The foraging and nesting behavior of four solitary-nesting bee species (Hymenoptera:Megachilidae) in the Gallatin Valley, Montana

by Peter Derek Jensen

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
I used trap-nests to study the behavior and distribution of four solitary bee species: Megachile relativa Cresson, Megachile rotundata (F.), Heriades carinata Cresson, and Coelioxys moesta Cresson. Megachile rotundata built nests that were typically filled with more cells and less empty space, while H. carinata nests had the fewest number of cells and included the most empty space of the three nesting species studied. The Megachile species made leaf and pollen foraging trips of similar duration, while H. carinata made foraging trips of shorter duration than the Megachile pollen collecting trips. Novel pollen records were noted for all three foraging species (including Caprifoliaceae and monocot pollen) and pollen use by M relativa was found to change with seasonal and geographical differences in available flora.

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APPROVAL

of a thesis submitted by

Peter Derek Jensen

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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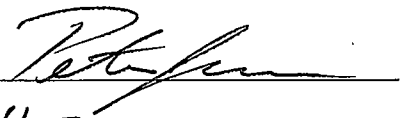
Signature 

Date 4·20·01

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ABSTRACT

I used trap-nests to study the behavior and distribution of four solitary bee species: *Megachile relativa* Cresson, *Megachile rotundata* (F.), *Heriades carinata* Cresson, and C*oelioxys moesta* Cresson. *Megachile rotundata* built nests that were typically filled with more cells and less empty space, while *H. carinata* nests had the fewest number of cells and included the most empty space of the three nesting species studied. The *Megachile* species made leaf and pollen foraging trips of similar duration, while *H. carinata* made foraging trips of shorter duration than the *Megachile* pollen collecting trips. Novel pollen records were noted for all three foraging species (including Caprifoliaceae and monocot pollen) and pollen use by *M. relativa* was found to change with seasonal and geographical differences in available flora.

INTRODUCTION

Some species of solitary bees in the family Megachilidae nest in hollow twigs or abandoned insect tunnels in dead logs (Krombein 1967). The nesting biology of these can be studied using predrilled wooden blocks called trap-nests. Trap-nests placed in habitats with appropriate floral resources will attract native populations and allow behavioral observations (Krombein 1967). In this study, I used trap-nests to examine the nesting biology of *Megachile relativa* Cresson, *Megachile rotundata* (F.), *Heriades carinatum* Cresson, and *Coelioxys moesta* Cresson in the Gallatin Valley of Southwest Montana. These species are similar in their nesting habits, with distinct differences exhibited by each species offering comparisons of nest characteristics and food resource utilization among species.

Objectives

The objectives of this study were 1) to describe and quantify nest construction patterns for *Megachile rotundata, Megachile relativa,* and *Heriades carinata* in trap-nests, 2) to determine the foraging rates and species specific pollen usage of *M. rotundata, M. relativa,* and *H. carinata*, 3) to describe the behavior of *Coelioxys moesta* at nests of *M. rotundata* and *M. relativa*, before, during, and after host parasitism and 4) to determine the distribution of *M. rotundata, M. relativa, H. carinata,* and *C. moesta* in an area encompassing the Gallatin Valley and the Bridger Mountains.

Megachile rotundata (F.)

The alfalfa leafcutting bee, *Megachile rotundata*, is a small bee, 7-9 mm long, that is indigenous to Eurasia (Stephen 1962). This bee was first collected in North America in 1947 (Krombein 1948). *Megachile rotundata* females, which are smaller than native *Megachile* have the distinguishing feature of a patch of silvery gray pollen-collecting hairs (called the scopa) on the underside of their abdomens. Most native leafcutting bees have a golden, tan, or black scopa (Richards 1984).

Much research has been done on the leafcutting bee since the late 1950's because of its economic importance in the alfalfa (*Medicago sativa*) seed production industry, which needs a pollinator with specific characteristics. An alfalfa flower is constructed so that the staminal column is held under pressure by interlocking projections from the keel and wing petals. When a bee lands on the keel, its legs often push the keel and wing petals apart, releasing the staminal column. When the flower is thus "tripped", the staminal column springs forward and hits the standard petal, rupturing the stigmatic membrane and releasing pollen from the staminal column (Free 1993). Because the stigmatic membrane must be ruptured to provide a liquid medium for pollen germination and growth (Armstrong and White 1935), a flower that is not tripped does not set seed. Although seeds may be set from self-pollination, more seeds are formed per pod and the seeds are larger when the flower is cross-pollinated (Free 1993). These requirements dictate that a pollinator be large enough to trip the flowers, and mobile enough to cross-pollinate the flowers.

Honey bees (*Apis mellifera* L.) are often ineffective alfalfa pollinators in many areas because of the construction of the alfalfa flower and the time of the flowering season (Bohart 1972). In many areas honeybees do not trip the alfalfa flowers, or are discouraged by the tripping action of the alfalfa flower, and avoid alfalfa. This results in some tripping estimates as low as 0.8% for honeybees (Stephen 1955). In contrast, the alfalfa leafcutting bee accepts alfalfa readily when it is the closest and most abundant pollen source (Stephen and Torchio 1961) and usually trips between 90 and 98% of the flowers it visits. Alfalfa leafcutting bees are also easy to manage because they can be reared gregariously in artificial nests and because their emergence can be manipulated to coincide with the first alfalfa bloom.

