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

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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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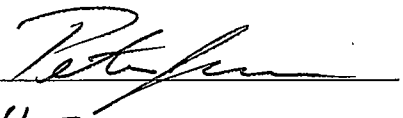
Signature 

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

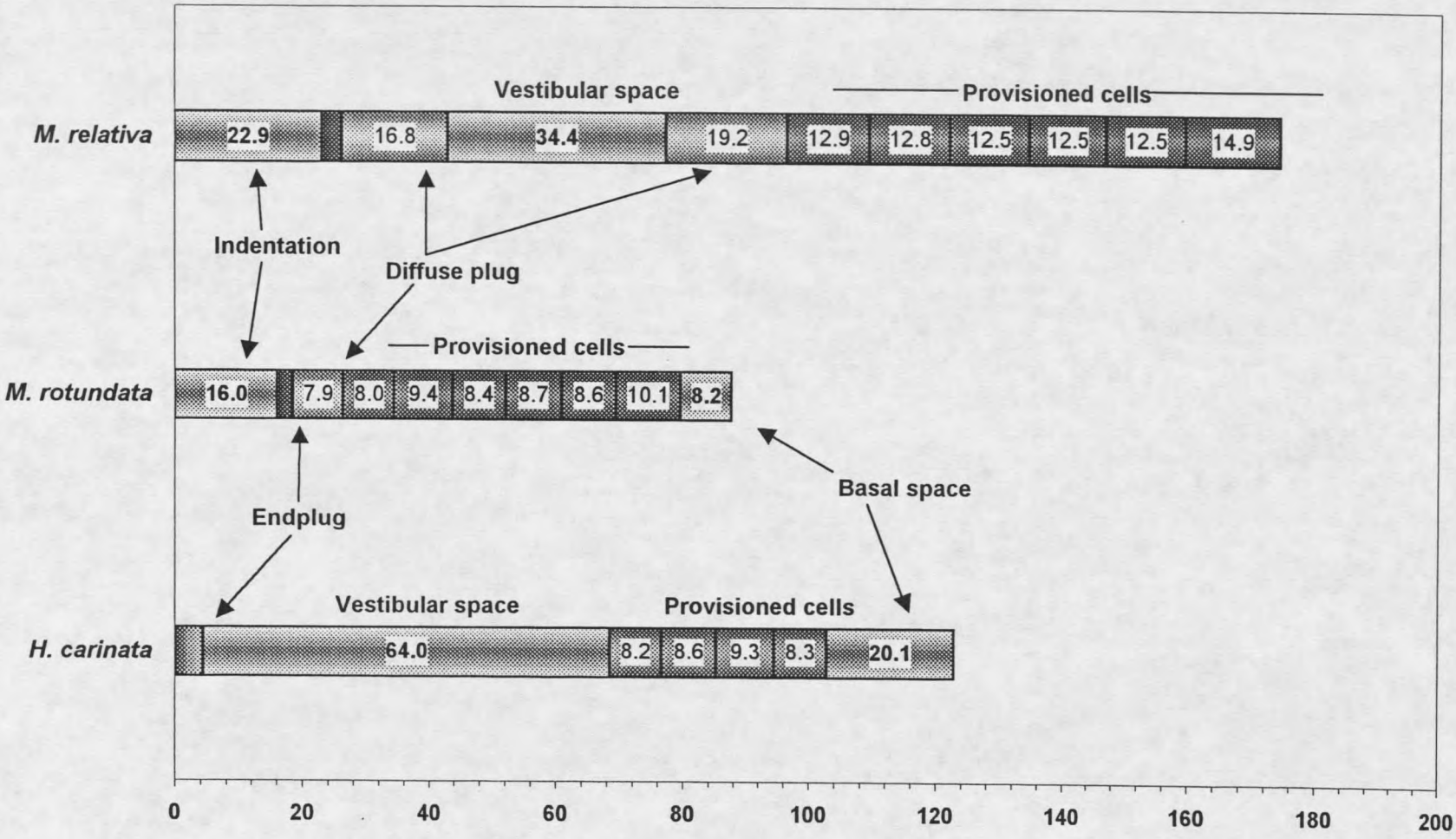

Figure 1. Typical Nest construction for *M. relativa, M. rotundata*, and *H. carinata.* Lengths in mm.

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.

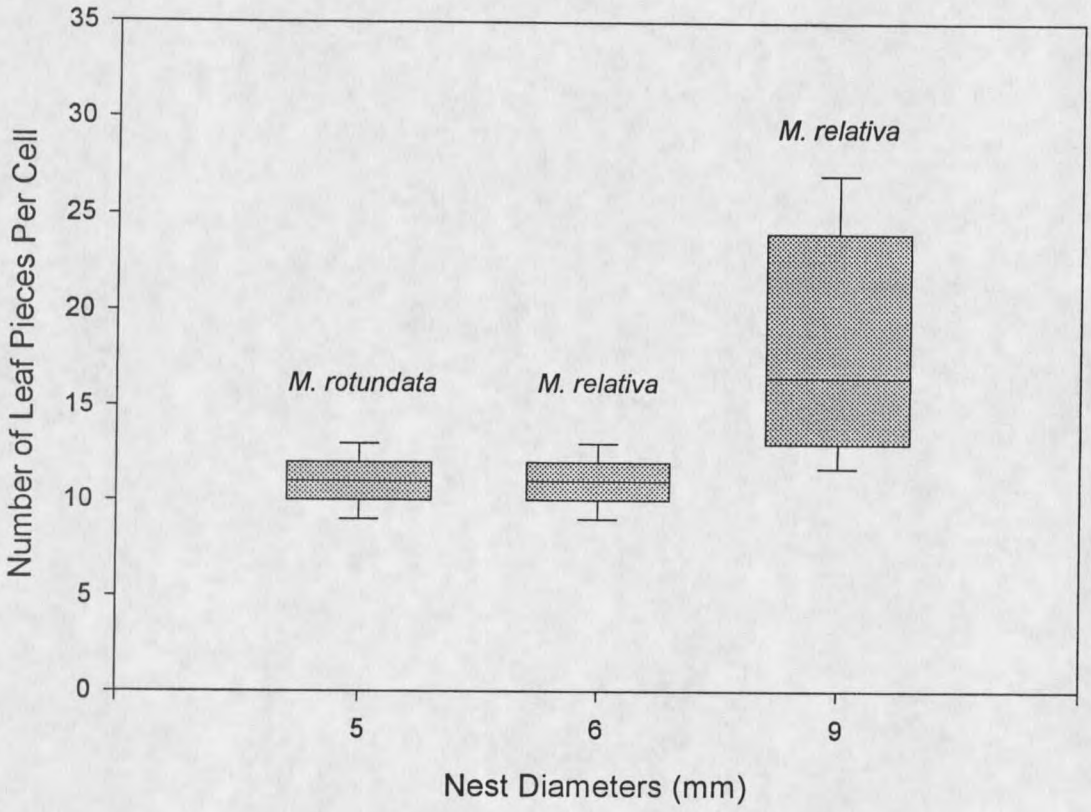

Figure 2. Number of leaf pieces used per cell cup for 5, 6, and 9 mm diameter nests. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

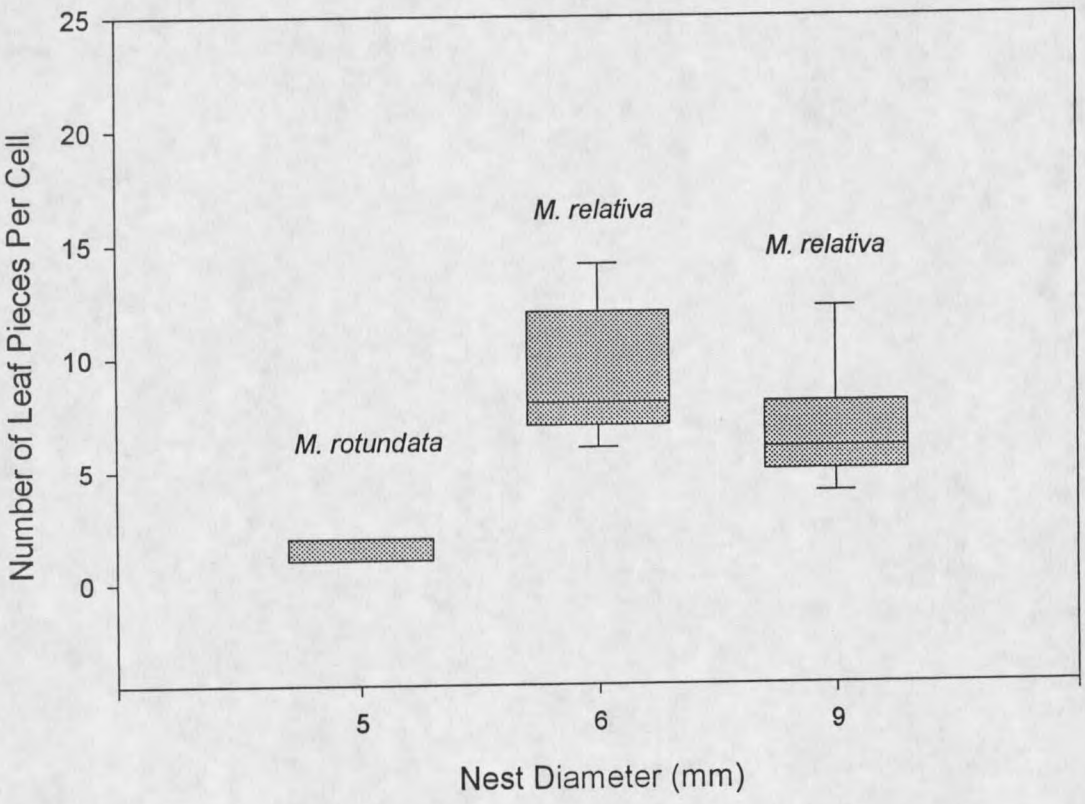

Figure 3. Number of leaf pieces used in cell cap for 5, 6, and 9 mm diameter nests. The boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles.

The number of leaf pieces used to cap each individual cell in the nest differed between the 6 (N = 74 cells) and 9 mm (N = 43 cells) diameter *M. relativa* nests and 5 mm (N = 30 cells) diameter *M. rotundata* nests. In this case, the number of leaf pieces in a cap differed between species. The 5 mm diameter *M. relativa* cell caps contained a significantly higher (Kruskal-Wallis, $P < 0.001$) number of leaf pieces than both the 6 mm and 9 mm diameter *M. relativa* nests (Fig 3). It is not surprising that the 6 and 9 mm *M. relativa* nests are not different because the diameter of the nest has no bearing on the number of leaf pieces that are required to cap a nest. It is surprising, however, to see the difference between the two *Megachile* species, as the difference suggests some strategy behind the additional leaf pieces found in *M. rotundata* cell caps.

