that will provide year-round floral resources, requiring a broader, more diverse plant community (Decourtye et al. 2010, Winfree 2010). While CRP plantings did not necessarily have high plant species richness, it is still possible that the community composition was sufficiently diverse in the timing and duration of flowering seasons to support multiple generations of the generalist *M. rotundata*. The native *M. brevis* would be less influenced by unwillingness to disperse. Michener (1953) discussed in length observations of *M. brevis* leaving a habitat patch mid-construction to more closely follow higher-quality pollen and nectar resources. Despite these differences, both of these generalist,

multi-generational bees are well primed for set-asides like those typically implemented by the CRP with minimal emphasis on planting native plant species.

Beyond conjecture, my findings of significantly higher nest abundance and *Megachile* reproductive output on CRP lands further suggests the possibility that resource abundance exceeded resource diversity as the primary limiting factor for the populations sampled. While we do not have measures of plant abundance, we know that disturbance is inherent to rangelands and relatively non-existent on CRP plots (as mandated by the USDA (U.S. Food Security Act 1985) as terms of enrollment), and that grazing decreases vegetation abundance (Alkemade et al 2012). The assumption of greater overall plant abundance on CRP sites is supported by our qualitative observations during the 2014 field season. In this case, bees may choose nesting sites with more overall resource abundance over those with a higher diversity of native flora.

Interestingly, *M. rotundata* was the only non-native species in our surveys, was found only in CRP fields, and has been managed as a pollinator of alfalfa seed crops (Pitts-Singer 2011), a dominant forb planted in many CRP seed mixes. While our CRP sites were rotational fallows previously used for intensive crop production, rangeland sites likely remained untilled, semi-native or semi-natural habitats. Thus *M. rotundata* populations may be more readily influenced by historical range than diversity in foraging resources, especially given the small radius of their foraging range and tendency to maintain a single nesting site over multiple generations (Pitts-Singer 2011). The high plant species diversity on rangeland sites could also suggest established preferences for diverse, native floral resources by native bees, which potentially buffers against introduced *Megachile rotundata*. However, given the low overall genus and species richness of our samples, this result can only be identified as a notable pattern within our study system, although future research could collect nests all season long to determine

if early season *M. rotundata* females nest in rangelands or if they are never a part of the bee community composition in these sites.

While my results provide important insights into the overall value of the CRP, it should be noted that they may be specific to the conditions of the one season during which we sampled. Plant and pollinator abundance can fluctuate dramatically from year to year (Winfree 2010) and future studies would benefit from addressing populations dynamics over time. A temporal comparison of bee and plant communities on range and CRP lands could account for year-to-year habitat shifts resulting from variations in temperature and precipitation to changes in management strategy, both on site and on surrounding lands. Additionally, by consistently providing nesting sites via trap nest installment over multiple seasons, a better understanding of the limitation of nesting habitat on agricultural land may be reached. If bee abundance and diversity increases over time, an argument could be made for the value of artificial nesting habitats for cavity-nesting bees on agricultural lands, especially in grasslands where woody, nesting substrates may be scarce.

The drawbacks of long-term studies of cavity-nesting bees largely stem from the necessity for over-wintering bees to emerge on-site in the following season to establish new generations. This limits the extent to which nests can be removed from traps for the purpose of species identification and performance measures. Indeed, with the exception of nest abundance and genus richness estimates, this study required invasive methodology that could not be used with an expectation of bee survival and reproduction. Overall, the biology and life history of cavity-nesting bees limits the kind of data that can be collected from any one method. Thus single-season studies such as ours must be carried out in conjunction with long-term research.

Regardless of time scale, assessments of cavity-nesting bee populations moving forward should include detailed plant community assays.

The necessity for long-term research especially applies to cavity-nesting bee populations on CRP land converted back to cropland after lease termination. Understanding how bee communities shift with the land conversion cycling inherent with long-term rotational fallows would help to inform future CRP policy regarding pollinator habitat. To mitigate potential declines in bee populations, the establishment of roadside edge and hedgerow habitats adjacent to re-established cropland could aid in ensuring a continued pollinator presence. This is particularly relevant in light of the recent introduction of CP-42 plantings. Sites that are leased and planted with the specific intention of supporting pollinator communities have the greatest potential for population growth and diversification while simultaneously having the highest risk for displacement of bee populations. This necessitates the development of policy accounting for the habitat loss that occurs following lease termination of CRP sites. The lack of follow-up assessing changes in environmental quality mid- and post-lease could be a major limiting factor in the efficacy of the Conservation Reserve Program and should be seriously considered for incorporation into current policies.