Both Stephen (1962) and Bohart (1962) reported that *M. rotundata* prefers alfalfa, but in the absence of alfalfa would also collect pollen from sweet clover (*Melilotus* spp.), Dutch clover (*Trifolium repens* L.), sunflower (*Helianthus* spp.), Russian thistle (*Salsola* spp.), *Eriogonum spp.*, and rabbit brush (*Chrysothaminus* spp.). Packer (1970) and Szabo and Smith (1970) showed that *M. rotundata* collected pollen from 21 of 100 plant species that were available and that alfalfa was not the preferred source of pollen from among these 21 plants. Horne (1995b) performed an experiment in southern Alberta that indicated the preferred pollen source was bird's-foot trefoil (*Lotus corniculatus* L.) and crown vetch (*Coronilla varia* L.), but that the highest level of nesting success was obtained using the moderately preferred sainfoin (*Onobrychus viciifolia* Scop.) pollen. Stephen and Torchio (1961) who observed the alfalfa leafcutting bee in an alfalfa field, described the bee as preferentially oligolectic. They claimed that although many other

plants were available beside the alfalfa field, pollen analysis showed only alfalfa pollen present in pollen samples collected from leafcutting bees. Nevertheless, *M. rotundata* can reproduce successfully on other crops such as red clover and white clover (*Trifolium* spp.) (Holm 1984), cicer milkvetch (*Astragalus* spp.) (Richards 1986), sainfoin (Richards and Edwards 1988), and wild lowbush blueberry (*Vaccinium* spp.) (Stubbs and Drummond 1997).

Cross-pollination of alfalfa and other crops occurs due to the position of the bee on the flower, as described by Vansell and Todd (1946). Since the bee always takes the same position on the flower, the staminal column always hits the bee beneath the head. This recurring pattern presents the opportunity for cross-pollination when pollen mixes on the bottom of the bee's head.

The life cycle of *M. rotundata* is fairly straightforward and lends itself to use in commercial alfalfa seed production. The leafcutting bee does not construct a burrow, but occupies pre-existing tunnels (Stephen and Torchio 1961). The cells in a tunnel are constructed with leaf cuttings obtained from one of many plant species usually in the Fabaceae or Polygonaceae family (Horne 1995a). The average cell is made with 4 to 5 leaf cuttings, stuck to each other and the walls of the tunnel with salivary secretions from the female. When preparing a cell, the female enters the nest headfirst with a leaf piece, arranges it, and then backs out of the nest and flies off to get another leaf piece. Klostermeyer and Gerber (1969) used an event recorder at nest entrances in Washington to monitor the duration of these trips. To complete a cell on average requires 15 leaf collecting trips of 318 seconds each, and 258 seconds spent in the nest between trips.

Once a cup-shaped structure is made from leaf pieces, the female starts to make nectar and pollen collecting trips. To provision a cell on average requires 17 trips lasting 894 seconds each and 204 seconds spent in the nest between each trip (Klostermeyer and Gerber's 1969). Because the combined trips average over 7 hours, a female can usually only provision one cell per day. The female deposits a single egg on the completed pollen and nectar mass in each cell. The cell is then capped with 3 to 10 small leaf cuttings the same size as the burrow (Stephen 1962) and another cell is started immediately in front of the completed cell in the nest. The females generally lay female eggs in the deepest third of the nest, and males in the outer two thirds of the nest (Gerber and Klostermeyer 1972, Richards 1984). When this pattern is disrupted it is generally evidence of nest usurpation, parasitism, or a multiply mated female (McCorquodale and Owen 1997). *Megachile rotundata* females generally prefer a burrow with an average diameter of 5.5-mm (Stephen 1961). Burrows up to 9.5-mm diameter are accepted by the bees, but are lined with more leaves than normal to reduce the size of the nest. These nests are still large however, and the pollen ball and the larvae might be up to twice the volume of those in normal nests (Stephen 1962). The number of cells constructed in a nest is a function of the length of the burrow, but usually ranges between 8 and 12 cells. Once all of the cells have been completed, the nest is then capped with 4 to 130 leaf cuttings (Stephen 1961). Females may construct up to 25 to 40 cells a year (Bohart 1962, Stephen 1961. Klostermeyer and Gerber (1969) recorded an average of 7.5-hours to complete a cell in Washington, but under ideal foraging conditions Richards (1984) recorded a completed cell in only 2.5 hours.

The time for egg and larval development varies with temperature. Richards (1984) noted that, at 15° C, it takes 15 days for the eggs to hatch, and 35 days for the larva to reach the prepupal stage. However, Stephen (1961) stated that, at 30° C, it takes only 2 to 3 days for the eggs to hatch and 11 days to reach the prepupal stage. More recently, Kemp and Bosch (2000) reported that it is not possible for the bees to complete development at a constant 18° C, but that the bees reach the prepupal stage in 11 to 13 at constant 26 or 29° C or at a variable 14:27° C treatment. The prepupa spins a cocoon to separate itself from its' own fecal pellets, and overwinters in this state (Richards 1984). Warmer temperatures during the prepupal stage stimulate some prepupa to complete development without diapause and emerge as a second generation later in the same season. Kemp and Bosch (2001) showed that temperature at the prepupal stage determines what percentage of a *M. rotundata* population will become nondiapusing, from 7 percent of the population at a constant 18° C during the prepupal stage, to 45 percent at a constant 32° C.

