

Disc size regulation in the brood cell building behavior of leaf-cutter bee, *Megachile tsurugensis*

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Abstract The leaf-cutter bee, *Megachile tsurugensis*, builds a brood cell in a preexisting tunnel with leaf discs that she cuts in decreasing sizes and assembles them like a Russian matryoshka doll. By experimentally manipulating the brood cell, it was investigated how she regulates the size of leaf discs that fit in the brood cell's internal volume. When the internal volume was artificially increased by removing a bulk of leaf discs, she decreased the leaf disc size, although increasing it would have made the leaf disc more fitting in the increased internal volume. As a reverse manipulation, when the internal volume was decreased by inserting a group of inner layers of preassembled leaf discs to a brood cell, she decreased the leaf disc size, so that the leaf disc could fit in the decreased internal volume. These results suggest that she uses at least two different mechanisms to regulate the disc size: the use of some internal memory about the degree of building work accomplished in the first and of sensory feedback of dimensional information at the construction site in the second manipulation, respectively. It was concluded that a stigmergic mechanism, an immediate sensory feedback from the brood cell changed by the building work, alone cannot explain the details of the bee's behavior particularly with respect to her initial response to the first manipulation. For a more complete explanation of the behavior exhibited by the solitary bee, two additional

behavioral elements, reinforcement of building activity and processing of dimensional information, were discussed along with stigmergy.

Keywords Nest building behavior · Brood cell · Sensory feedback · Stigmergy · Reinforcement · Haptic memory · Leaf-cutter bee

Introduction

Studies of nest building behavior in bees and wasps have a history of nearly a century, and many of them quantify nest structures and analyze behavioral sequences and proximate cues involved (Fabre 1915; Michener 1953; Hasenkamp 1974; Eickwort 1975; Smith 1978; Maeta 1979; Brockmann 1980; Downing and Jeanne 1988a,b; Torchio 1989; Jeanne 1996). However, detailed studies on the behavioral mechanism in building brood cells are relatively few because of the small size of the brood cells for which experiments are difficult to be performed (Fabre 1915; Altevogt 1955; Klostermeyer and Gerber 1969; Hasenkamp 1974; Downing and Jeanne 1988a).

Recent studies focus on the question as to how dimensional information about the nest structures either cues individual builders (Karsai 1999; Theraulaz and Bonabeau 1999; Bonabeau et al. 2000) or is exchanged among them (Jeanne 1996; Jeanne and Bouwma 2002; Seeley 2002; Britton et al. 2002) to execute a correct behavioral step during on-going building activities. The information cuing is called “stigmergy,” the original theory of which has been developed to understand the building behavior of social insects (Grassé 1959). According to the theory, the coordination and regulation of building activities do not depend on the individual builders themselves but are

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mainly achieved by the stimulus–response sequences interplayed between the individuals and the nest structure. In other words, they simply respond to a stimulus from the nest structure changed by an immediately preceding step of their building activities (Theraulaz and Bonabeau 1999).

Besides the benefit of applying stigmergy to the systematic investigation of complex building behaviors of social insects, its application to solitary species needs precautions. It tends to overlook some properties of behavior, for examples, reinforcement of building activities and processing of dimensional information, both of which typically take place within individual builders and are important in the regulation of their building behavior. Because a solitary builder must finish each of the complex building steps to fulfill the goal, completion of a cell, on one hand, the building behavior itself can be a potential source of reinforcing stimuli (Herrnstein 1977; Hughes et al. 1989). On the other hand, processing of information about the brood cell dimensions and determining the size properties of building materials potentially influence the quality of her work. This influence is particularly significant when she builds the brood cell that requires a high degree of geometric precision (Hasenkamp 1974; Batra 1984; Roubik 1989; Kim 1992).

Leaf-cutter bees are particularly suitable for nest manipulation experiments because their brood cells can be easily manipulated in caged environments and the dimensions of the brood cells and leaf discs can be quantified (Kim 1992). Under natural conditions, a builder cuts individual leaves into small discs (called “discs” hereinafter), lines them together to build a cylindrical brood cell (called “cell” hereinafter), in which she stores a mixture of pollen and nectar, lays an egg, and caps the cell. She repeats these activities several times to complete a series of cells in tunnel-like natural cavities such as hollow twigs and holes in a tree trunk.