Taken together, the results of this study suggest that CRP plantings are clearly beneficial to bees, and CRP management may have widespread implications, especially as the value of the CRP extends beyond policy-specified goals. For pollinators, the CRP may provide essential nesting and resource-rich habitat, while simultaneously buffering against decreased crop production due to pollinator declines. Fortunately, pollinator conservation persists as a point of mutual interest for proponents of land restoration, biodiversity, and intensified agricultural output alike. Given this potential for support from biophilic and anthropophilic conservationists,

increased popularity of policies for habitat restoration and pollinator protection like the CRP could provide a platform to enable further wild bee research. Critics of the funding necessary to facilitate programs like the CRP and studies like ours can be counteracted with realistic, long term expectations for a decrease in managed pollinator costs. Additionally, concerns surrounding the threats to food security of enacting rotational fallow policy can be countered with the long-term benefits of soil mitigation *and* pollinator establishment. Thus, preserving and maintaining a diverse and abundant community of bees provides long lasting benefits that extend to all trophic levels while directly benefiting humans.

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APPENDIX I.

Supporting Statistical Information

Figure A1. Total nest abundance is plotted, pre-analysis, by land type and serves as an illustration in support of the decision to eliminate the CRP site, SB, from all final analyses. SB's single nest is labeled as an outlier.

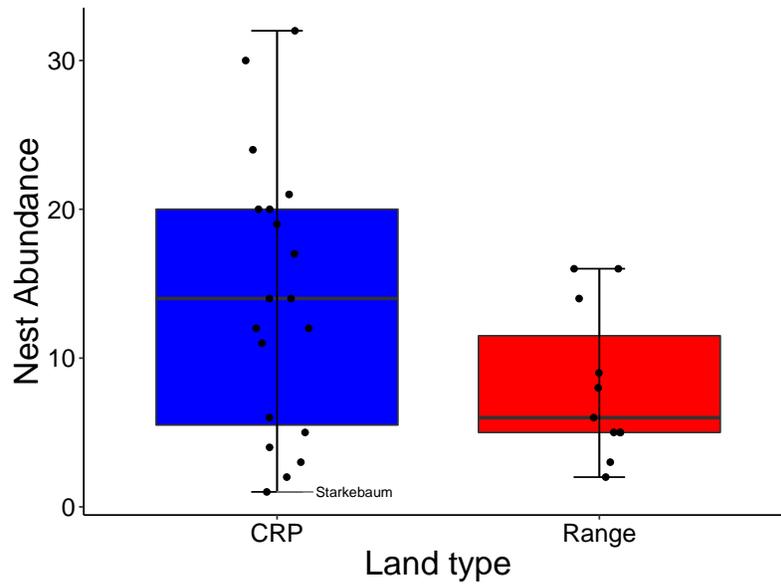
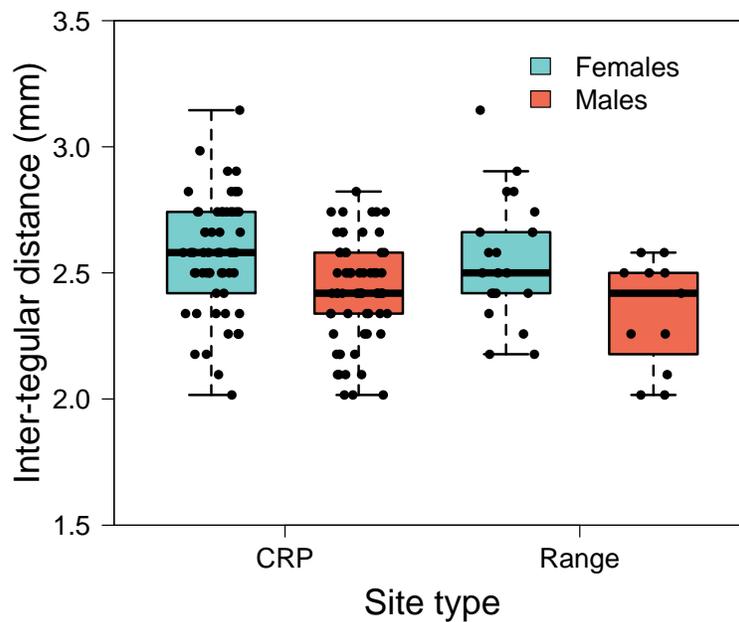


Figure A2. Male *Megachile brevis* were consistently smaller than females in both site types.