Typically the bees begin to emerge in early June, but emergence continues until the end of June (Stephen and Torchio 1961). The adults emerge in the opposite sequence to that which they were laid in each tube. For example, the last cell formed is the first to emerge, then the second last cell formed. This continues in succession, except in cases where a bee matures early and in the process of emerging chews its way through and destroys its' nestmates (Stephen 1962).

Emerging adults are generally in the ratio of two males per female. The males mature earlier, emerge first, and wait for the females to emerge so they can mate. Mating

often occurs as females are emerging, or during one of the females' frequent rest stops, frequently occurring in direct sunlight (Stephen 1962). Females usually mate only once (Hobbs 1967), but males may mate several times (Richards 1984). Females thus retain enough sperm in their spermatheca to fertilize all of the eggs that they will lay in their lifetime (Richards 1984). The females are larger than the males and have an oval shaped abdomen that ends in a sting, and has conspicuous rows of pollen-collecting hairs called a scopa. The males have a straight-sided abdomen, no scopa, and an abundance of yellow hair on their face (Richards 1984). A newly emerged female feeds on nectar and pollen during which time the eggs develop after mating, and then selects a nest site and begins constructing cells. The nesting habits of these bees are distinctly gregarious as the females show a preference to nest close to conspecifics, and will accept very crowded nesting conditions (Kukovica 1966, Tepedino et al. 1994). The females return to the nesting tunnels at night, and remain active for almost two months. Conversely, the males remain in the field overnight, and are reduced to 50% of their numbers 15 days after emerging (Richards 1984).

Alfalfa leafcutting bees require a minimum temperature of 21° C for flight and 18° C for development unless specifically bred for colder temperatures. Apparently, the bees are remarkably plastic as shown by Holm (1984) who studied a bee population that adapted to the cold windy climate of Denmark within five years. Even if they are bred for colder temperatures, however, many environmental variables associated with higher temperature, heat units, and mean actual temperature have a significant positive effect on bee productivity and cell quality (Richards 1996). The bees have a long foraging life (9

weeks) and thus remain active sometimes until late September (Bohart 1972). They are most efficient for the first half of the summer, after which behavior and reproduction often becomes erratic and much less efficient (Stephen 1961). If the nests are warm throughout the summer, it is possible to have a second generation emerge in late summer (Stephen 1961, Kemp and Bosch 2001). This second generation varies in efficiency and represents a loss for owners in northern North America. In Montana the second generation provides no pollination service since the alfalfa seeds will not set before the frost, and the second generation is not very efficient at constructing new cells due to a lack of resources and cooling temperatures (Richards 1984). In warmer production areas, the second generation is considered beneficial, providing an extended growing season by continuing pollination once the first generation has dwindled (Parker et al 1987).

Alfalfa leafcutting bees are rapid fliers, but have a limited range that is generally less than 250 meters from the nest site when forage is available (Stephen 1962). This, however, allows them to return to the nest with leaf cuttings within as little as 10 seconds. Pollen collecting trips can last anywhere from 2 to 15 minutes (Stephen 1962, Klostermeyer and Gerber 1969). The short foraging range benefits growers since the bees only pollinate the alfalfa belonging to the owner, and the bees are not lost to insecticides in neighboring fields (Stephen 1961). Females visit flower after flower, collecting pollen and nectar from 11 to 15 flowers per minute. The males visit flowers for nectar only and seldom trip flowers (Hobbs 1967).

Megachile relativa Cresson

Megachile relativa is very similar to *M. rotundata*, but is native to North America and is distributed from the Northwest Territories to the East Coast, and south to California and Georgia (Krombein et al 1979).

Interestingly, however, this species has received little attention in the literature beyond mention by Krombein (1967), a report on its' biology in Wisconsin by Medler and Koerber (1958), and more recently its nesting ecology in Michigan (Strickler et al. 1996).

The life cycle of *M. relativa* is almost identical to that of *M. rotundata*. Adults emerge in mid-June in Michigan and the females begin nest construction (Strickler et al. 1996). Females prefer somewhat larger nests, of a diameter ranging from 5.5 to 6.25 mm (Medler and Koerber 1958, Strickler et al. 1996). A basal space, or a base of circular leaf pieces frequently precede the first cell. *Megachile relativa* nests also frequently contain diffuse plugs, vestibular spaces, and indentations. Diffuse plugs are leaf pieces pushed together in sequence, but not chewed or packed together. Vestibular spaces are empty spaces between the outermost provisioned cell and the end plug. Indentations are spaces between the outermost leaf of the end plug (or "cap") and the entrance to the nest. Cells are constructed on average with 10 oblong leaf pieces, capped with 3 circular leaf pieces and have a completed length of about 11 mm. An average of 4.2 cells are built in nests with a depth of 142 mm , although up to 14 cells have been reported (Strickler et al. 1996). Medler and Koerber (1958) estimated that females completed one or two cells per day.

In Wisconsin, the egg hatches after 2 to 3 days and the larva passes through five instars over 12 to 14 days while consuming the provisions and spinning a cocoon under field conditions. Larval development can be reduced to 6 to 8 days by increasing the temperature up to a constant 27° C (Medler and Koerber 1958). The pupal stage can also be reduced from 18 days in the field to 6 days at 27° C. The adults do not emerge immediately upon eclosion, but remain quietly within the cell for three to four days while their integument hardens. *Megachile relativa* is reported to be bivoltine in Michigan and in Wisconsin (Medler and Koerber 1958, Strickler et al 1996).