Foraging Data

The 5,362 observations made of females arriving at nests or departing nests included 4,562 of *M. relativa*, 478 of *M. rotundata*, and 322 of *H. carinata*. Observations were recorded as leaf or pollen collecting trips only for the *Megachile* Species, because I was unable to see what type of cargo the *H. carinata* females were carrying (Table 3).

Table 3. Number of observations for each cargo type.

	M. relativa	*M. rotundata*
Pollen	339	36
Leaf	1155	71
Total	1494	107

The number of *M. relativa* observations are especially high since the females of this species nested in abundance in both the 1999 and 2000 field seasons. *Megachile*

rotundata was observed at the Post Farm experimental alfalfa seed-plot field site only in the 2000 field season. Observations were made at this site because no *M. rotundata* females were nesting at the NHF or RCF sites. Although the number of bees was not limited, observations were very difficult to make, even with the aid of a video camera, as the amount of activity at a large bee board is visually overwhelming. Few observations were made of *H. carinata* due to low numbers of this species nesting at both the NHF and RCF sites in 1999; no females were observed at these sites in 2000.

I used a Kruskal-Wallis test to ensure that leaf and pollen collecting trip durations were distinct in *M. relativa* ($P < 0.001$) and *M. rotundata* ($P < 0.001$). While the different leaf and pollen trip durations were expected within the *Megachile* species, the two distinct trip durations were not expected for *H. carinata,* because I did not distinguish any differences in trip purpose while observing the females and did not record any pitch collecting trips (Fig. 4). The amount of time spent in the nest after each trip type was also different for *M. relativa* ($P < 0.001$) and *M. rotundata* ($P = 0.012$), but not for *H. carinata* ($P = 0.60$)(Table 4).

Table 4. Mean task duration (seconds) ($\pm$ SE) for *M. relativa, M, rotundata,* and *H. carinata.*

	M. relativa	*M. rotundata*		*H. carinata*
Leaf Trip Duration	75 ± 2	101 ± 14	Trip Duration	475 ± 27.6
Duration of time in nest between leaf trips	103 ± 3	83 ± 8	Duration of time in nest between trips	109 ± 8
Pollen Trip Duration	656 ± 16	672 ± 88		
Duration of time in nest between pollen trips	152 ± 8	121 ± 17		

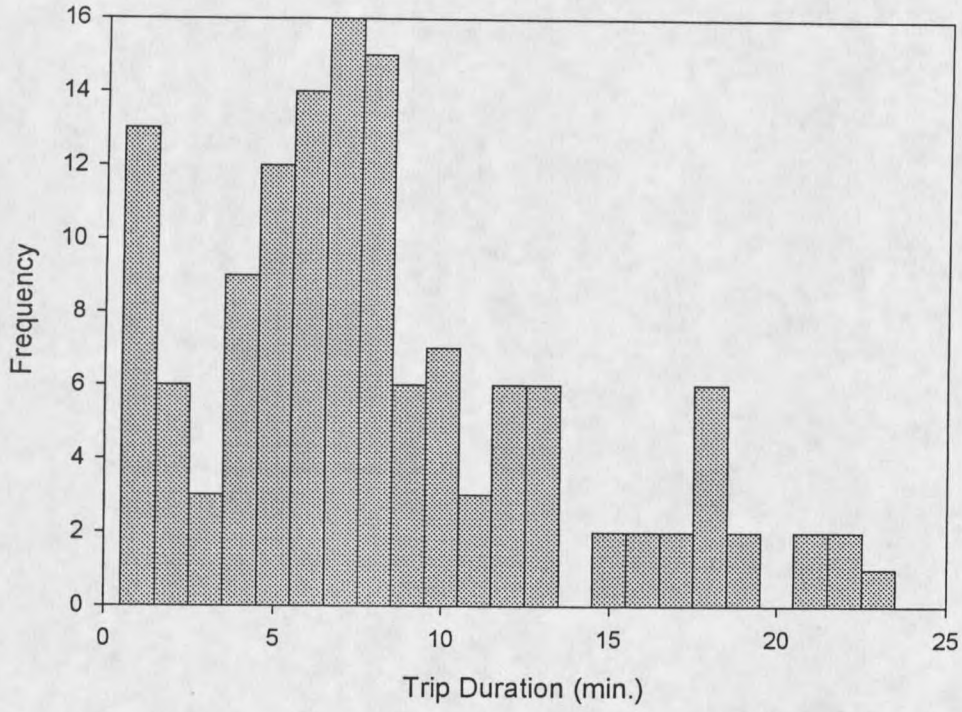

Figure 4. Distribution of *H. carinata* trip duration.

The durations of trips did not differ significantly between *M. relativa* and *M. rotundata* for either leaf (P = 0.25) or pollen (P = 0.70) collecting trips. The amount of time the females spent in the nest after a leaf collecting trip was marginally significant (P = 0.08) between the *Megachile* species, but the duration of time in the nest after a pollen collecting trip was not significantly different (P = 0.22).

Heriades carinata trip duration was not compared with the *Megchile* species because the purpose of the trips was not clear. While I can speculate two different trip types are contained within the *H. carinata* foraging data, I cannot make any conclusions without observations including trip purpose. In addition, the mean duration of the trips are different enough from the *Megachile* species' that entirely different functions are possible. *H. carinata* is the smallest of the three bee species, and might require the

shortest duration of time to collect pollen. If this is the case, then the shorter of two possible trip types might be for gathering pollen and pitch, while trip durations at the upper end of the range might simply be for feeding.

Pollen Types Within Nests

The 200 cells dissected to obtain pollen samples included 159 from 44 *M. relativa* nests, 26 from 19 *M. rotundata* nests, and 15 from 11 *H. carinata* nests. The samples originated from several sites (Table 5). Within these nest cells, I was able to categorize the pollen used into 11 categories at the level of plant family (or above): 1) Asteraceae, 2) Brassicaceae, 3) Caprifoliaceae, 4) Fabaceae, 5) Fagaceae, 6) Hydrangiaceae, 7) Oleaceae, 8) Rosaceae, 9) Tiliaceae, 10) unknown non-grass monocot, and 11) unknown other. Among the Asteraceae, I could distinguish at least three types: 1) *Cirsium* spp. (thistle), 2) *Taraxacum* spp. (dandelion), and 3) unknown Asteraceae. Similarly, the Fabaceae could be divided into 1) *Lotus* spp. (birdsfoot trefoil), 2) *Medicago* sp. (most likely all *Medicago sativa*, alfalfa), and 3) unknown Fabaceae. At least two types of non-grass monocot pollen were distinguishable based on pollen grain size, so are hereafter referred to as small and large monocot. It is important to note that some cells contained nothing but monocot pollen, which rules out the possibility of accidental introduction of monocot pollen in the provisions.

Table 5. Number of dissected cells from each site.

	M. relativa	*M. rotundata*	*H. carinata*
W. Bozeman	112	-	5
SE Bozeman	44	-	-
Post Farm	-	26	-
Rocky Creek	-	-	10

Although pollen samples were often from different sites, the types of pollen used by *M. relativa, M. rotundata,* and *H. carinata* overlapped strongly and all three species were polylectic (Fig. 5). Among the 15 pollen types found in *M. relativa* nests, 9 were also found in *M. rotundata* nests, whereas 7 were found in *H. carinata* nests. However, a chi-square test using the categories Asteraceae, Fabaceae, Monocot, and "other" pollen rejected ($P < 0.001$) the null hypothesis of homogenous pollen prevalence between the three species. The results of the chi-square analysis are not surprising considering that the three species may have different pollen preferences and most of the samples were collected from different sites for each species (Table 5). The larger number of pollen types found in *M. relativa* nests could very well be due to the larger number of cells sampled for this species.

I found several differences in pollen prevalence when comparing the two sites that were the source of the *M. relativa* samples (Fig. 6). Relative to the W. Bozeman site, cells at the SE Bozeman site had lower prevalence of monocots, *Lotus* sp., and *Taraxacum* sp. and a greater prevalence of the unknown Asteraceae, *Medicago* sp., and Caprifoliaceae (the latter which may be *Symphoricarpos*, being absent from the W. Bozeman samples). A chi-square analysis using the categories Asteraceae, Fabaceae, Monocot, Caprifoliaceae, and "other" pollen rejected ($P < 0.001$) the null hypothesis of homogenous pollen prevalence between the two sites. The significant differences in pollen prevalence are most likely due to differential pollen availability between the two sites. For example, the West Bozeman site is adjacent to agricultural land, whereas the