Distribution Specification

We specified a log-transformed normal distribution for models of total nest abundance and *Megachile* nest abundance, a negative binomial distribution for *Megachile* cell abundance within nest, and a Gaussian distribution for *Megachile* body size (IT-distance).

Figure A3. Counts of genus (a) and species richness (b) were non-normally distributed and homoscedastic by land-use type, requiring the use of a non-parametric analysis of means for genus richness by land-use type

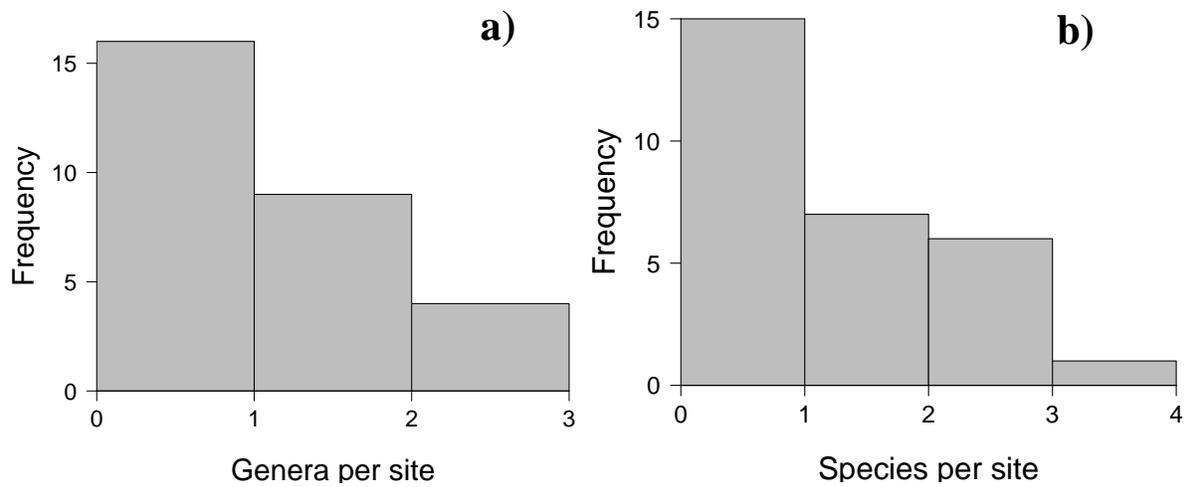


Figure A4. Both total nest abundance (a) and *Megachile* nest abundance (b) had log normal distributions ($X^2 = 355.83, 304.31, p = 0.463, 0.452$, respectively).

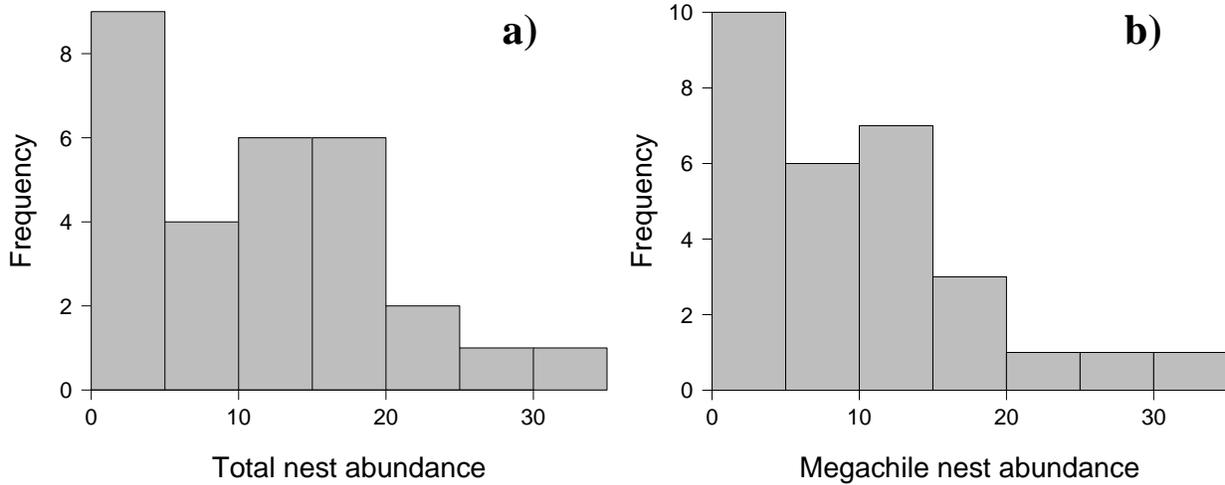


Figure A5. Our counts of *Megachile* cells had a Gamma Poisson distribution ($X^2=304.31, p=0.4519$), verified with a Pearson's chi-square analysis of goodness-of-fit.