Although no sex ratios were reported, the sex data from Medler and Koerber (1958) pertaining to the cell sequence in completed nests showed a female bias. These data also showed that female eggs are preferentially placed in inner cells of the nest. Strickler et al. (1996) reported average female weight to be 43 mg, whereas that for males who emerge earlier is only 32 mg.

Heriades carinata Cresson

Heriades carinata ranges through most of the United States from Quebec south to Georgia and British Columbia south to Arizona and Texas. The only existing literature is a short note by Rau (1922), a detailed study performed by Matthews (1965), and some notes from Krombein (1967).

H. carinata is a small black bee with a gray scopa and a life cycle very similar to that of *Megachile rotundata,* except that the nest partitions are made with pitch instead of leaf pieces.

Matthews (1965) listed 6 plants species on which he observed flower visitation. However, he noted that many of these plants may have been only nectar sources because pollen analysis revealed that provisions were almost exclusively made up of sumac (*Rhus typhina* L.) pollen. Pollen gathering trips in Michigan averaged 597 seconds with 52 seconds spent in the nest to deposit the pollen, and pitch collecting trips lasted 287 seconds.

A comparison of *Heriades carinata* populations in Michigan and Oregon revealed an average of 4.6 cells and 6.4 cells per nest, respectively (Matthews 1965). The nests often contained a basal space, as well as a vestibular space and an indentation. The end plug is much thicker than the cell partitions, and may contain debris in addition to pitch.

Coelioxys moesta Cresson

Bees in the genus *Coelioxys* are cleptoparasites (brood parasites) primarily in the nests of *Megachile* species (Baker 1971). *Coelioxys* females enter a nest while the host female is foraging and oviposits by piercing the provision and the leaf lining and depositing an egg in the resulting slit. One end of the parasite egg touches the pollen mass, but the majority of the egg is located between the leaf layers of the host cell (Graenicher 1905). The first instar is very brief and seldom seen. The second and third instars are very characteristic with long mandibles that are used to kill the host larva, and any other parasitic larvae that may be present in the cell. The fourth and fifth instars are similar to the host larvae in both size and habit (Baker 1971). The adults emerge at the same time as the host bees from unparasitized cells within the same nest.

Known hosts of *Coelioxys moesta* include, *Megachile centuncularis* (L.), *M. concinna* Smith, *M. frigida frigida* Smith, *M. relativa* Cresson, and *M. texana* Cresson.

METHODS

Description of Sites

Study sites were all located in Montana's Gallatin Valley and were selected because of accessibility, diversity, and low human traffic. At each site I placed one set of trap-nests with multiple nest hole sizes (see below). The following is a brief description of each site:

1) MAD River N (111°32'W, 45°46'N): This site is located approximately 30 km south of Three Forks, Montana. The trap-nests were located directly under limestone cliffs, about 20 m from the Madison River, shaded by cottonwoods (*Populus deltoids* Marsh.).

2) MAD River S (111°32'W, 45°46'N): The trap-nests were located 30 m from the Madison River on a level floodplain, 20 m from limestone cliffs, shaded by cottonwood trees.

3) MAD Gully (111°32'W, 45°46'N): The trap-nests were located halfway down the length of a 1.3 km gully, shaded by Juniper (*Juniperus* sp.).

4, 5) HHS, HHN (111°24'W, 45°55'30"N): The Horseshoe Hills site is 8 km north of Logan, Montana. The trap-nests were separated by approximately 1 km each. The North and South trap-nests were located within 10 m of a seasonal stream, shaded by cottonwoods. Vegetation primarily consists of cottonwood, junipers, Yellow Sweetclover (*Melilotus officianalis* (L.) Lam.), Canada thistle, (*Cirsium arvense* (L.) Scop.).

6) HH Gully (111°24'W, 45°55'30"N): The trap-nests were located in a small dry gully, shaded by Junipers. This site was approximately 1 km east of the other two Horseshoe Hills sites.

7) RB Stream S (111°39'W, 45°35'N): The Red Bluff site is located 8 km east of Norris, Montana. The stream trap-nests were located 10 m from a stream, in a long canyon, shaded by junipers.

8, 9) RB Marsh N, RB Marsh S (111°39'W, 45°35'N): The North trap-nests were located immediately beside a stream in a marshy area surrounded by rangeland. The South trap-nests were located 1 km farther south in the marsh on the edge of dry rangeland.

10) Olson (111°04'W, 45°36'N): This site is 8 km south of Bozeman, Montana, at the base of the Hyalite Canyon drainage. The trap-nests were located 30 m from a large stream and 200 m from a sprawling neighborhood containing ornamentals. Trap-nests were shaded by scrubby cottonwoods

11) W. Bozeman (111°04'W, 45°40'N): This site is at the Montana State University Horticulture Farm on the west end of Bozeman. The trap-nests were located in an abandoned ornamental orchard, shaded by dogwood (*Cornus stolonifera* Michx.). The orchard was surrounded by agricultural test plots and rangeland.

12) SE Bozeman (111°04'W, 45°40'N): This site is in a SE neighborhood in Bozeman. These trap-nests were located in a residential backyard with many ornamentals. The trap-nests were 150 m from a weedy industrial storage yard and 300 m from a wooded (cottonwood) stream.

13) Battle Ridge (111°05'30"W, 45°36'N): This site is 30 km north-east of Bozeman, Montana in the Bridger Mountains. The trap-nests were located in a Douglas fir (*Pseudotsuga menziesii* (Mirbel) Frango) forest, montane area, shaded by junipers.