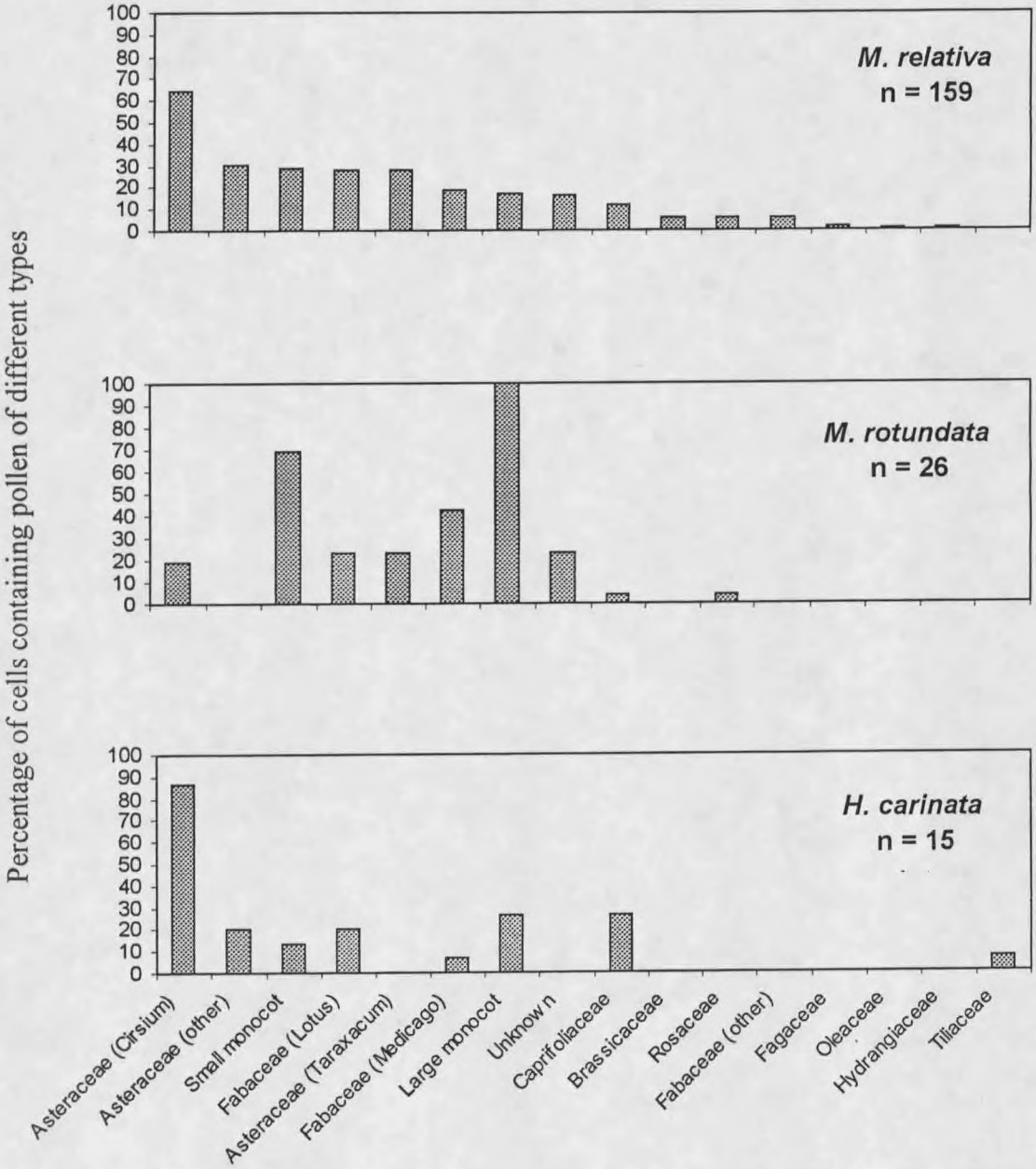

Figure 5. Species comparison of pollen use

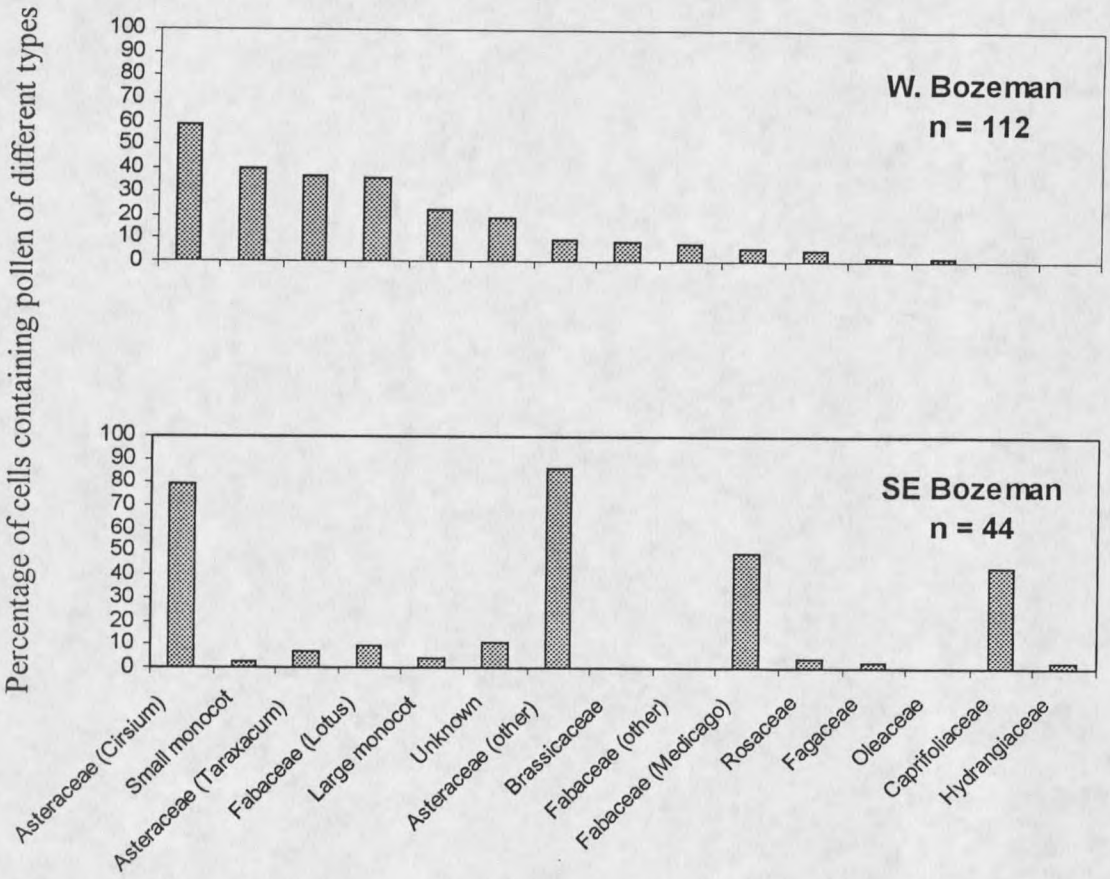

Figure 6. *M. relativa* site comparison of pollen use.

SE Bozeman site is in a neighborhood with ornamental plants, near a wooded creek and a weedy industrial storage lot.

No single pollen type dominated the *M. relativa* samples, although the three types of Asteraceae were among the five most prevalent pollen types found in cells of this species. Comparison of pollen data from 1999 and 2000 for *M. relativa* reveals broad similarities with a few minor differences (Fig. 7). The unknown Asteraceae was the second most prevalent pollen type collected in 1999, but ranked only sixth in 2000. Monocot pollen was also less prevalent in 2000. A chi-square test using the categories

Asteraceae, Fabaceae, Monocot, and "other" pollen by year rejected ($P = 0.039$) the null hypothesis of homogenous pollen prevalence between the two field seasons. Differences in pollen prevalence between years may be due to the shorter length and drought conditions of the 2000 field season. Differences between years in the presence or absence of the less prevalent pollen types could also be due to sampling error.

The two unknown monocots were the most prevalent pollen types in nests of *M. rotundata*; the large type of monocot pollen was found in all 26 cells examined (Fig. 5). This is somewhat surprising because *M. rotundata* is a widely used commercial pollinator of alfalfa (*Medicago sativa*)(Richards 1984), a field of which was located just several meters from the nests of the bees. *Medicago* was the third most prevalent pollen among the *M. rotundata* cells.

As with *M. relativa*, thistle (Asteraceae: *Cirsium* spp.) pollen was the most prevalent type in the cells of *H. carinata*, being found in the vast majority of cells. Minor differences between *M. relativa* and *H. carinata* include the presence of Tiliaceae pollen The similarity in the prevalence of different pollens in samples taken from larval frass and provisions of *M. relativa* (Fig. 8) indicates that timing of sampling (i.e., pre- vs. post-ingestion) did not greatly affect the results. The only difference was the absence of Oleaceae pollen from the frass samples, even though there were four times as many frass samples than provision samples.

In comparing pollen types with cells containing healthy and dead larvae, I found no clear indication that a particular pollen type was associated with higher larval mortality (Fig. 9). However, a chi-square test using the categories *Cirsium*, Asteraceae,

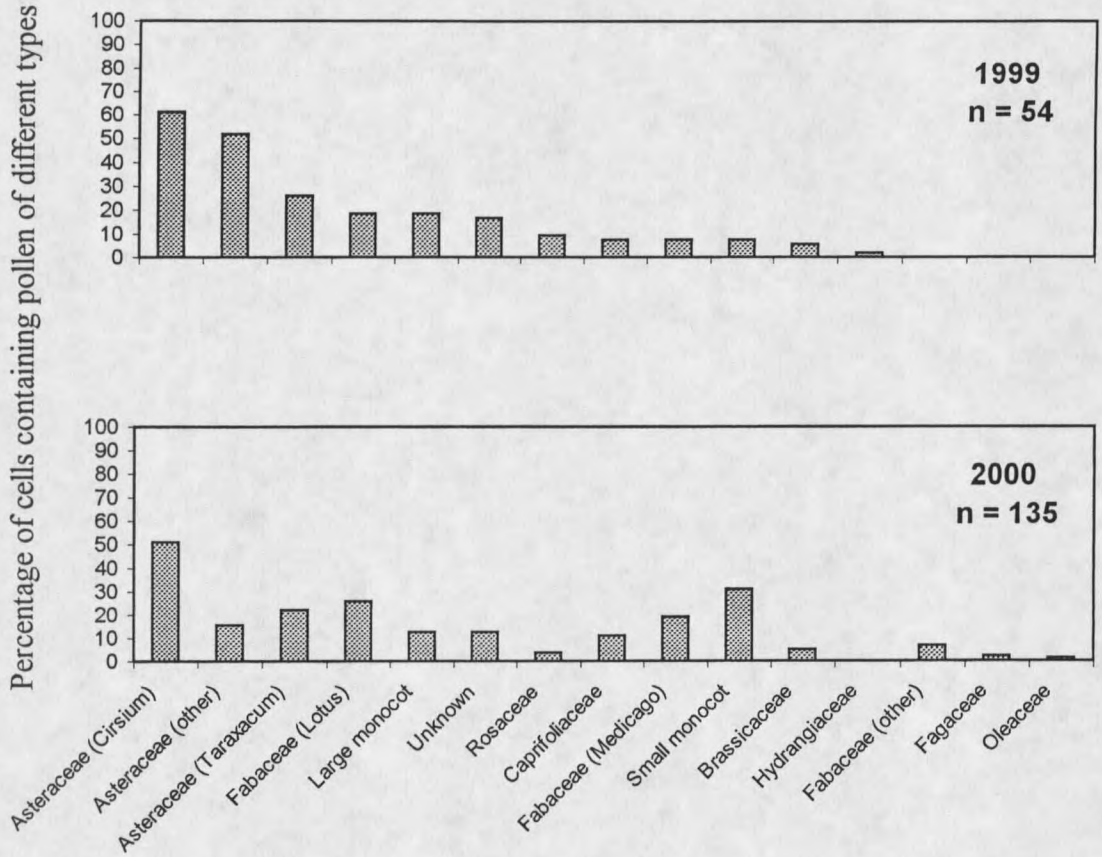

Figure 7. *M. relativa* year comparison of pollen use.