In the present study, I present the results from a set of manipulations performed on some dimensional elements of a cell built by a leaf-cutter bee, *Megachile (Megachile) tsurugensis* Cockerell, to investigate the mechanism behind the building behavior. The bee builds the cell by lining its interior with a series of discs and adjusts the disc size to its decreasing internal volume as layers of discs accumulate within (Kim 1992). Then, I present a working hypothesis; that is, she may adopt either one or both of two control mechanisms. In one, the changing internal state of the animal regulates output. This could be some memory about the degree of building work accomplished. In the other, she attends only to the immediate sensory feedback from the construction site as in the stigmergy theory. Finally, I discuss the interpretation of results in the light of the hypothesis with respect to the possible roles of reinforcement of building activities and information processing of the cell dimensions and disc size in the bee’s on-going building behavior.

Materials and methods

Collection and rearing of bees

I collected foraging females of *M. tsurugensis* at Shishizuka field at about 5 km east of Tsukuba Science City during summer months. I marked groups of one to four individuals of these bees with a dot of colored enamel paint on the dorsal part of the thorax before introducing them into cages for experiments. After individual females started nesting, experimental manipulations were performed. Females that did not engage in nesting behavior for more than 1 week or when the experiments were finished were discarded. Therefore, one to four females were constantly nesting inside each cage during the experimental period.

Two separate cages of the same dimensions (length=2.0 m×width=1.8 m×height=1.7 m) made of wooden frames and nylon nets (mesh size=1 mm) were built inside a vinyl house (7.0×4.0×2.3 m) at the National Agricultural Research Center in Tsukuba Science City. As food resources for the bees, I provided flowering plants of *Coreopsis drummondii*, *Cosmos sulphureus*, *Cosmos bipinnatus*, *Caryopteris incana*, and *Caramintha nepeta* grown inside the cages and maintained constant blooming by an automatic drip irrigation system. To supplement these floral resources, pollen grains collected by the honey bee were smashed between two plastic plates, and the pollen particles were placed on a plastic dish at 30 cm above the ground by a wooden pole in each cage. To provide leaf materials for the bees, I maintained a constant supply of five to ten stalks of rose, *Rosa hybrida*, each having seven to ten leaves of about 3–7 cm long in a water-filled flask in each cage. To provide artificial nest sites for the bees, I made cylindrical tunnels of 10 cm (8.5 or 9 mm internal diameter) drilled through straight-grained pine sticks (13 cm long and 2.5×2.5 cm). These tunnels were placed inside a wooden station (20×10×5 cm) installed at 160 cm above ground near a western corner of the cages. The entrance of the tunnels faced east, so that the bees could engage in nesting activities as early as possible each morning.

Manipulations

I performed four different cell manipulations for a total of 15 individual bees. Manipulation 1, 2, and 3 were performed in the tunnels of 8.5 mm, and Manipulation 4 was performed in those of 9.0-mm diameters. To analyze the size of discs, I collected the disc data from the same individual cell and compared the sizes between the manipulation and control. To analyze the number of discs used for building a cell and the size of cells in general, I selected for each manipulated cell a control cell for comparison. The control cell was any unmanipulated cell

adjacent to a manipulated cell. If there were two adjacent control cells sandwiching the manipulated cell along the linear cell sequence, an average value was taken. Because there is some difference in cell size between male and female progeny and cells are usually larger for female than male progeny in *Megachile* species (Kim 1992), a manipulated and its control cell within each progeny sex were compared by pairwise analyses. During the experiments, I observed the bees' activities inside tunnels using a penlight whenever possible. After the experiments, I measured head widths of the bees to a nearest 0.01 mm by an ocular micrometer.

Specific manipulations

Manipulation 1

An individual female under normal conditions lines discs of decreasing sizes toward the innermost layer of a cell, but she starts with a few initial discs that are often in irregular sizes. Thus, after she lined the first ten discs and while she was collecting the 11th disc, I removed its inner layers made of seven discs, leaving only three outermost discs intact, which essentially increased the cell's internal volume. This manipulation allowed her to fill in the lost parts with new discs as if the cell returned to an early building stage. She resumed building behavior starting with the 11th disc and completed the entire cell. I compared the size of some selected discs from the same cell to analyze her response to the manipulation. I preserved the removed inner layers in a refrigerator (4°C) to be used in Manipulation 3.