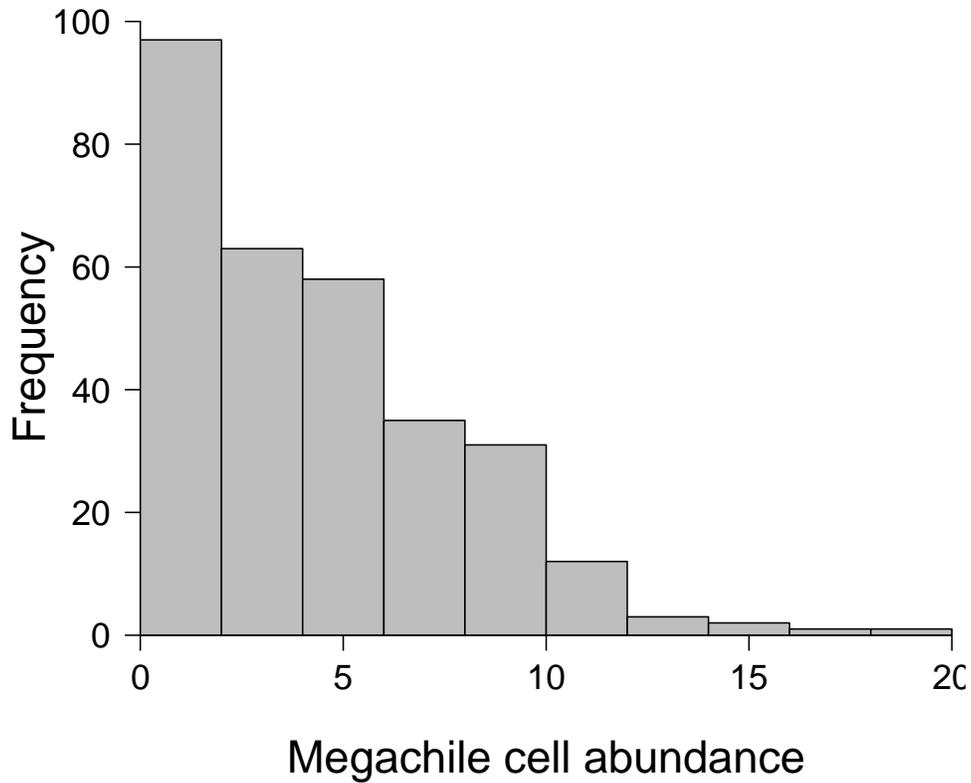


Figure A6. Female ITD was normally distributed ($W=0.9828$, $p=0.3648$), verified with a Shapiro-Wilk test for normality.