14) RCF (111°57'W, 54°40'N): This site is 4 km east of Bozeman, Montana. The trap-nests were located 20 m from stream, along the weedy border of a cultivated field, shaded by lilac (*Syringa vulgaris* (L.)).

15) Fort Ellis (111°57'W, 54°40'N): This site is 3 km east of Bozeman, Montana. The trap-nests were located in the wooded/weedy border of a sheep pasture, 50 m from a farmhouse with ornamentals. Shaded by Douglas fir.

16) Grassy Mountain 2.5 and 3.5 (110°51'W, 45°49'N): This site is 16 km north-east of Bozeman, Montana in the Bridger Mountains. The trap-nests were located 4 and 5.6 km up a logging road on a mountainside in a logged Douglas fir forest, shaded by juniper. There is a marshy area 30 m below the GM 3.5 site.

17) BFH (110°58'30"W, 45°42'30"N): The Bozeman Fish Hatchery is located 3 km east of Bozeman. The trap-nests were located behind the mechanical shed, 5 m from a stream, shaded by cottonwoods. The fish hatchery lot has many ornamentals and weedy borders.

18) Fulker (111°15'W, 45°52'N): This site is 8 km east of Manhattan, Montana. The trap-nests were located 5 m away from a small stream, shaded by large cottonwoods, and 40 m from a farm house surrounded by pasture.

19) Post Farm (111°04'30"W, 45°40'N): This site is 3 km west of Bozeman. The bee boards and trap-nests were located 50 meters from a small stream, and between two seed

alfalfa test plots with weedy borders. Shade was provided by a shelter built for the bee boards by Ruth O'Neill, who also placed the *M. rotundata* at the site. Bees were originally purchased from Mennie Bee Farms Inc. (Parkside, Saskatchewan).

Four of these sites, (W Bozeman, SE Bozeman, RCF, and Post Farm) were selected for behavioral studies due to proximity and nesting species.

Trap-Nesting Methodology

Bees constructed nests in trap-nests placed at our sites during each summer. Three types of traps were used at the sites. The first trap-type consisted of pine boards drilled to a depth of 15 cm with diameters: 4.8, 6.4, 7.9, 9.5, and 11.1 mm. I drilled 6 to 10 holes of one diameter in each board. I then inserted cardboard tubes with diameters of 3.2, 3.7, 4.6, 5.9, 7.5, 8.0, and 9.0 mm into the holes. This provided an easy way to remove a capped nest and enabled us to provide a consistent number of available nest holes, as well as an efficient method of storing and incubating the completed nests.

Five boards with holes of equal diameter were held together with a large hose clamp, which also held the "block" of boards to a 6 x 12 x 48 cm long wooden board. Three or four blocks with different hole sizes were mounted on the wooden board that was then bound to a fence post at the site. Blocks with larger holes were mounted higher on the post with four inches separating each block. The blocks were all oriented with the holes facing southeast to receive direct insolation each morning (Stephen 1962).

The second trap type consisted of the same pine boards, this time with 15 cm grooves routed in the side. Three nest diameters were cut: 6.3, 9.5, and 12.5 mm. A

plexi-glass sheet (3 mm thick) was then fitted and fastened with black electrical tape to the grooved side of the board. This provided a transparent surface to view nest construction, measure nest dimensions, and observe adult emergence. These boards were mounted in the same fashion to 6 x 12 x 48 cm long wooden boards on fence posts. The transparent side of the boards faced inwards in the blocks to ensure that light did not disturb the nesting females. The hose clamps holding the blocks together could be easily opened in the field to allow quick nesting observations through the plexi-glass.

The third type of trap was used at the Post Farm site and consisted of grooved, mated, expanded polystyrene laminate boards 9.5 cm deep manufactured by Beaver Plastics (Edmonton, Alberta) used in commercial seed alfalfa operations (Richards, 1984). These traps were used only because *M. rotundata* was so rare at the other two sites, and the ongoing alfalfa experiment being conducted by Ruth O'Neill and Sue Blodgett provided *M. rotundata* nests for observation and measurement.

Behavioral observations

Nest Structure

I measured the diameter, cell length, space length, vestibule length, plug length, and indentation length for 50 completed nests of the 3 species. Measurements were taken in a different way for each of the trap-nest types. Because *H. carinatum* nested only in the cardboard tubes, these nests were cut open along one side and rolled open to examine the nest structure. *Megachile rotundata* nests were visible when the grooved polystyrene boards were separated. Basal space, vestibular spaces, nest plugs, indentation, and cell length measurements were taken with the cells in sequence, and leaf count data was

collected as the cells were dissected to obtain pollen samples. *Megachile relativa* nests were all obtained from the routed nests, and the nest dimension measurements were made through the plexi-glass. Again, leaf counts were taken as the cells were dissected to obtain pollen samples.

Foraging Behavior

All four species were observed during nest construction, provisioning, or parasitising cells. Observations were made daily while I was sitting just below the nests, out of the flight path of approaching females. When large numbers of females were active, a video camera was used to record activity. Each female arrival and exit from the nest was recorded along with any visible cargo the female was carrying. Any unique or rare behavior or interactions were also noted. A series of observation intervals were tested before settling upon an ideal interval of two hours to obtain the most useful data while allowing me to remain attentive.

The plexi-glass trap-nests were checked daily and progress marked on the glass to record any nest building progress and determine a cell construction rate.

Megachile relativa and *Coelioxys moesta* females were observed inside the nests and their behavior recorded *ad libitum*.