Manipulation 2

As a modified version of Manipulation 1, I repeatedly removed discs from a cell. Immediately after the bee lined the cell with the first two discs, I removed the second disc and allowed her to add another disc, which again I removed. I repeated this disc removal five times; thus, until this point, only one disc lined the tunnel. After the fifth removal, I let the bee line discs 2 to 5. Then, I removed the sixth position disc five times and left her line discs 6 to 9. Finally, I removed the tenth position disc five times and allowed her to complete the cell. Through this manipulation, I removed a total of 15 discs from three discrete layers of the cell. I compared the size of removed and unremoved discs of the same cell to analyze her response to the manipulation.

Manipulation 3

As a reverse treatment of Manipulation 1, after the bee lined the cell with the first three discs, I grafted the cell with

the inner layers made of seven discs obtained from Manipulation 1, by carefully inserting the latter to the cell. The manipulation significantly decreased the internal volume of the resulting cell. This created the condition as if the cell advanced to a later building stage. I allowed her to resume building behavior starting from the fourth disc and to complete the entire cell. I compared the size of some selected discs from the same cell to analyze her response to the manipulation.

Manipulation 4

In normal building behavior, both the cell's internal diameter and length are gradually decreased by the bee's disc-lining activity. To examine how the diameter cue alone may affect the bee's response, I artificially decreased only the diameter without changing the length of the cell. Immediately after the bee lined the cell with the first seven discs, I squeezed the half-made cell by inserting a piece of cardboard pad (10×15×1 mm) between a part of the cylindrical wall of the cell and the tunnel. This manipulation decreased the internal cell diameter by approximately 1 mm along one third of the cell's cross-sectional perimeter where the cardboard pad was inserted. Then, I allowed her to resume building activities with the eighth disc and to complete the cell.

Collection of cells

After the manipulation experiments, I harvested cell-filled nest sticks and kept them at 25°C for 2 weeks, during which larval development and cocoon spinning occurred. Then, I split open the nest sticks to carefully separate individual cells in a linear arrangement (Fig. 1a). I sequentially removed discs by a pair of flat-tip forceps, starting with the outermost one lined first by the bee and exposed a cocoon inside. Then, I sandwiched the discs between strips of paper towels, soaked in hot water (60–70°C) for 10 min, and flattened them on a transparent plastic sheet to make a Xerox copy (Fig. 1c). I measured the disc length and width and cocoon length and diameter to the nearest 0.01 mm by a digitized caliper. The external dimensions of the cocoon estimate the cell's internal dimensions because the last instar larva spins a cocoon tightly on the inner surface of the cell.

Data analysis

I analyzed all data with Statistica 6.1 for Windows (StatSoft, Tulsa). For the analyses of disc number and cocoon dimensions, for which the normality of the data was not ensured, I performed the Wilcoxon signed-ranks test between the treatments across the same individual bees. To