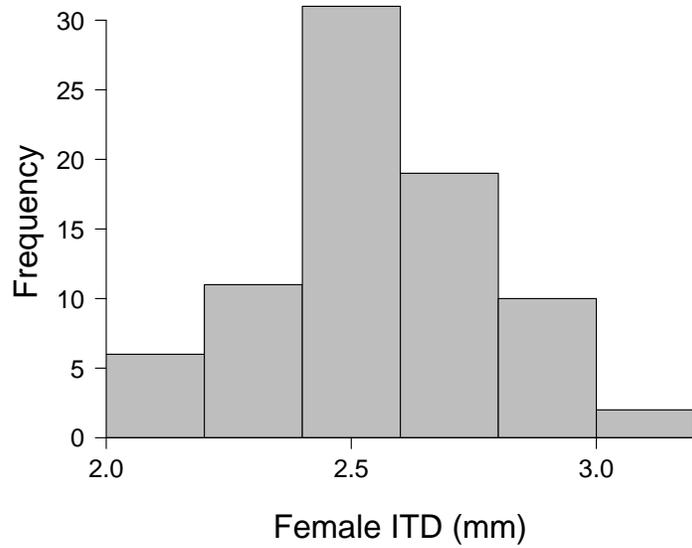
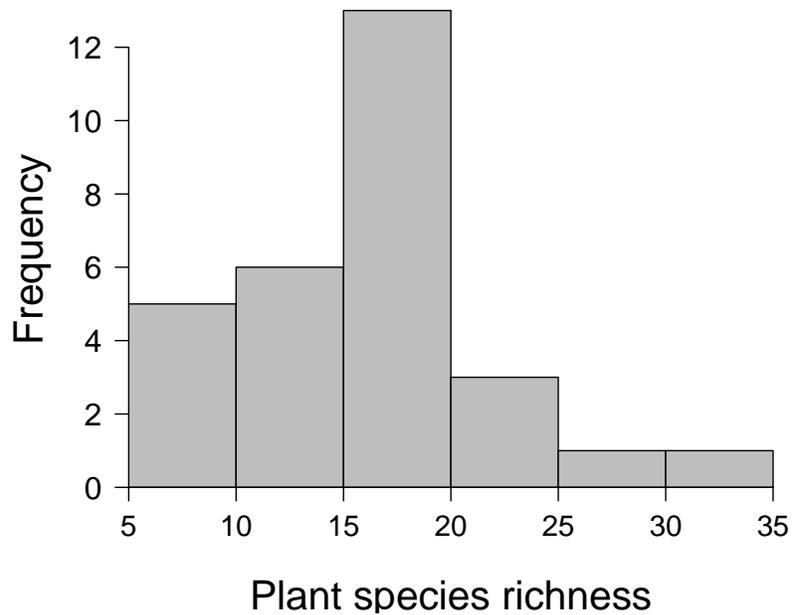


Figure A7. A Shapiro-Wilk test confirmed the normal distribution of plant species richness ($W=0.963$, $p=0.39$).



Model Selection

Table A1. “Model” indicates the response variable being tested, “K” is the number of parameters (covariate + response), “AICc” is the small-sample AIC score, “ Δ AICc” is the change in AIC score relative to the model with the highest likelihood, “ModelLik” is the relative likelihood of a model given the data, and “LL” is the log-likelihood of the model.