Medicago, Fabaceae, Monocot, Caprifoliaceae, and "Other" pollen rejected (P = 0.001) the null hypothesis of related pollen prevalence between the three larval conditions. Because the healthy and dead larvae are drawn from the same population of cells, it follows that the pollen prevalence should be homogenous. The chi-square analysis suggests that although pollen prevalence between the healthy and dead larvae look similar, they are in fact not homogenous. Pollen types in the smaller sample of moldy

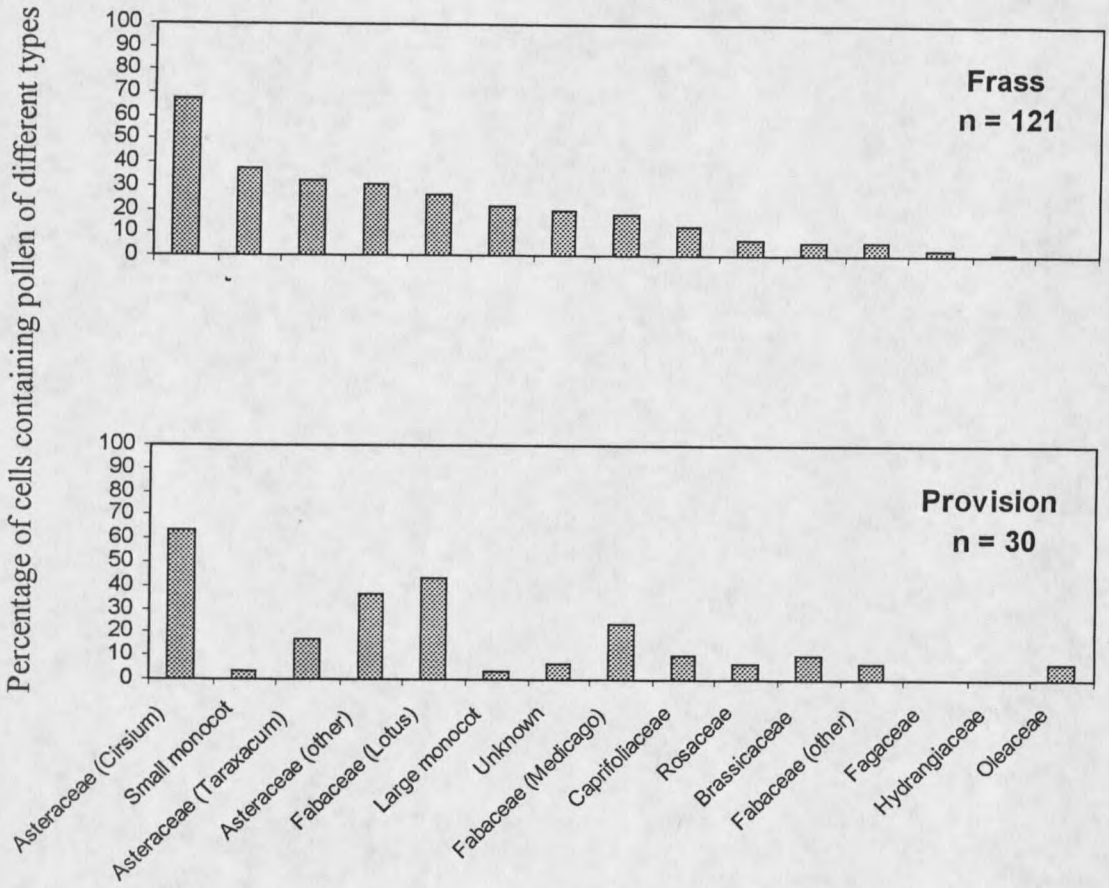

Figure 8. *M. relativa* source comparison of pollen use

cells suggest an association of mold with a higher prevalence of *Medicago* and Caprifoliaceae. However, when we examine the data from the SE Bozeman site, where all of the moldy cells originated, this does not appear to be the case (Fig 10). A chi-square test using the categories Asteraceae, Fabaceae, Caprifoliaceae, and "other" pollen did not reject ($P = 0.80$) the null hypothesis of homogenous pollen prevalence between the moldy larvae and the SE Bozeman site. Thus at this time, larval mortality cannot be firmly associated with particular types of pollen in the provisions.

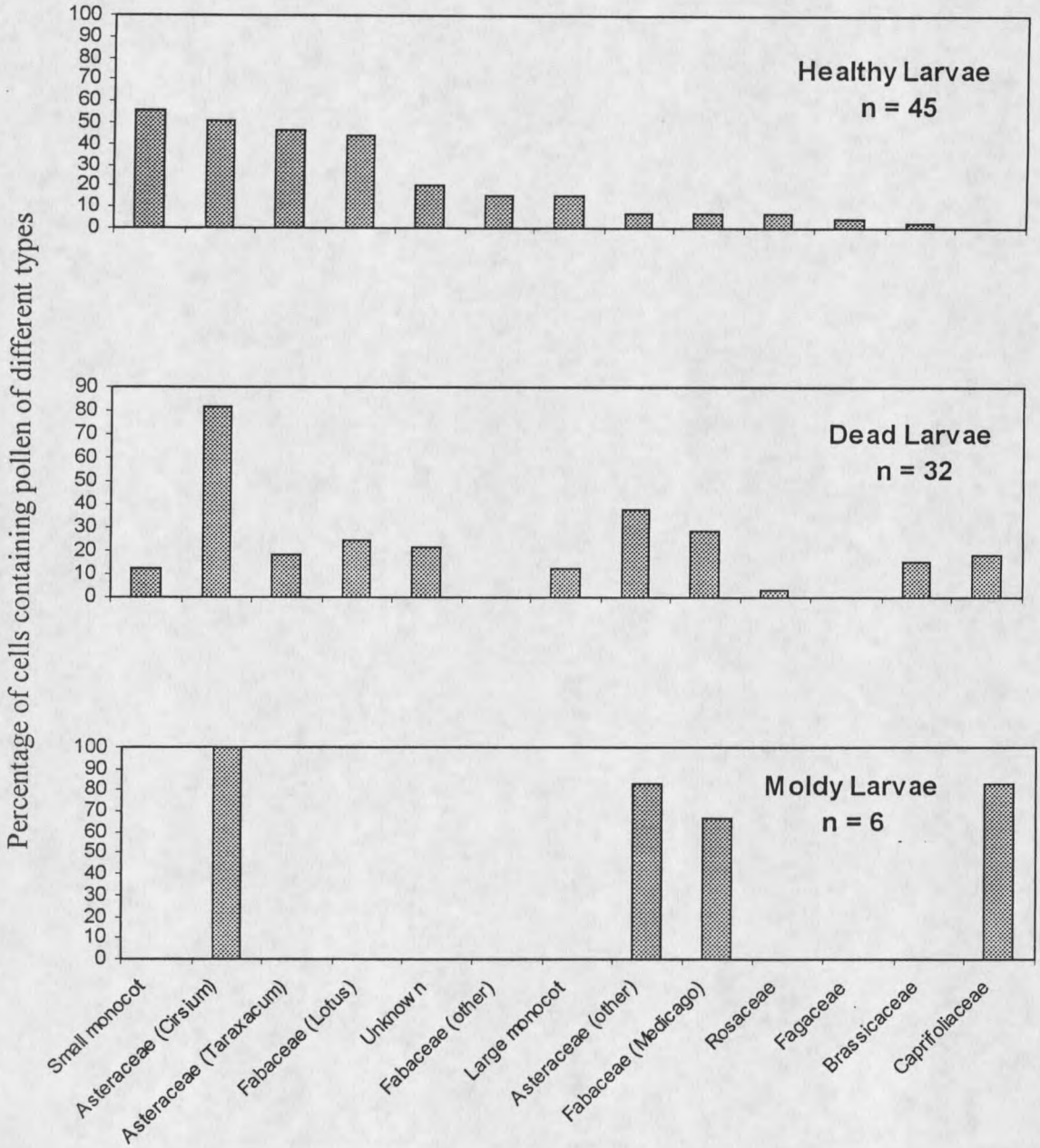

Figure 9. *M. relativa* larval condition comparison of pollen use

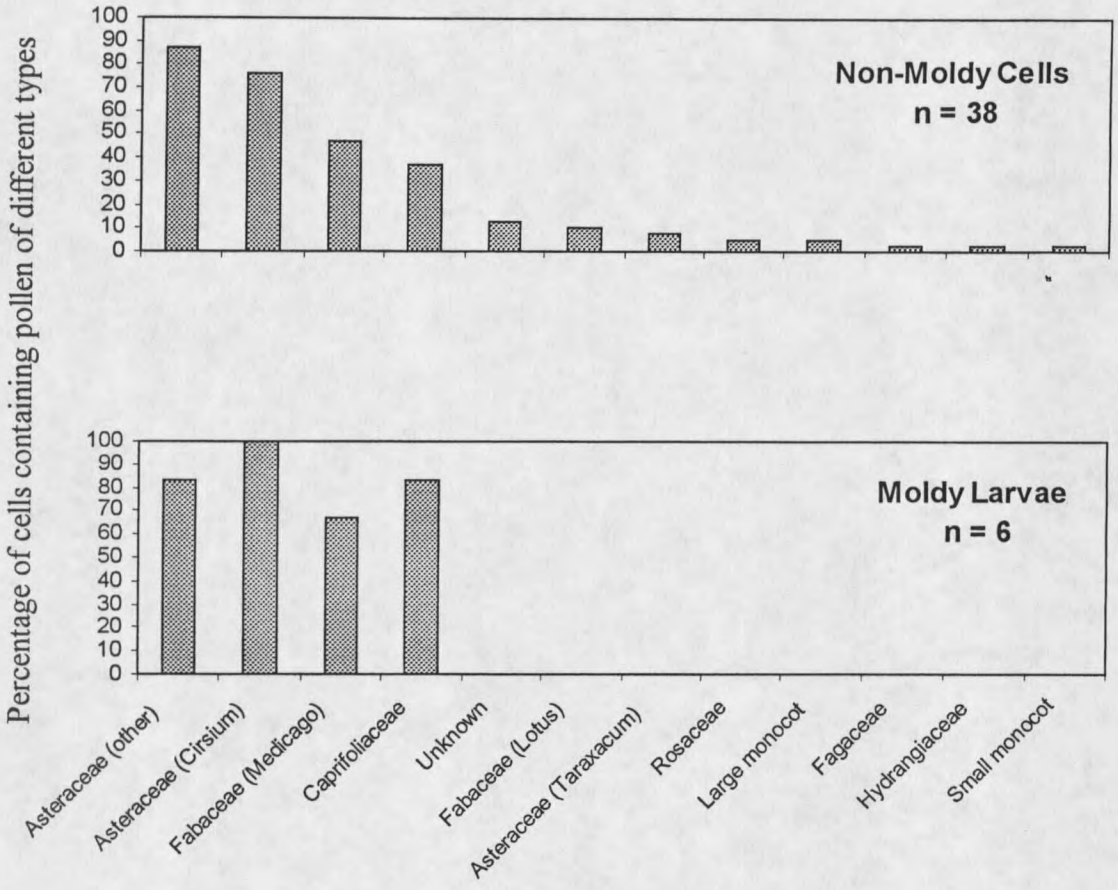

Figure 10. Distinguishing *M. relativa* SE Bozeman site from moldy larvae pollen use.

Larval Health and Parasitism

While dissecting the *Megachile* species cells to retrieve pollen samples, I recorded the condition of the larva in each cell. I recorded the larval condition for 128 *M. relativa* cells and 30 *M. rotundata* cells (Table 6).

I expected to find *Coelioxys moesta* in the *M. relativa* nests because I observed many parasitism attempts by *C. moesta* females at the trap-nests. *M. rotundata* is listed

as a host, but I did not observe any *C. moesta* at the bee boards at the Post Farm site. Indeed, I did not find any *C. moesta* in the *M. rotundata* cells that I dissected. This may

Table 6. Percent larval mortality for *M. relativa* and *M. rotundata*.