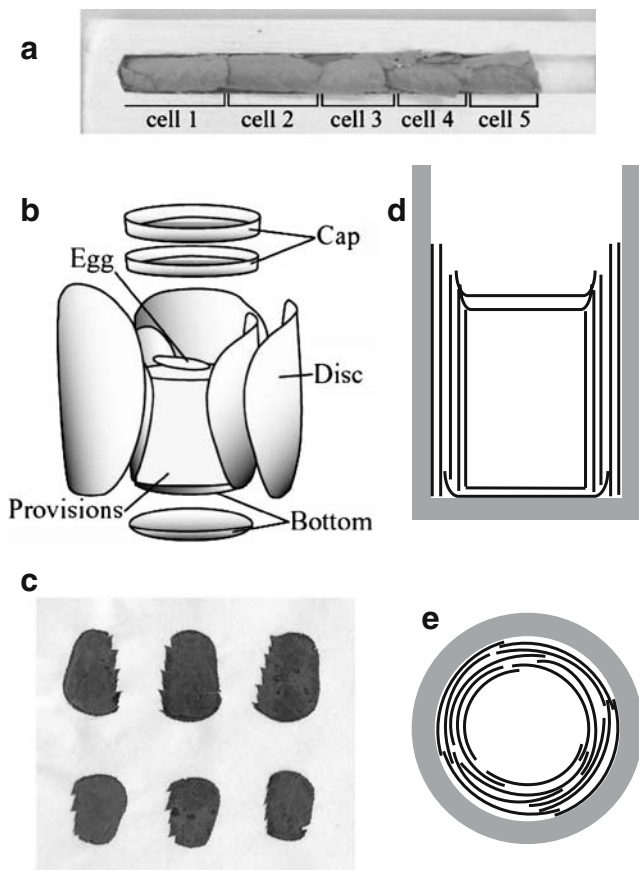


Fig. 1 Cell architecture of nests in an artificial tunnel. **a** A nest split open to expose a series of five cells lined with discs. **b** A diagram showing a typical cell composed of several innermost leaf pieces that are circular discs for the bottom and the cap and oval discs for the wall. These compartments are tightly attached together to form a cylindrical container where the provisions and an egg are laid. **c** Some larger (*upper row*) and smaller (*lower row*) discs obtained from outer and inner cell layers, respectively. **d** A diagram showing a longitudinal section of a cell. The rectangular space inside represents an entire room where provisions and an egg are laid and a cocoon is later formed. The *shaded area* represents a tunnel wall and a bottom. **e** A diagram showing a cross-sectional view of a cell. The circular space inside represents the same room described in **d**

analyze disc size data, when normality of the data was ensured, I performed either paired *t* test or analysis of variance (ANOVA) between some selected disc positions of the same cell across the same individual bees. When normality was not ensured, I used the Wilcoxon matched-pairs test. For a certain manipulation, I performed regression analysis to obtain slopes of the curves for disc size against disc position number. Sample size (*N*) is the number of bees tested unless otherwise indicated. With regard to the correlations between the bee's body size and disc number, cocoon diameter, or length, I performed the Spearman rank correlation test because normality of the data was not ensured for these parameters, but I performed a simple correlation test for the correlation between the bee's body size and the disc size.

Results

General observation and basic cell structures

Among the 15 individuals of *M. tsurugensis* experimented, six individuals successfully built cells with all of the four manipulations, and nine other individuals had some but not all of the manipulations. Their cell-building behavior consisted of a sequence of stereotypical activities: walking back and forth and antennating within an existing cell just before exiting a nest, cutting a disc from a leaf outside the nest, and bringing it into the cell to line it against its internal wall. The speed of completing one cell including lining, provisioning, laying an egg, and capping the cell was somewhere between zero and two cells per day, largely depending on weather conditions. Among those individuals that were excluded from the experiments because of unsuccessful nesting, some individuals (*N*=3) occasionally entered neighbors' nests with or without a disc. When the nest owner returned, aggressive interactions occurred either inside or outside the tunnel (*N*=4 observations).

Each cell was composed of the bottom, the wall made of layers of discs, the cap and the cell contents, the provisions collected from pollen and nectar, and an egg (Fig. 1b). Figure 1d and e show a longitudinal and cross-sectional view of a typical cell assembled with a series of discs, respectively. The bee started from a bottom of a nesting tunnel, brought in one by one the first few oval discs, and laid them to form a cylindrical wall covering an entire space where a new cell was being built (Fig. 1d). She assembled these discs whose long axis aligned parallel to the tunnel axis, so that the two sides of the adjacent discs overlapped each other (Fig. 1e). She continued this wall-building activity with progressively smaller oval discs several times and braced them with a round disc in a dovetailed manner at the bottom of the cylinder (Fig. 1b,d). Then, she resumed the wall-building activity using even smaller oval discs until reaching the innermost layer of the wall and again braced them with a round disc to finish a cylindrical container. As a result, the longitudinal view of the cylinder shows that a layers of oval discs covering the wall extended from the bottom to an entrance area, which was somewhat tapered inwardly by laying of increasingly smaller oval discs to the innermost layer (Fig. 1d). The inward tapering is important because it helped the bee snugly push in a round disc to cap the entrance after provisioning a food mass and an egg. This inward tapering also gradually delimited and determined the internal length of a cell when it reached the innermost layer covered by the last few small oval discs, where the cap later sat on (Fig. 1b, d).