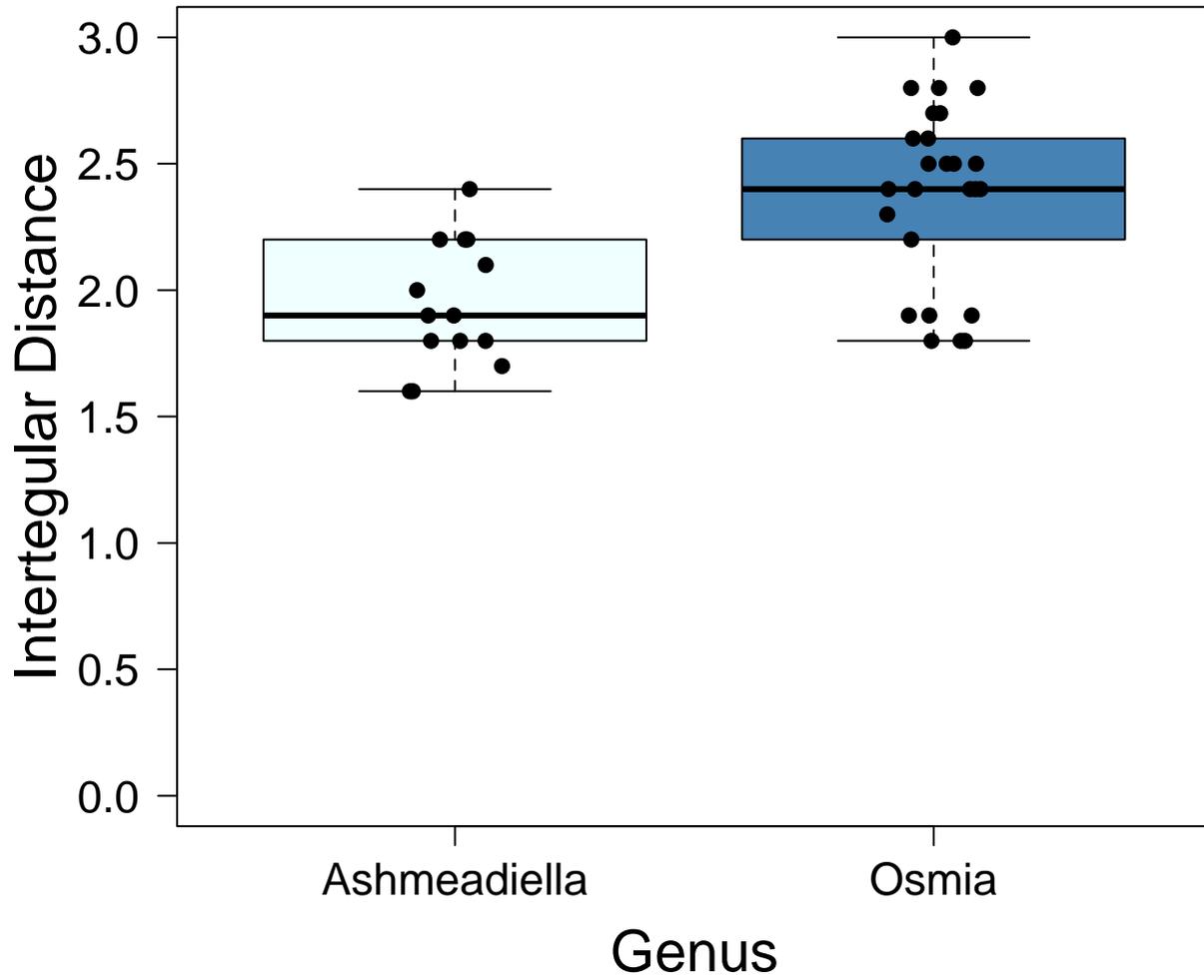
Mechanism model comparison parameters						
Model	K	AICc	Δ AICc	ModelLik	AICcWt	LL
Nest Abundance						
4	2	22.9426	0.0000	1.0000	0.5868	-7.9913
3	2	25.2673	2.3247	0.3128	0.1835	-9.1537
2	3	25.2991	2.3565	0.3078	0.1806	-7.8162
1	4	27.9091	4.9664	0.0835	0.0490	-7.6502
Megachile Nest Abundance						
4	2	22.8058	0.0000	1.0000	0.4470	-7.9229
2	3	23.5880	0.7822	0.6763	0.3023	-6.9607
1	4	25.2532	2.4474	0.2941	0.1315	-6.3222
3	2	25.4484	2.6425	0.2668	0.1193	-9.2442
Megachile cells per site						
2	3	1526.203	0.000	1.000	0.3864	-759.0345
4	2	1526.637	0.434048	0.8049	0.3111	-760.2784
1	4	1528.053	1.849915	0.3965	0.1533	-758.9254
3	2	1528.106	1.903247	0.3861	0.1492	-761.0130
Female IT span						
3	2	16.4464	0.0000	1.0000	0.5190	-3.9529
4	2	17.9525	1.5062	0.4709	0.2444	-4.7060
2	3	18.5984	2.1520	0.3410	0.1770	-3.8882
1	4	20.7741	4.3277	0.1149	0.0596	-3.8037

Table A2: Best fit model coefficients for plant species richness analyses

Response	Parameter	Estimate	Std. Error	z value	Pr(> z)	
Total Nests						
	Intercept	1.0636	0.0778	13.661	1.21e-13	***
	Range	-0.2400	0.1264	-1.895	0.0689	
Megachile Nests (1)						
	Intercept	1.0196	0.0777	13.13	3.1e-13	***
	Range	-0.3343	0.1261	-2.65	0.0133	***
Megachile Nests (2)						
	Intercept	1.2391	0.1813	6.835	2.96e-07	***
	Range	-0.2770	0.1315	-2.106	0.045	*
	Plant rich	-0.0149	0.0111	-1.336	0.193	
Megachile Nests (3)						
	Intercept	1.1861	0.1876	6.322	1.29e-06	***
	Range	0.4956	0.7400	0.670	0.509	
	Plant rich	-0.0113	0.0116	-0.971	0.341	
	X	-0.0422	0.0398	-1.061	0.299	
Megachile Cells (1)						
	Intercept	1.7750	0.0993	17.884	<2e-16	***
	Range	-0.2083	0.1045	-1.994	0.0462	*
	Plant rich	-0.0104	0.0066	-1.573	0.1158	
Megachile cells (2)						
	Intercept	1.6361	0.0446	36.678	<2e-16	***
	Range	-0.2574	0.1000	-2.574	0.0101	*
Response	Parameter	Estimate	Std. Error	t value	Pr(> z)	
Female IT span						
	Intercept	3.0130	0.1181	25.517	<2e-16	***
	Plant rich	0.0084	0.0067	1.242	0.225	