Mortality Factor	*M. relativa*	*M. rotundata*
Coelioxys moesta	5	0
Mellitobia chalbyii	29	7
Toryimidae	2	3
Dermestidae	1	0
Mold	5	0
Healthy	38	90
Unknown	20	0

be a function of the lesser number of dissected cells, or simply sampling error. However, I suspect from observing how skittish the *C. moesta* females were at the *M. relativa* trap-nests, that the sheer size of the bee board and the amount of activity at the Post Farm site may have been daunting enough to reduce the levels of *C. moesta* parasitism.

Mellitobia chalybii, and the torymid parasitic wasps are almost never seen at the trap-nests due to their size, but also due to their method of crawling around from nest to nest in small cracks in the wood. Parasitism levels for these wasps may be inflated due to the storage of the trap nests at room temperature. Because the emergence time is only 17 days (Johansen and Eves 1966) these wasps hatch in great numbers long before their hosts, and infest any unprotected nests nearby. Infestation of neighboring nests would also occur naturally between nearby nests, but the contamination of nests from different sites or collection dates that occurs upon storage in the lab would not.

The high level of unknown mortality in *M. relativa* in my samples may be due to several factors including dehydration, heat, provision toxicity, or simply unviable larvae.

Distribution Data

I recorded the emergence of 492 Megachilid bees over two years in trap-nests from 18 sites in Gallatin County in Montana. Species collected from nest tubes included, *Megachile relativa, Megachile rotundata, Megachile pugnata, Heriades carinata, Coelioxys moesta, Coelioxys funeraria, Osmia lignaria, Stelis montana, Stelis subemarginata, Hoplitis albifrons argentifrons, Hoplitis fulgida fulgida, Hoplitis robusta,* and *Hoplitis spoliata.* The bees that emerged in 1999 came from the first season of trap-nesting which included only 9 field sites in the Gallatin Valley (Table 7). The bees that emerged in 2000 came from the original 9 sites, and an additional 9 sites, distributed through the Gallatin Valley and in the Bridger Mountains (Table 8).

I used principle components analysis (PCA) to examine the relationship between sites based on the species diversity that I recorded from nest tube emergences and with the prediction that sites located close together geographically should be similar in species diversity. The 1999 PCA plot shows a correlation between the three Red Bluff sites; with a smaller distance between the data points on the plot indicating the degree of similarity between sites (Fig. 11). The first and second principle components cumulatively account for 62% of the total variance in the 1999 data. The 2000 PCA plot does not show the same relationships (Fig. 12), although the Red Bluff sites are still relatively close together. There are several unexpected similarities between sites in the 2000 PCA plot (GM2.5 and RCF, or GM 3.5 and Olson), but the general trend shows a geographic relationship, with westerly sites (RB, MAD, HH) tending to the left of the plot, and the easterly sites (GM, RCF, Battle Ridge) on the right. The first and second principle

components cumulatively account for 56% of the total variance in the 2000 emergence data.

Table 7. Emergence numbers for *M. relativa, M. rotundata, H. carinata, C. moesta,* and four additional genera at the 1999 field sites.

	M. relativa	*M. rotundata*	*H. carinata*	*C. moesta*
MAD N	3	19	0	4
MAD S	4	1	0	0
MAD gully	5	17	0	0
HHS	0	0	0	0
HHN	14	21	0	0
HHGully	28	4	0	4
RB Stream	12	0	0	0
RB Marsh N	4	0	0	0
RB Marsh S	3	0	0	0
Total	73	62	0	8
	Megachile spp.	*Hoplitis* spp.	*Osmia* spp.	*Stelis* spp.
MAD N	0	0	0	0
MAD S	0	0	0	0
MAD gully	0	0	6	0
HHS	0	0	0	0
HHN	0	0	0	0
HHGully	0	0	0	0
RB Stream	0	0	0	0
RB Marsh N	0	0	0	0
RB Marsh S	0	0	0	0
Total	0	0	6	0

Table 8. Emergence numbers for *M. relativa, M. rotundata, H. carinata, C. moesta,* and four additional genera at the 2000 field sites.

	M. relativa	*M. rotundata*	*H. carinata*	*C. moesta*
MAD N	7	2	10	7
MAD S	6	0	42	6
MAD gully	0	0	0	6
HHS	0	0	0	0
HHN	0	0	1	1
HHGully	0	0	0	0
RB Stream	0	0	9	3
RB Marsh N	0	0	0	0
RB Marsh S	0	0	0	1
Olson	9	1	0	0
W. Bozeman	30	1	17	12
Battle Ridge	4	0	0	21
RCF	0	0	1	1
Fort Ellis	0	0	0	0
GM 2.5	1	0	0	0
GM 3.5	0	0	0	5
BFH	0	0	0	0
Fulker	3	13	50	0
Total	60	17	120	63
	Megachile spp.	*Hoplitis* spp.	*Osmia* spp.	*Stelis* spp.
MAD N	0	0	5	0
MAD S	0	0	0	0
MAD gully	0	0	6	0
HHS	0	0	0	0
HHN	0	0	1	0
HHGully	0	0	0	0
RB Stream	9	0	1	0
RB Marsh N	0	0	0	0
RB Marsh S	0	0	0	0
Olson	0	7	0	0
W. Bozeman	0	1	8	1
Battle Ridge	0	10	0	0
RCF	0	8	0	2
Fort Ellis	0	0	0	0
GM 2.5	0	14	0	1
GM 3.5	0	5	0	0
BFH	0	0	0	0
Fulker	4	0	0	0
Total	13	45	21	4

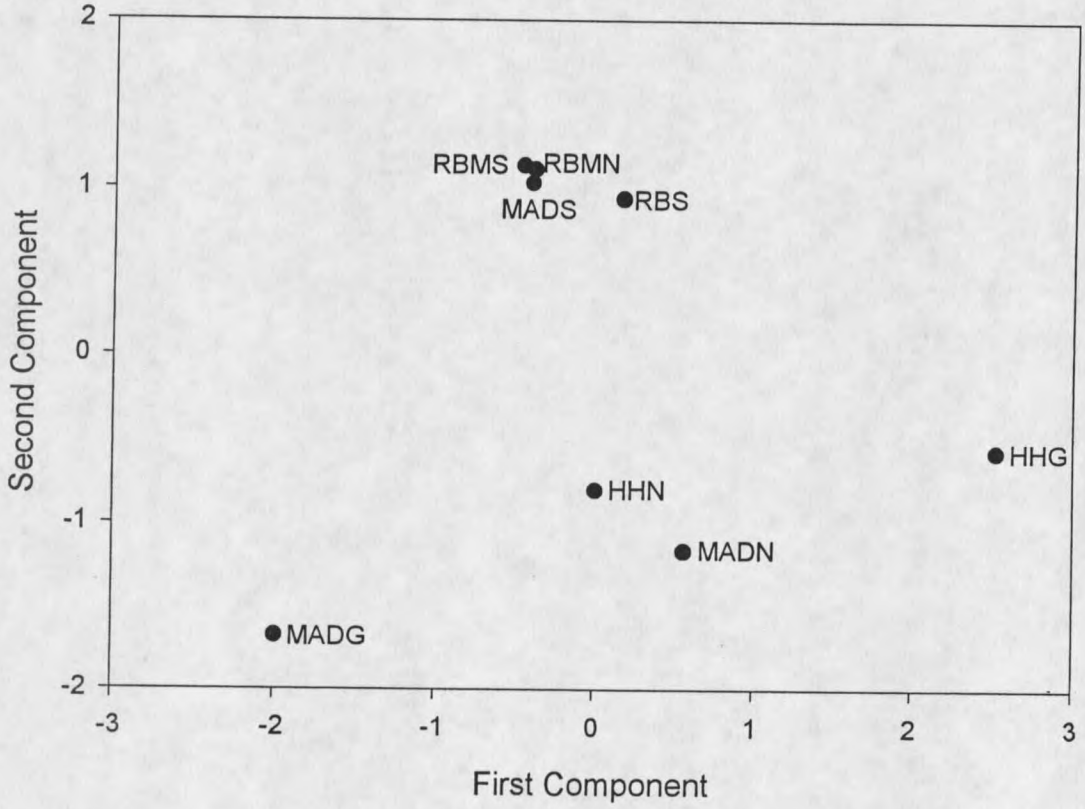

Figure 11. Principle components analysis plot of 1999 field sites

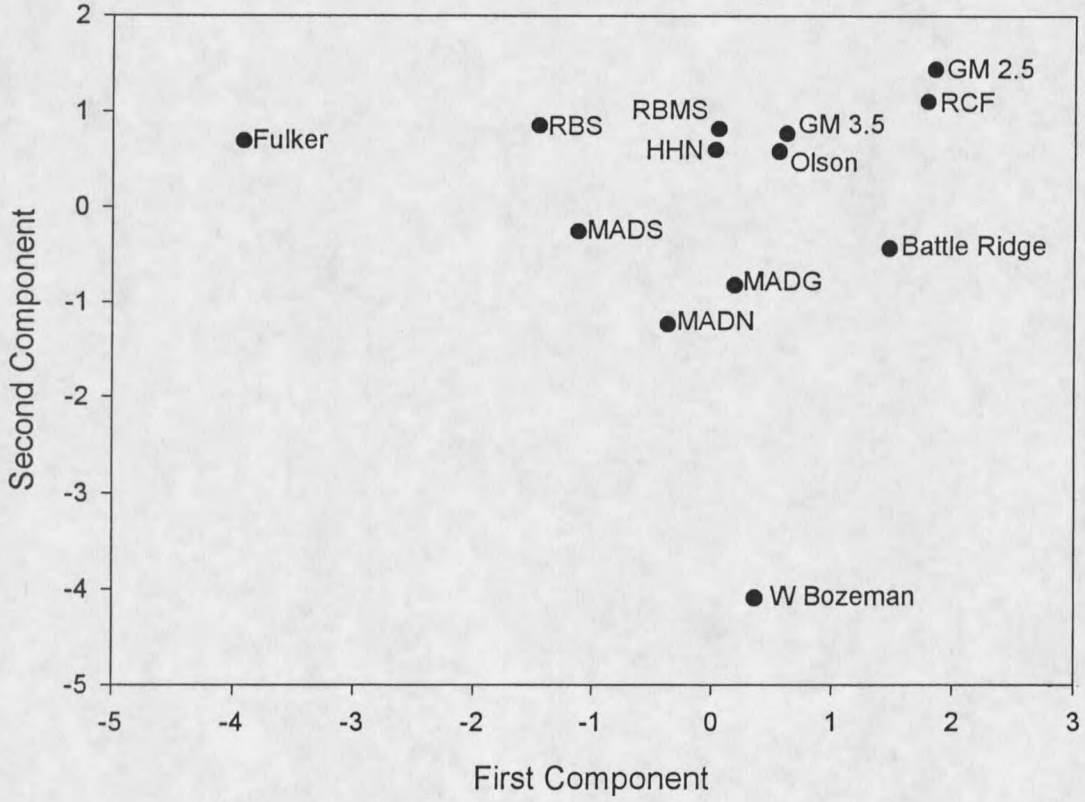

Figure 12. Principle components analysis plot of 2000 field sites.