Pollen Types Within Nests

Throughout the field season, I collected and pressed flowers in the proximity of the nests (at the W. Bozeman, SE Bozeman, and RCF sites) every three weeks. I sampled in a 200 m radius when possible and collected any visible flowers. The pollen from these

specimens was used to create a reference collection using the methods described by Moore et al. (1991) and Sawyer (1988) with slight adaptations described below. Pollen was taken from the anthers and placed in an Eppendorf tube with 2 ml of distilled water and one drop of Safranin. The sample was allowed to sit for 24 hours and then centrifuged at 3000 rpm for five minutes. The dye was poured off and the pellet re-suspended in water for a second rinse. After a second centrifugation, the supernatant was poured off, and the pellet re-suspended in two drops of water. The sample was placed on a slide and allowed to dry. Euparal was used as a mount and the slides were sealed with clear nail polish. Slides were examined under a Nikon phase contrast light microscope at 400X.

Samples were obtained from the nests in two ways. The first was to insert the wooden end of a cotton swab stick in the nest and twist it in the exposed outermost cell provision while the female was out gathering pollen in the field. The second method involved dissecting the nest in the lab and taking a pollen sample from either the uneaten provision, or the frass left by the developing larva (Strickler et al.1996). Sample type was recorded to check for differences in samples due to maceration or digestion. The condition of the larva in each cell was also recorded during the dissection of the nest and categorized as healthy, dead, or moldy. Moldy larvae are of course dead, but pathogenic mold may be a mortality factor that is be related to the pollen chosen by the foraging female, and it was easily distinguished from other mortality factors.

The entire slide was examined and any pollen types that comprised more than 1% of the sample were recorded. I did not record the pollen types in very small quantities

because they were probably not due to foraging by the female, but rather pollen mixing on the flowers or another source. Pollen grains were identified by comparison with reference slides. General characters were verified using Kapp (1969). Reference slides were kept in the laboratory of Kevin O'Neill at Montana State University.

Because this study was performed in conjunction with a diversity study, I was only able to dissect cells that were built in the plexi-glass traps that were exclusive to this experiment. Nests built in soda straws could be dissected only after adults were allowed to emerge. Because the year 2000 adults had not emerged before this paper was submitted, I was only able to examine pollen for more than one season in *M. relativa* (1999 – 2000) nests, but not in *M. rotundata* or *H. carinata* (1999 only).

Larval Health and Parasitism

Upon dissection of the *M. relativa* cells to obtain pollen samples, any parasitism, or larval mortality was recorded, in order to provide an account of larval mortality in the native *M. relativa* population. Although not from a native population, larval mortality for *M. rotundata* was also recorded. These data revealed the occurrence of *Coelioxys moesta* because even *Coelioxys* larvae that do not complete development leave behind characteristic cast skins from the second and third instars (Graenicher1905), and in some cases the slit formed by the ovipositing female is still visible in the cell lining.

Larval condition was not recorded for *H. carinatum*, because the larvae were all allowed to develop to adults for consideration in a simultaneous diversity study. In many cases, I was unable to tell from the nest remains if a larva had emerged or not.

Distribution

Traps at each of the 19 sites were checked every 1 to 14 days for nests with a finished end plug (capped). Capped, cardboard-lined nests were immediately replaced with an empty cardboard tube, and filled transparent boards were replaced when more than 50% of the available tubes were filled. The cardboard tubes were labeled with the site and collection date and stored in perforated plastic bags placed in ventilated 24 x 36 cm plastic tubs according to site. The nests were held at room temperature in the lab until October to allow the emergence of any second generation bees, and then incubated at 8° C for five to seven months during the winter to initiate diapause. Nests were removed from cold storage in April each year to allow the adults to emerge before the start of the next field season. Emergence dates were recorded for all specimens. Specimens were sent to Dr. Terry Griswold at the Utah State University Bee Lab in Logan Utah for identification. Voucher specimens were placed in the insect collection at Montana State University in Bozeman.

Statistical methods

Minitab release 13.1 was used for all of the statistical analysis (Minitab Inc. 2000). Nonparametric tests were used for all of my data because data sets were usually small and not always normally distributed. Chi-square tests were used to compare all of the pollen usage data across species and within all of the *M. relativa* pollen type categories. The Kruskal-Wallis test was used to compare both inter and intra-species cell length, the number of leaf pieces used in cell cup or cell cap construction, and the

duration of different foraging trip types. Mood's Median Test was used to compare the percent reproductive and non-reproductive space used by each species in the nest. Finally, I used Principle Components Analysis to examine for similarities in the Megachilid bee fauna among sites.

I used transition matrices to organize and arrive at an average nest structure for the nests of *M. relativa, M. rotundata,* and *H. carinata.* Using all of the structures that I recorded from each *M. relativa* nest, I listed each structure along the top and left side of the matrix. The matrix was then filled with relative probabilities reflecting how often one structure followed another in the nests. For example, if I look at the diffuse plug character, I tabulate how many times a diffuse plug was followed by a space divided by the number of *M. relativa* nests, and record it at the intersection of the diffuse plug row and the space column. Then I tabulate how many times a diffuse plug is followed by an end plug divided by the number of *M. relativa* nests, and record it at the intersection of the diffuse plug row and the end plug column. To interpret the matrix and get the structure of a typical nest, I simply followed the relative frequencies greater than 0.5 down the rows. The same procedure was used for *M. rotundata* and *H. carinata* nests using all of the structures found in their respective nests.