Additionally, in order to differentiate between mud nests of *Osmia* and *Ashmeadiella*, I compared the body sizes of adult individuals found within nests. *Ashmeadiella* cells were initially differentiated by their smaller appearance compared with *Osmia* cells. To support this choice of methodology, I used a chi-squared analysis to compare the IT-distances of the two genera (see Performance: Body Size section), using 15 *Ashmeadiella* and 25 *Osmia* spp. individuals. The analysis supported my hypothesis that *Ashmeadiella* bees are significantly smaller than *Osmia* ($t_{35.0} = -4.55$, $p < 0.001$, Figure 7). Because I did not find any adult *Ashmeadiella* or *Osmia* on rangeland sites, I did not need to incorporate land type as a random factor. Additionally, I assumed that the significant difference in body size between the two genera would be maintained between the two site types and thus I could preserve my methods for nest identification across the two treatments for land-use.

Figure A8: *Ashmeadiella bucconis* are significantly smaller than *Osmia spp.* and subsequently construct smaller cells and nests.



APPENDIX II

Cavity-Nesting Bee Biology

Cavity-nesting bees make up about a third of Colorado's wild bee diversity (Scott et al. 2011). While genera of cavity-nesters vary in their life histories and biology, certain characteristics are maintained throughout. All cavity-nesters are solitary- females construct and provision individual nests independent of males and other females. All bees are haplodiploid, resulting in haploid males developing from unfertilized eggs and diploid females developing from fertilized eggs (Scott et al. 2011). Additionally, from what we know, cavity-nesting bees choose both the sex and to some degree the size of their offspring (Seidelmann 2014). Generally proterandrous, females oviposit fertilized (female) eggs first and unfertilized (male) progeny last (Pitts Singer and Cane 2011). Consistently dioecious, female bees tend to be larger than males, regulated by the quantity of nectar/pollen provision provided by the mother, who also typically possesses specialized pollen-collecting morphology for nest construction that is reduced or absent in males (Scott et al. 2011).

Upon emergence, a female cavity-nesting bee will typically mate with a male waiting at the entrance of her nest. She then immediately seeks a floral resource to feed herself with nectar and facilitate egg development by feeding on pollen (Torretta et al. 2012). While males are opportunistically polygynous (Seidelmann 1999), females typically mate once before beginning nest construction (Pitts-Singer and Cane 2011). Females spend most of their life foraging for nesting sites, nest-building materials, nectar, and pollen to provision their larvae. Every nest contains a series of end-to-end brood cells with enough provisions in each cell to facilitate complete larval development (Roulston and Cane 2000). Most bees deposit only one egg per cell, with a few exceptions (see *Lithurgopsis apicalis*) (Scott et al. 2011). Female brood cells take

longer to emerge and are located at the back of the nest, while the faster-developing males emerge from cells near the nest's entrance (Pitt Singer and Cane 2011, McCorquodale 1993). While not all cavity-nesters have the same number of generations per flowering season, at least one generation of progeny overwinter within the nest as a post-defecating, diapausal larva or prepupa. At the beginning of the following season, diapause is triggered by photoperiod, temperature, or precipitation cues (Danforth 1999, Stephen and Osgood 2014).

Nest characteristics of bee genera

There are 1,478 described *Megachile* species (Pitts-Singer and Cane 2011), 59 of which occur natively in Colorado. The nesting behavior of *Megachile* is the most well-documented and understood of the genera observed in our nests. *Megachile* larvae have a largely liquid diet, which is reflected in the relative effort a female puts into collecting nectar as opposed to pollen. Additionally, *Megachile* larval provisions contain twice as much nectar as pollen (Pitts-Singer and Cane 2011). *Megachile* males receive approximately 17% fewer provisions than females, given their shorter development time and smaller body sizes (Pitts-Singer and Cane 2011).

Megachile spp. females use leaf and petal fragments in their nest construction, regardless of the specificity of foraging preferences across the genus. *Megachile* use scissor-like mandibles to cut circles from the margins of a leaf or petal. Each disk is then transported to the nesting site (Pitts-Singer and Cane 2011) and used to construct and cap off end-to-end brood cells (Moisset 2011). The edge of each new leaf piece is chewed to create a sticky pulp that binds the plant material together to line the walls of the cavity (Pitts-Singer and Cane 2011). The basal end of each cell, excluding the first cell built farthest from the nest opening, is embedded in the concave end of the preceding cell (Torretta 2012).