Additional Behavior Notes

Megachile relativa

Nest Construction. Once a nest has been chosen, the female starts to build a cell. Leaves are cut first in oblong shapes. The female lands on a leaf and starts to cut down and back relative to her body position. As the cutting proceeds, the leaf section that she is cutting away begins to fall away from the leaf. She remains on the leaf-piece, grasping one edge of the leaf with her legs and continuing her cutting. When the piece finally disconnects, she takes flight as it drops and carries the leaf back to the nest. The leaf is carried in a curved shape, with the lowest edges held in her mesothoracic legs and the upward curve closest to the body. Leaf pieces were taken from a variety of plant species including *Medicago sativa* L., *Lotus corniculatus* L., *Potentilla fruticosa* L., *Spiraea betulifolia* Dall., and *Verbascum thapsis* L. I observed leaf cutting within 10 m of the nest, and also identified leaf pieces from the nests that I dissected.

The female lands at the nest, grasping the nest entrance with her prothoracic legs. Because the leaf is occupying the mesothoracic legs, she often makes several attempts at landing. Once she has landed and walked inside the nest, she heads straight to the back of the nest. At the back of the nest, she walks sideways around the circumference of the nest tunnel. Eventually, she releases her grasp on the leaf and crawls around the curvature of the leaf, so that she is now on the concave face of the leaf. If the leaf piece is one of the first of the cell, the female pushes it far enough back into the nest so that one end of the oblong shape creates a backing for the cell that she is building. This piece

abuts either the back of the tunnel (if it is part of the first cell of the nest) or the apical portion of the previous provisioned cell. Subsequent leaf pieces are not pushed back as far and cover only the side of the cell. In order to secure the leaf in place, she chews the edges of the leaf and secretes a substance that may be salivary in origin (as described for *M. inermis* Provancher by Stephen (1955)). This was visible, even through plexiglass, as dark and wet borders on the leaf pieces. Once the leaf has been secured, she backs out of the nest and flies away to get another leaf piece. The leaf edges that have been chewed not only stick to the sides of the cell, but also provide points of attachment for ensuing leaves. I could not see the females through the plexiglass once they had constructed more than one layer of leaves, so I do not know if any additional smoothing or chewing occurs before the provisioning of the cell begins.

Cell Provisioning. Once the cell has been built the female begins to collect pollen to provision the nest. Upon returning from a foraging trip, the female lands at the nest and walks in headfirst. At this point, she deposits nectar in the pollen mass. The female then turns around, either by somersaulting in the nest or by backing out to the nest entrance and turning to back into the nest. She backs up to the incomplete cell and uses mesothoracic and metathoracic legs to scrape the pollen off her scopa. Prior to leaving the nest I observed many females stopping to groom themselves from front to back. Others continue walking while continuing to scrape and kick remaining pollen from their bodies, leaving a faint trail of pollen clumps in the nest. Once the female reaches the nest opening she flies away in search of more pollen.

When the cell has been provisioned, a single egg is oviposited on the pollen mass, which sits at the basal end of the cell. On several dozen occasions I noted that behavior of the female changes noticeably at the time of oviposition, from long pollen collecting trips to repeated 3 to 10 second trips with no clear purpose. Many of these trips are simply the female leaving the nest, flying away and turning in the air and returning immediately. Cell capping begins shortly after the egg has been deposited. These leaf pieces, which are circular rather than oblong as before, begin to take on a concave shape as they are placed one in front of the other, and provide a perfect curved surface upon which to begin the next cell.

The point in time when one cell ends and another begins is difficult to pinpoint. The females simply change from gathering circular leaf pieces to oblong leaf pieces. This transition is sometimes also marked by many trips to neighboring holes. Short trips, quickly entering nearby holes for 5 seconds, leaving, and then checking another. The end of this searching is marked unceremoniously with the female returning to the original nest with an oblong leaf cutting.

Nest Usurpation. I did not observe any direct nest usurpation, but did see one female pulling the leaves out of a finished nest one by one and letting them drop to the ground in front of the trap-nest blocks. The female did not stop at the first finished cell, but pulled out and dropped a developing larva and continued pushing provisions and more leaves out of the nest. Discarding of larvae is also commonly observed at nests of *M. rotundata* (Ruth O'Neill, personal communication). I removed this nest tube to

preserve the finished nest for the diversity study, and the destructive female immediately began a new nest in that location.

In another instance, I observed a female pulling out paralyzed Lepidopteran larvae from a eumenine wasp nest. This female was nesting in another hole nearby, but would periodically return to the eumenine nest to pull out more larvae and mud. She ceased pulling out larvae on the second day, and the nest was quickly finished and capped by the eumenine female. Once capped, the nest was left alone.

Nest Modification. On several occasions (N = 4), I observed *M. relativa* females through the plexiglass nest that were facing the back of the tube and walking backwards towards the entrance while scraping the tube surface. While doing this, each female scraped small wood splinters from the sides of the nest. In each case, they did not haul these splinters out of the nest, but simply left them lying in the nest and continued smoothing. The debris accumulated and was incidentally pushed out of the nest by the females during provisioning trips, but no specific effort was observed to carry debris out of the nest.

Guarding. On several occasions I observed a bee sitting in the entrance facing out of a nest hole. The female would sit and periodically groom until another bee arrived or departed from a nearby nest hole. At this point, the sitting female would fly out of her nest and chase the other bee momentarily before returning to the nest hole again. Upon returning, the female rotated around the circumference of the nest hole, and poked her head in and out of the entrance. Then she would wait at the entrance until another bee

flew nearby. When I checked the occupancy of the hole on previous days, I found that these aggressive females were new occupants. It may be possible that this is behavior used to establish a new nest, and was only observed on a few occasions due to chance.

Response to Parasitism. I also observed the reaction of a female *M. relativa* to a recent visit from *Coelioxys moesta.* The usual response is an apparently extended amount of time spent headfirst in the provisions after returning from a provisioning trip (N = 10). This is followed by the usual turn and pollen deposition, but then a change occurs in the behavior. Instead of a regular trip the female will either make a quick return visit or remain in the nest for many minutes waiting at the entrance. The change in behavior can be illustrated best by a specific example from July 25th, 2000. The female *M. relativa* female was making approximately 15 minute pollen collecting trips, with about 2 minutes spent inside the nest unloading nectar and pollen. The *C. moesta* female deposited an egg in the nest while the host female was away. When the host female returned from collecting pollen her behavior inside the nest did not change, and she left on another trip. This time she returned after only 3 minutes, and stayed only for 1.5 minutes. She then left on another collecting trip. The *C. moesta* returned and parasitized the nest again. When the host returned she unloaded pollen in a usual manner and departed. But she returned after only 4 minutes and sat in the nest waiting for 20 minutes. During this time, the *C. moesta* female made two attempts to parasitize the nest again, and was repelled by the host. When the host did leave, the parasite female entered the nest 3 minutes later, but did not oviposit. The parasite female entered the nest once again after another 3 minutes. When she turned to oviposit she pushed her abdomen deep into the provision

and worked it around for 58 seconds (most oviposition events that I observed took less than 10 seconds). When she turned around to do the usual headfirst checking or concealing, she again took about 50 seconds longer than usual. She emerged from the nest covered in nectar, so much so that she had to sit on the front of the nest and fan her wings for 10 seconds before she could fly away. The host returned from a regular length (15 min) pollen trip. Instead of the usual 1 minute of depositing nectar, she had her head in the provisions for 10 minutes. When she emerged from the cell, her thorax was covered in big balls of pollen and nectar. She groomed for 6 minutes, then turned and deposited pollen, and then groomed again for 5 minutes. Then she left the nest, but only for 35 seconds, returning to sit at the nest entrance for 2 minutes. She left again for 15 seconds, returning for only 10 seconds. She made nine more similar trips before finally staying in the nest for 2 minutes, possibly ovipositing, then making four more very short trips and visits before returning with a leaf. She then continued to cap the cell.

It appears from this account that the *Megachile* females are sometimes able to detect the activity of parasitic species, and are able to respond to this threat with behavioral changes.

Heriades carinata

The *H. carinata* species also exhibits the characteristic cell provisioning cycle. Upon establishing a new nest, a distinct searching behavior was observed (N = 7) by these bees. Nest scouting trips were taken after a tube had been capped. The bees returned to the capped nest, and then searched several holes nearby. When a new nest

was finally chosen, foraging trips started immediately. However, during the first few trips it would take the female several seconds of hovering in front of the nest block to find the new nest. The length of time spent hovering slowly diminished upon subsequent collecting trips, until the female was flying directly to the new nest hole.

Although I could clearly distinguish pollen and leaf collecting trips for *M. relativa,* it was difficult to distinguish pollen and pitch collecting trips for *H.* carinata except during nest capping and establishment. This was perhaps due to increased difficulty in seeing a ball of pitch, or to intermittent pitch collecting behavior. I was, however, able to observe globs of pitch visible at the nest entrance during the pollen collecting trips. It seemed that the amount of pitch increased as the pollen provisioning continued, but I did not quantify this in any way. Matthews (1965) also noted pitch at the cell entrance and, while he observed the female using some of this deposited pitch to build a cell partition, he explained that it was a rare occurrence. He described this deposited pitch, not as a reservoir, but as a marker delineating the amount of nest space that the female was planning to use. Matthews recorded that the deposit invariably ended up being the base of the end plug, regardless of what it was used for during the rest of the nest construction process.

Cell partition construction occurred during a very obvious lapse in the pollen collecting activity usually lasting close to an hour. The female would suddenly stop collecting pollen, and would be visible only momentarily as she backed towards the nest entrance and then disappeared again into the nest. The pitch visible at the entrance diminished in quantity very obviously during this time. Pollen collection resumed

immediately after this break, indicating a new cell cycle had begun. Matthews (1965) watched *H. carinata* in transparent traps in Michigan and reported that distinct pollen and pitch collecting trips were made, and that pitch collecting trips were made during the construction of the cell partitions. It is possible that the same behavior was exhibited by the bees that I was observing, however, since no *H. carinata* nested in transparent traps in my study, I was unable to differentiate between pollen collecting trips and pitch collecting trips.

Coelioxys moesta

The cell cycle for this parasite is very different from the provisioning *Megachile* species. The female sits and waits on a nearby vantage point (e.g. a leaf nearby), flies into a nest, deposits an egg, and then goes back to sitting and waiting. The egg deposition and larval development have been well documented by others (Eves et al. 1980, Graenicher 1905, Graenicher 1927).