RESULTS

Nest Structure

Basic Structure and Nesting Materials

The three species constructed nests with different dimensions, cell numbers, and used different types or amounts of nesting materials. *Megachile relativa* nest cells consisted of capsules constructed by the nesting female from circular pieces of leaves excised from nearby vegetation. The mass of pollen, on which a single egg is laid, is deposited in the basal (cup) portion of the cell. Before beginning a new cell, the female uses leaf pieces to build a cap on the provisioned cell. In this species the average number of provisioned cells was 6.05 $\pm$ 0.67 ($\pm$ SE) and they are contiguous (Fig. 1). The space between the last provisioned cell and the entrance of the nest was occupied by some combination of empty space, diffuse plugs made of loosely associated leaf pieces, and dense plugs made of tightly-packed leaf pieces. Using the transition matrix (Appendix A) I arrived at a typical *M. relativa* nest containing provisioned cells followed by a diffuse plug, a vestibular space, a second diffuse plug, an end plug, and finally an indentation (Fig. 1).

Megachile rotundata nests were made of the same basic materials as *M. relativa* nests, but their overall structure differed slightly. On average they contained 6.67 $\pm$ 0.41 ($\pm$ SE) provisioned contiguous cells. In addition, the space between the last cell provisioned and the entrance of the nest was occupied by a single diffuse plug followed by a densely packed end plug at the nest entrance. This basic structure was also observed in each one of the hundreds of *M. rotundata* nests (also from the Post Farm site)

examined by Ruth O'Neill (personal communication). I used the same transition matrix procedure as with *M. relativa* to arrive at a typical nest structure for *M. rotundata*. The nest has a (basal) space between the innermost end of the nest tunnel and the innermost cell. The average nest contains cells followed by a diffuse plug, a chewed and packed end plug, and then an indentation at the nest entrance (Fig. 1).

Heriades carinata nests were different from those of *M. relativa* and *M. rotundata* in several different ways. The first is that the cells were not encapsulated with leaf pieces, but rather partitioned with thin walls of tree sap. Second, the average nest contained only 4.67 $\pm$ 1.0 ($\pm$ SE) provisioned contiguous cells. Third, the empty spaces between the basal end of the nest and the first cell, and between the last cell and the plug at the nest entrance (vestibular) were often very long, taking up the majority of the available space in the nest. Using the same transition matrix procedure, I arrived at a typical nest structure for *H. carinata* consisting of a basal space, provisioned cells, a vestibular space, and then a thick end plug at the nest entrance (Fig. 1).

Nest Cell Numbers and Dimensions.

In nest cell cavities with diameters of 6 and 9 mm (all 150 mm long), completed *M. relativa* nests contained a mean ($\pm$ SE) of 6.05 $\pm$ 0.67 cells (N = 22 nests; range: 1-11 cells), or approximately 0.4 cells per cm of the nest tunnel. *Megachile rotundata* nests, all of which were constructed in cavities with diameters of 5 mm (all 95 mm long) housed a mean of 6.67 $\pm$ 0.41 cells (N = 9; range: 5-9 cells), or approximately 0.7 cells per cm of the nest tunnel. *Heriades carinata* nests, all built in 4 mm diameter, 150 mm