Megachile rotundata were the only non-native species in our sample and were introduced to the Americas as a managed crop pollinators of *Medicago sativa* (alfalfa). *M. rotundata* do not typically have a wide foraging distance, preferring to stay in close range of their nesting site and preferring agricultural landscapes. Multiple studies provide evidence this unwillingness to disperse from a site of emergence within an agricultural study (Tepedino 1983; Frohlich et al. 1983; Barthell et al. 1998), potentially explaining the absence of *M. rotundata* individuals in our rangeland sites). These bees exhibit facultative bivoltinism or multivoltinism, producing several generations per season and having a relatively short development time. *Megachile rotundata* almost exclusively use leaves in nest construction (Pitts-Singer and Cane 2011), requiring 14-17 leaf discs to complete nest construction. Additionally, *M. rotundata* are widely polylectic, preferring to forage on mass blooms of alfalfa but exhibiting little host plant preference later in the season (Pitts-Singer and Cane 2011).

Megachile brevis is a ubiquitous species across the Great Plains and are native to Colorado and essentially widespread across especially the western United States (Michener 1953). A vast majority of what is known of *M. brevis* is taken from Michener's extensive three-year study of Kansas *M. brevis*, published in 1953. *Megachile brevis* have a wide foraging range and are ready-dispersers, willing to abandon a nest mid-construction to find a higher quality resource patch. *Megachile brevis* can have up to four generations per season, facilitated by its generalist preferences (polylectic). This species has generally been observed using both leaves and petals in nest construction, using stiffer leaf material as structural support and petals as cell filler (Packer 1987, Michener 1953).

While little research exists on the nesting behaviors of *M. onobrychidis*, this species was, until recently, considered to be a subspecies of *M. brevis* (Scott et al. 2011). Additionally, *M.*

onobrychidis is widely acknowledged as a leaf-cutting species that accepts trap-nests, thus allowing us to group any potential *M. onobrychidis* nests under the general category of *Megachile* (Sheffield et al. 2011).

Finally, *Megachile montivaga* (formerly *Megachile helianthi*) (Scott et al. 2011) collect floral tissue for nest construction (Sheffield et al. 2011). These bees are reported to preferentially nest in soil, but exclusively use flower petals when nesting in cavities (Sheffield et al 2011).

Osmia bee nesting behavior is extensively documented and easily identifiable from other cavity-nesting genera. North America *Osmia* nest in pre-existing cavities and use mud to line cell walls. Cell partitions are constructed using finely-masticated leaf tissue (Cane et al. 2007, Scott et al. 2011). *Osmia* mason bee morphology indicates their preference of nest substrate. Facial horns are used by female *Osmia* to polish mud packed against cell walls during nest construction (Cane et al. 2007). Approximately 84% of *Osmia* species are reported to have this nesting behavior (Cane et al. 2007), which was consistent with our observations of mud cells containing identifiable, adult *Osmia*. Thus we were confident in our ability to identify empty nests as *Osmia* in the absence of a mature bee.

Lithurgopsis apicalis, oligolectic foragers on the *Opuntia* cactus (Rozen and Hall 2014; Scott et al. 2011), create elongated cells that parallel their large body size. *L. apicalis* nests are easily identifiable by their cells, which were largely comprised of prominent pollen provisions (Rozen and Hall 2014) (See Figure). There is no evidence that *Lithurgopsis apicalis* utilize any material other than loosely pack pollen in their nest construction (Rozen and Hall 2014). Large, elongate *Opuntia* pollen is easily distinguishable and differs from the hard-packed provisions of other species. *L. apicalis* are unique in their deposition one or two eggs per cell on discs of more

loosely packed pollen placed on opposite sides of the cells that serve as early instar provisions for developing larvae (Rozen and Hall 2014)

The genus *Ashmeadiella* is native to the western Great Basin and is closely related to *Osmia*. Little is known about *Ashmeadiella* nesting behavior and biology, but their use of cavities as nesting sites is well documented. Their small size allows them to nest in twigs and *Ashmeadiella* in our traps were found in smaller bamboo and pine cavities. *A. bucconis* has been largely observed visiting flowers in a tribe of the Asteraceae family, making them specialists in their foraging preferences (Michener 1939). In conjunction with their close relatedness to *Osmia*, cells observed in conjunction with adult *Ashmeadiella bucconis* were made from small amounts of mud and dirt and contained small, densely packed larval provisions.