There are several different phases in locating and parasitizing a nest. The first is finding a site that is being used by provisioning bees. This phase is evident because *C. moesta* females fly very close to the nest, landing lightly enough to drag the tip of the abdomen on one of the edges of the entrance. I observed the abdomen dragging on several dozen occasions, but I was not able to see anything remaining in the nest after an abdomen dragging. It could be a scent marking behavior that allows the parasite to return and continue checking possible nests. I did not read any other accounts of the abdomen dragging.

A second phase that I observed on many (N > 30) occasions, involves repeated hovering in front of the trap-nest blocks. Very close to the entrances, but not touching the nests. The parasite lands occasionally, perhaps at previously marked entrances. Some of the time, the parasite will briefly enter a nest, but sometimes she will simply land at the nest entrance momentarily, touching the front edges with her antennae. No abdomen dragging occurs at this time.

Another phase in this searching behavior involves sitting near the nests (within a meter, but not usually on the nest block) and watching. The parasite will occasionally fly up to the nest block and hover in front of several holes, but more often it seems the parasite is watching for a particular bee. It is difficult to determine if the parasite is watching for a single bee, since the parasites are not always observed at their vantage points. Unless they are observed visiting the same nest more than once, it is difficult to ascertain if one nest is engaging their attention more than another. On a couple of occasions, I observed the parasitic females entering three or four neighboring nests and backing out again after only 5 seconds, finally coming out of the last nest headfirst and after 10 seconds. The parasite appeared to know the general area, either visually or chemically, but examined several nests before ovipositing. Conversely, I also watched parasites fly directly into a particular nest immediately after a host female departed. This occurred after minutes of waiting while other possible host bees came and went, but did not elicit any interest from the waiting parasite.

More support for the hypothesis that *C. moesta* females are watching a certain nest comes from their choice of when in the host provisioning cycle to oviposit.

Oviposition occurs only during the provisioning stage of the megachilid cell cycle. It also seems that the oviposition only occurs near the end of the provisioning cycle, within an hour (three final pollen trips) of the cell capping. It was not uncommon for the *C. moesta* female to make several oviposition trips during this window of opportunity.

DISCUSSION

Nest Construction

The direct comparison of the average number of cells per nest between *M. relativa* (6.05 ± 0.67) and *M. rotundata* (6.67 ± 0.41) is not completely appropriate as *M. rotundata* was nesting in shorter nest tubes. Because *M. rotundata* is smaller than *M. relativa*, and had significantly shorter cell lengths, it follows that the species should be able to fit many more cells in a nest tube than the larger bee. This is supported by the comparison of the space usage within the nest. *Megachile rotundata* had the highest amount of reproductive space in the nests compared with the two other species in this study. Indeed, Stephen (1961) reported that the number of cells per nest averages between 8 and 12 in 15 cm deep nests for *M. rotundata* compared to the 6.67 cells in my nests.

Strickler et al. (1996) reported that *M. relativa* in Michigan built nests with a mean (± SD) of 4.2 ± 2.5 provisioned cells with a range of 1 to 12 cells per 14.2 cm nest. While slightly shorter than the nests in this study, the average number of cells per nest is much lower than the 6 that I observed, but with a similar range and standard deviation. This difference may be due in part to a much higher sample size, as Strickler's study was performed over a six-year period (N = 1123).

Following the trend of a smaller bee able to construct more cells per nest, one would expect *H. carinata* to have the highest average number of cells per nest. In this study, I found the exact opposite, with *H. carinata* having only 4, the lowest average

number of the three species I observed. The reason for this reversal lies in the use of the nest space by this species. *Heriades carinata* had the highest percentage of non-reproductive space in the nests that I measured. Matthews (1965) reported an average number of 6.4 cells per completed nest in Oregon, and 4.6 in Michigan in variable length tubes. While these slightly higher numbers may be the result of larger sample sizes, my results are very similar to the Michigan average.

Megachilids usually follow a pattern of building larger cells at the basal end of the nest to accommodate larger amounts of provision for female cells. This results in larger (female) progeny that take the most time to develop (Gerber and Klostermeyer 1972). In my intra-species comparison of the first four cell lengths I found a significantly longer basal cell in both *M. relativa* and *M. rotundata*. However, I am skeptical of this result because it is only the first basal cell that proved longer. I would have expected to see the second and third cell average lengths larger than the first as was observed by Gerber and Klostermeyer for *M. rotundata* (1972) or tailing off towards a significantly shorter length, and not simply dropping as they did in my data. This pattern could be because of a choice I had to make between trimming the cells upon removal from the nest to get the most accurate cell length measurement or to dissect and count the number of leaf pieces that were used to construct each cell. I chose to dissect the cells to count leaf pieces since I was not examining sex ratios, and as a result the longer basal cell length that I found may be an artifact of the measurement method.

Analysis of *H. carinata* nests did not pose the same dilemma. The cells are partitioned but not lined, and therefore the cell lengths are immediately visible. In

addition, none of the four basal cell lengths were significantly longer, so a comparison is moot. Matthews (1965) did not describe any differences in cell length between male and female cells.

In comparing the number of leaf pieces used to construct the cell and cell cap between *M. relativa* and *M. rotundata* I had to consider nest diameter. *Megachile relativa* nested in both 6 mm and 9 mm diameter nests, while *M. rotundata* nested in 5 mm diameter nests. I found that while *M. relativa* in 6 mm nests and *M. rotundata* in 5 mm diameter nests did not have different numbers of cell leaf pieces, the 9 mm diameter nests did have significantly more leaf pieces. Stephen (1962) noted that *M. rotundata* used more leaf pieces to line larger diameter nests in order to reduce the volume of the cell, so the *M. relativa* in this study are likely doing the same. *M. rotundata* used significantly more leaf pieces to cap cells than the *M. relativa* females in either nest diameter. In considering methods of preventing parasitism, it makes sense that, although *M. rotundata* has the least amount of non-reproductive space in the nest in the form of diffuse plugs or vestibular spaces, additional leaf pieces capping each cell might perform the same function, while using little additional space. Strickler et al. (1996) speculated that *M. relativa* created nests with long diffuse plugs and spaces to finish a nest quickly and put an end plug on the nest, when usurpation was common or when floral resources are scarce. It would be interesting to measure and compare the non-reproductive space of *M. relativa* nests constructed in times of plentiful and scare resources to test this hypothesis.

Foraging

Foraging trip lengths for either leaf pieces or pollen provisions were not different between *M. relativa* and *M. rotundata*. Although these two species were observed at different sites, the sites were separated only by 8 km and experienced similar environmental conditions. The duration of leaf collecting trips was not significantly different between *M. relativa* and *M. rotundata,* which is not surprising considering that it involves finding a suitable leaf-cutting (which are abundant nearby for both species), and returning to the nest. Klostermeyer and Gerber (1969) reported leaf-collecting trips with a mean duration of 318 seconds for *M. rotundata*, quite a bit longer than, but not outside of the duration range of the leaf collecting trips that I recorded. I suspect the differences are due to available nest construction material (vegetation) and weather conditions. Stephen and Torchio (1961) recorded leaf-collecting trips as low as 10 seconds in Oregon, which was also in the range of leaf collecting trip duration that I recorded.

I expected *M. rotundata* to make shorter pollen collecting trips than *M. relativa,* because they were surrounded with resources and the smaller scopa on *M. rotundata* should fill with fewer pollen collecting trips. However, my data showed that although a dense stand of alfalfa grew in close proximity, *M. rotundata* also foraged on many other pollen sources (some not as close by), which may help to explain longer foraging trips than hypothesized. Klostermeyer and Gerber (1969) recorded average pollen-collecting trips of 894 seconds for *M. rotundata* in Washington, again much longer than the average pollen-collecting trip duration that I recorded.

The amount of time that the *Megachile* females spent in the nest after a leaf collecting trip was different, but the time spent in the nest after a pollen collecting trip was not. This suggests that the *Megachile* species perform similar tasks after pollen gathering trips, but different tasks after leaf gathering trips. While the nest construction of *M. relativa* and *M. rotundata* are not very distinct, they may be manipulating the leaf pieces in a different manner or are experiencing different handling constraints to require a different duration of time in the nest after a leaf collecting trip.

H. carinata had a mean foraging duration between the leaf gathering trip duration and the pollen gathering trip duration of the other two species. The trip duration is probably skewed due to the fact that the foraging data are a combination of pollen and pitch gathering trips. However, Matthews (1965) reported pollen-gathering trips with an average duration of 597 seconds, and pitch collecting trips with and average duration of 287 seconds, which when combined would average very close to the overall mean trip duration of 476 seconds that I observed. This species was smaller than either of the *Megachile* species, and I would have expected it to have the shortest foraging trip duration of the three species I studied. Since I do not have specific pollen gathering observations I cannot confirm whether this is actually the case. However, the mean pollen gathering trip of 597 seconds that Matthews (1965) reported is shorter than the pollen gathering trips of the two *Megachile* species (656 and 672 seconds) that I recorded. More observations of *H. carinata* need to be made to determine if pollen and pitch gathering trips are comparable to those recorded by Matthews (1965).

Included in my *H. carinata* foraging data are some trips of very long duration. Klostermeyer and Gerber (1969), as well as Michener (1952), suggested that some megachilid bees make trips specifically for feeding in addition to gathering provisions or nest construction materials. While these trips were easily identified in my *Megachile* species observations, they were not removed from the *H. carinata* data. Thus, the foraging mean duration that I have recorded may be further skewed by long trips when the female is feeding in addition to collecting pollen for larval provisions.

Pollen Use

For *M. relativa* cells I was able to identify 15 distinct groups of pollen belonging to eight different families; four of the types I could identify to genus. Medler and Koerber (1958) listed 23 species in seven families based upon flower visitations of *M. relativa* on flowers in Wisconsin, although some of these may represent nectar collection rather than pollen foraging. Strickler et al. (1996) identified pollen using reference slides from four plant families in Michigan by examining provisions with a light microscope (Table 9).

While all of the families listed in Table 9 are present in Montana (Dorn 1984), the identification of pollen using light microscopy is limited to comparison with reference slides (Dr. Matt Lavin, personal communication), and therefore limited by samples that I gathered at the field sites. However, although I was limited to identifications within my reference collection, I reported novel pollination records for *M. relativa* (Caprifoliaceae, Fagaceae, Hydrangeaceae, Oleaceae). This is due to the fact that very few studies have

used pollen identification to determine what pollen *Megachile* spp. bees are collecting. In light of this fact, perhaps it should not be surprising that *M. relativa* is foraging on at least two types of non-grass monocot plants. Krombein et al. (1979) reported *M. relativa* flower visitation on the Iridaceae family. I did not see or collect any Iridaceae from my field sites for my pollen reference collection, however, *Sysyrinchium* spp. are reported to be inconspicuous because they are small and their flowers open only in bright sunshine (Nelson 1977).