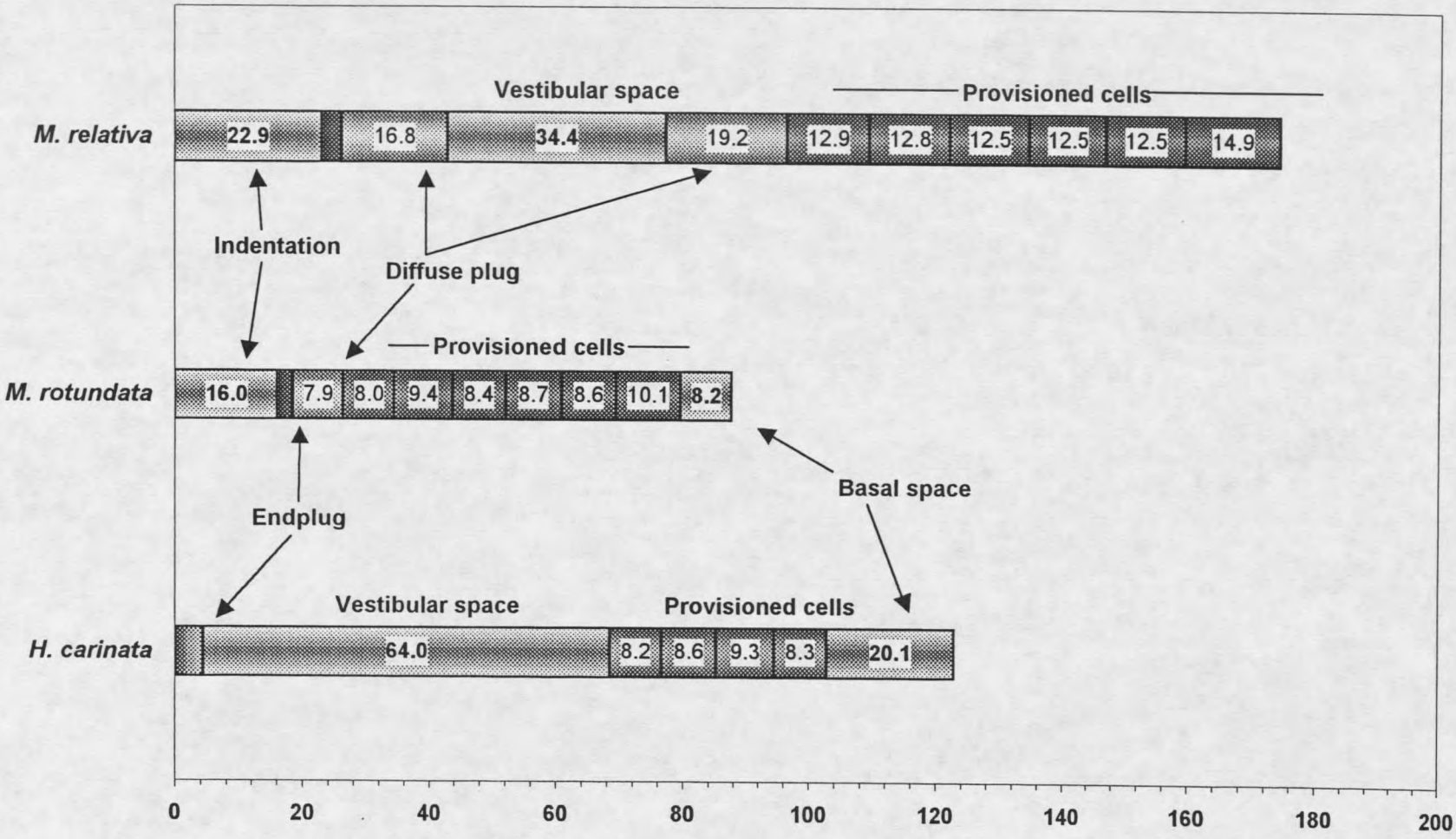

Figure 1. Typical Nest construction for *M. relativa, M. rotundata*, and *H. carinata.* Lengths in mm.

long cavities, contained only 4.67 ± 1.0 cells per nest on average (N = 10; range: 2-9 cells per nest), or approximately 0.31 cells per cm of nest tunnel.

Because the three species differ in body size, I anticipated that they would build cells of different length. Comparison of cell lengths, using just the first four cells built in each nest (because the average *H. carinata* nest contained only 4 cells) revealed significant differences among species (Table 1) (Kruskal-Wallis test, $P < 0.0001$).

Table 1. Mean cell length (mm) of basal four cells in nests of *M. relativa, M rotundata,* and *H. carinata.*

	M. relativa		*M. rotundata*		*H. carinata*	
	Mean ± SE	N	Mean ± SE	N	Mean ± SE	N
Cell 1	14.9 ± 0.45	31	10.1 ± 0.54	9	8.3 ± 0.70	10
Cell 2	12.5 ± 0.34	27	8.6 ± 0.50	9	9.3 ± 0.60	10
Cell 3	12.5 ± 0.50	22	8.7 ± 0.29	9	8.6 ± 0.68	5
Cell 4	12.5 ± 0.42	19	8.4 ± 0.44	9	8.2 ± 0.37	5
Total	13.2 ± 0.24	99	8.9 ± 0.25	36	8.7 ± 0.33	30

An intra-species Mann-Whitney comparison of the cell lengths showed that while the first cell was significantly longer than the next three combined for *M. relativa* ($P < 0.0001$) and *M. rotundata* ($P = 0.0065$). *Heriades carinata* did not construct nests with the same trend, and none of the first four cells was significantly longer than the others (Mood's Median Test, $P = 0.32$).

The comparison of percent reproductive space (how much of the available space in the nest is occupied by provisioned cells) within the nest was performed using Mood's Median Test. It showed that *M. relativa* and *M. rotundata* did not have significantly different ratios of reproductive space in the nests, while *H. carinata* had significantly less reproductive space ($P < 0.001$). The same test was used to compare the percentage of

non-reproductive space (i.e. the amount of available space in the nest occupied by plugs, vestibular spaces, and basal spaces). As expected, *H. carinata* had the highest amount of non-reproductive space in the nest (P = 0.003), while *M. relativa* and *M. rotundata* did not have significantly different percentages of non-reproductive space (P = 0.94). These differences seem to be due to the large amount of empty space that *H. carinata* leaves in the nests (Table 2).

Table 2. Percent nest space (± SE) devoted to different structures.

	M. relativa	*M. rotundata*	*H. carinata*
Provisioned cells	39.5 ± 1.7	55.1 ± 3.5	17.33 ± 2.0
Empty space	22.9 ± 5.0	25.5 ± 3.5	51.8 ± 7.0
Plugs[A]	18.4 ± 2.9	9.9 ± 1.5	3.0 ± 0.3

[A] diffuse or densely-packed leaf material for *Megachile*, plant sap for *Heriades*.

Number of Leaf Pieces per Cell

The number of leaf pieces used to construct cell cups differed among 6 (N = 74 cells) and 9 mm (N = 42 cells) diameter *M. relativa* nests and 5 mm (N = 29 cells) *M. rotundata* diameter nests. Cells within 9 mm *M. relativa* nests were constructed with a significantly greater number (Kruskal-Wallis, P < 0.001) of leaf pieces than both 6 mm *M. relativa* and 5 mm *M. rotundata* nests (Fig. 2). This difference was expected, *M. relativa* females needed to fill the extra space in a 9 mm nest with extra leaf pieces or the provisions and larvae would be much larger than a larvae in a 6 mm diameter cell. It is interesting to note that the 6 mm *M. relativa* and 5 mm *M. rotundata* nests do not have significantly different numbers of leaves.