I was able to identify pollen in nine distinct groups for *M. rotundata* belonging in four families (Asteraceae, Caprifoliaceae, Fabaceae, and Rosaceae), two groups of non-grass monocot pollen, and some unidentified pollen (Table 9). While this range of pollen use is not surprising (Stubbs et al. 1994, Small 1997), the high usage of monocot pollen is interesting as the *M. rotundata* nests were nesting in a shelter between alfalfa plots and amidst fairly abundant red clover. I was surprised to find that, while some of the cells contained pollen from dicot families, 100% of the cells contained one or more types of monocot pollen. Indeed, some of these cells contained nothing but monocot pollen. Again, while many studies have reported *M. rotundata* pollen preference or foraging records, they have been based upon flower visitation (Fairey and Lefkovitch 1991, Fairey et al. 1989, Krombein et al. 1979, Richards 1986, 1987, 1991, 1995, Richards and Edwards 1988, Stephen and Torchio 1961, Small et al. 1997, Stubbs and Drummond 1994).

Heriades carinata collected pollen from eight groups that I was able to discern, belonging in four families (Asteraceae, Caprifoliaceae, Fabaceae, and Tiliaceae), and two

groups of non-grass monocot pollen. Matthews (1965) reported flower visitation records of six families, but pollen analysis revealed "almost entirely" Anacardiaceae pollen in the cells from Michigan. Because there was no sumac at either the W. Bozeman or RCF site, it is not surprising that I did not find Anacardiaceae pollen in the *H. carinata* samples. The presence of monocot pollen in some of the *H. carinata* nests may have farther-reaching implications than monocot pollen in either of the *Megachile* species' nests. Since *H. carinata* is considered to be a "primitive" Megachilid (Matthews 1965) the behavior of this species may indicate that foraging on monocots for cell provisions is widespread behavior in the rest of the Megachilid family.

The difference in pollen prevalence between the two sites where *M. relativa* nested has pollination management implications. It may be that the composition of surrounding vegetation has an impact on the pollen that is collected by *M. relativa*. *Cirsium* spp., *Taraxacum* spp. and Fabaceae were present in abundance at both the W. Bozeman site and SE Bozeman site, yet they were collected in different ratios in nests at each site. While this difference may be due to sampling error, they imply that megachilid beekeepers must be conscious of the vegetation surrounding a crop that they wish to have pollinated.

I believe that the differences in pollen prevalence in samples between 1999 and 2000 reflects the plasticity of *M. relativa* foraging behavior. The 1999 field season was a very productive and long season, with observations starting at the end of June and ending at the end of August. In contrast, the 2000 field season was unseasonably hot and dry, with observations all occurring within the month of July. With this in mind, the

Table 9. Pollen records for each species by author.

Family	*M. relativa*			*M. rotundata*			*H. carinata*		
	Krombein et al. 1979*	Strickler et al. 1996	This study	Krombein et al. 1979*	Stubbs et al. 1997	This study	Krombein et al. 1979*	Matthews 1965*	This study
Dicot Families									
Anacardiaceae							X	X	
Apocynaceae	X						X	X	
Asclepiadaceae				X			X	X	
Asteraceae	X	X	X	X	X	X	X	X	X
Boraginaceae	X			X					
Brassicaceae	X		X						
Capparaceae							X		
Caprifoliaceae			**X**			**X**			**X**
Ericaceae					X				
Euphorbiaceae				X					
Fabaceae	X	X	X	X		X	X	X	X
Fagaceae			**X**						
Gentianaceae	X								
Geraniaceae	X								
Hydrangeaceae			**X**						
Hydrophyllaceae	X			X					
Lamiaceae							X	X	
Oleaceae			**X**						
Onagraceae	X								
Polemonaceae	X								
Polygonaceae				X			X		
Ranunculaceae	X								
Rosaceae	X		X		X	X	X		
Salicaceae					X				
Scrophulariaceae	X			X			X		
Solanaceae	X								
Tiliaceae									**X**
Urticaceae	X								
Vitaceae							X		
Monocot Families									
Iridaceae	X								
Unknown (non-grass)			**X**			**X**			**X**

*These records are based upon flower visitation and may not indicate pollen gathering.

differences in pollen use make more sense. The 1999 season is characterized by higher levels of pollen from the Asteraceae and fewer pollen types than the 2000 pollen use. Considering the environmental stress of the 2000 season, it makes sense that the

differences in pollen use between families are not as great, yet pollen was used from more families, possibly as some sources disappeared.

Pollen use levels were compared between frass samples and provision samples to determine if digestion could be disproportionately removing any pollen types from the samples. The lack of Oleaceae pollen in the frass samples and presence in the provision samples suggests that this may be the case. However, with the very low levels of Oleaceae even in the provision samples it is possible that none of the frass sampled cells contained any pollen from the Oleaceae family.

I compared pollen samples from nests of healthy, dead, and moldy larvae to determine if any pollen could be detrimental to *M. relativa* larvae. The pollen samples from dead or moldy larvae cells have high amounts of *Cirsium* sp., Asteraceae, and Caprifoliaceae in comparison with the pollen from cells containing healthy larvae. These differences are not necessarily causal, but the fact that I found significant differences warrants further study of provision pollen content related to larval mortality.

While I uncovered previously unreported pollen use by all of the three families through pollen identification, I believe further study is needed to elucidate the presence of monocot pollen in such high levels in these megachilid nests. While Krombein et al. (1979) reported flower visitation on Iridaceae for *M. relativa*, a confirmation of monocot pollen usage by *M. relativa, M. rotundata,* and *H. carinata* would indeed be novel. A second reference collection needs to be made, sampling an even wider variety of plants to try to identify the unidentified pollen that I collected from the provisioned cells. The presence of monocot pollen raises questions about the efficacy of these bees as possible

pollinators of crops, as well as the role of pollinators in general. I suggest that unless obvious matching reference-pollen samples are collected, provisions should be examined with a scanning electron microscope to identify pollen beyond the limitations of reference matching.

Larval Health and Parasitism

The mortality levels that I recorded from cell dissections were certainly not irregular. Eves et al. (1980) reported that levels of 10% parasitism have been reported for *Coelioxys* species in *M. rotundata* operations in Idaho. While I did not record any *Coelioxys moesta* parasitism of *M. rotundata*, I believe this may have been the function of a small sample size. The *Coelioxys moesta* females that I observed on the trap-nests at the W. Bozeman site were very easily scared away by bees coming and going from the nests. While *Coelioxys* sp. females have been observed at the Post Farm bee boards (Ruth O'Neill, personal communication), I think that the sheer numbers and amount of activity at the *M. rotundata* nests may also have reduced *C. moesta* parasitism levels.

I believe that the high levels of parasitism that I recorded for *Melittobia chalybii* were artificially inflated by the trap-nest storage in the laboratory. Johansen and Eves (1966) reported that while *M. chalybii* is not a serious pest of *Megachile rotundata* commercial operations, but it is a common laboratory pest. The capacity of this wasp to crawl through very small spaces made it impossible to control in the plexi-glass traps, and definitely caused higher parasitism levels in my *M. relativa* samples.

The high levels of unknown mortality in *M. relativa* may be explained by the trap-nests themselves. Apparently newly drilled nests have a tendency to absorb moisture from the nests and dry out some of the nests (Dr. Frank Drummond, personal communication). While not all of the nests built in my new plexi-glass traps were dehydrated, this may explain a good deal of the unknown mortality that I found in the *M. relativa* nests. I cannot estimate what percentage of the nests were dehydrated because a larva that dies due to some other cause and subsequently dries up, appears exactly the same as a larva that has died due to insufficient humidity.

Distribution

The principle components analysis of the emergence data may be misleading. The very low diversity in the 1999 emergences (some *M. relativa* and *M. rotundata* but few other species) yielded a plot based on very few numbers, and 62% of the variance explained by the first two principle components, although not especially high, is probably inflated. The 2000 emergence data is better because of the greater number of species, and the larger geographical spread of the field sites. I was surprised and encouraged by the presence of three species of *Stelis,* which are rarely collected (Dr. Terry Griswold, personal communication). The principal components analysis of the 2000 emergence data provided a plot that shows a slight geographical trend; the western sites tend to the left of the plot, while the eastern sites tend to the right. This pattern may be significant as the western sites are located in drier, more open areas with warm season vegetation, while those to the east are closer to the mountains and have cool season vegetation. It

follows that the bee diversity would reflect these ecological differences. The first two principle components accounted for 56% of the variability in the data. This is low enough to suggest that there are more than two underlying dimensions that are responsible for the correlation between sites.

It is important to note that the principle components analysis of the bee emergence data is a preliminary step in the overall analysis in a trap-nesting study being performed by Dr. Kevin O'Neill and I. Further analysis will include bee and wasp species that emerged from the trap-nests in additional to the 2001 emergences, each of which will provide more data to allow more meaningful principle components analysis.

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APPENDIX A

TRANSITION MATRICES

M. relativa - transition matrix 24 nests

	basal space	cell	diffuse plug 1	vestibule	diffuse plug 2	endplug	indentation
back	0.08	0.92	0.00	n/a	n/a	n/a	n/a
cell	n/a	n/a	0.83	0.00	n/a	0.08	0.08
diffuse plug 1	n/a	0.00	n/a	0.45	n/a	0.35	0.20
vestibule	n/a	0.00	n/a	n/a	0.88	0.11	0.00
diffuse plug 2	n/a	0.00	n/a	0.00	n/a	0.88	0.12
endplug	n/a	n/a	n/a	n/a	n/a	n/a	1.00

Average *M. relativa* nest has cells then diffuse plug, vestibule, and a diffuse to chewed final plug with a space on the end of the tunnel

M. rotundata - transition matrix 10 nests

	basal space	cell	diffuseplug	vestibule	finalplug	indentation
back	0.90	0.10	0.00	n/a	n/a	n/a
basal space	n/a	0.90	0.10	n/a	n/a	n/a
cell	n/a	0.00	0.90	0.10	0.00	n/a
diffuse plug	n/a	n/a	n/a	0.00	1.00	0.00
finalplug	n/a	n/a	0.00	n/a	n/a	1.00

Megachile rotundata has an average of a basal space, then cells followed by a plug, no vestibular spaces on average and a space on the end

H. carinata - transition matrix 10 nests

	basal space	space	cell	Membranous plug	vestibule	final plug	indentation
basal space	0.00	0.90	0.10	0.00	0.00	0.00	n/a
space	n/a	n/a	1.00	0.00	0.00	0.00	n/a
cell	n/a	n/a	0.00	0.30	0.40	0.20	0.10
vestibule	n/a	n/a	0.00	n/a	n/a	1.00	0.12

Average *H. carinata* nest has a basal space, cells, a vestibule and then a final pitch plug