The disc size had a positive relationship with bees' head widths; that is, larger bees cut larger discs than smaller bees (*N*=11 bees, first cells in 8.5-mm tunnels, *N*=121 discs, *r*=

0.25, $P=0.0566$; those in 9.0-mm tunnels, $N=146$ discs, $r=0.29$, $P=0.0004$). When the disc size was compared between the two tunnel diameters, there was no difference in disc length ($N=45$ discs composed of first five discs of the first cell built by nine individual builders in tunnels of two different diameters, 18.1 ± 3.8 mm in the 8.5-mm tunnel and 17.6 ± 2.9 mm in the 9.0-mm tunnel; paired t test: $t=0.797$, $P=0.4296$), but the discs used in the tunnel of the smaller diameter had a narrower width than those in the larger diameter ($N=45$ discs composed of first five discs of the first cell built by nine individual builders in tunnels of two different diameters, 10.3 ± 1.5 mm in the 8.5-mm tunnel and 11.4 ± 1.7 mm in the 9.0-mm tunnel; paired t test: $t=-3.768$, $P=0.00049$).

The number of discs used for individual cells had no relationships with bees' head widths (Spearman rank correlation: $N=11$ bees, first cells in the 8.5-mm tunnel, $r_s=-0.22$, $P=0.5138$, those in the 9.0-mm tunnel, $r_s=-0.16$, $P=0.6408$). Regardless of the body size of the bees, they used fewer discs to build a cell in tunnels of the smaller diameter than in those of the larger diameter ($N=9$ bees, 11.4 ± 1.7 discs, first cells in the 8.50-mm tunnel; 13.4 ± 2.2 discs in the 9.0-mm tunnel; Wilcoxon matched-pairs test: $z=2.52$, $P=0.0117$).

With regard to the cell's internal dimensions, there was no relationship between cocoon sizes and the bees' head widths (Spearman rank correlation: $N=10$ bees, first cells in the 8.5-mm tunnel, diameter: $r_s=-0.31$, $P=0.3852$, length: $r_s=0.17$, $P=0.6495$; first cells in the 9.0-mm tunnel, diameter: $r_s=-0.28$, $P=0.4310$, length: $r_s=0.36$, $P=0.3101$). The cell's internal dimensions did not differ between the two different tunnel diameters: cocoon diameter ($N=10$ bees, 6.68 ± 0.24 mm for first cells in the 8.5-mm tunnel; 6.88 ± 0.25 mm for those in the 9.0-mm tunnel; Wilcoxon matched-pairs test: $z=0.76$, $P=0.4446$), cocoon length ($N=10$ bees, 12.72 ± 1.27 mm for first cells in the 8.5-mm tunnel; 12.86 ± 0.99 mm for those in the 9.0-mm tunnel; Wilcoxon matched-pairs test: $z=0.56$, $P=0.5751$).

Manipulation 1

After a cell's internal volume was increased by removing a bulk of discs from the cell, the bees did not increase the disc size but kept the subsequent discs even smaller than the size of the original disc before the manipulation (Fig. 2a, one-way repeated-measures ANOVA: planned comparisons, (1) $F_{1, 12}=10.18$, $P=0.0078$, (2) $F_{1, 12}=2.44$, $P=0.1446$, (3) $F_{1, 12}=6.70$, $P=0.0237$). Because the bees cut an 11th disc before entering the manipulated cell and receiving no dimensional information of the cell, I discounted it from data analysis. Therefore, the disc at position 11 in the figure actually represents the 12th disc cut by the bees. The total number of discs used for the manipulated cell was comparable to that of the control cell

(Fig. 4, Wilcoxon matched-pairs test: control vs manipulated, $N=12$ pairs, $z=1.64$, $P=0.0994$). The internal length of the manipulated cells was shorter than that of the control cell (Fig. 5, Wilcoxon matched-pairs test: control vs manipulated, $N=12$ pairs, $z=2.04$, $P=0.0409$), but the internal diameter was similar between the manipulated and control cells (Fig. 5, $z=0.51$, $P=0.6101$).

Manipulation 2

When a series of five discs were sequentially removed from the three disc layers of a cell, the bees kept constant the disc size at each removal. The size difference between the

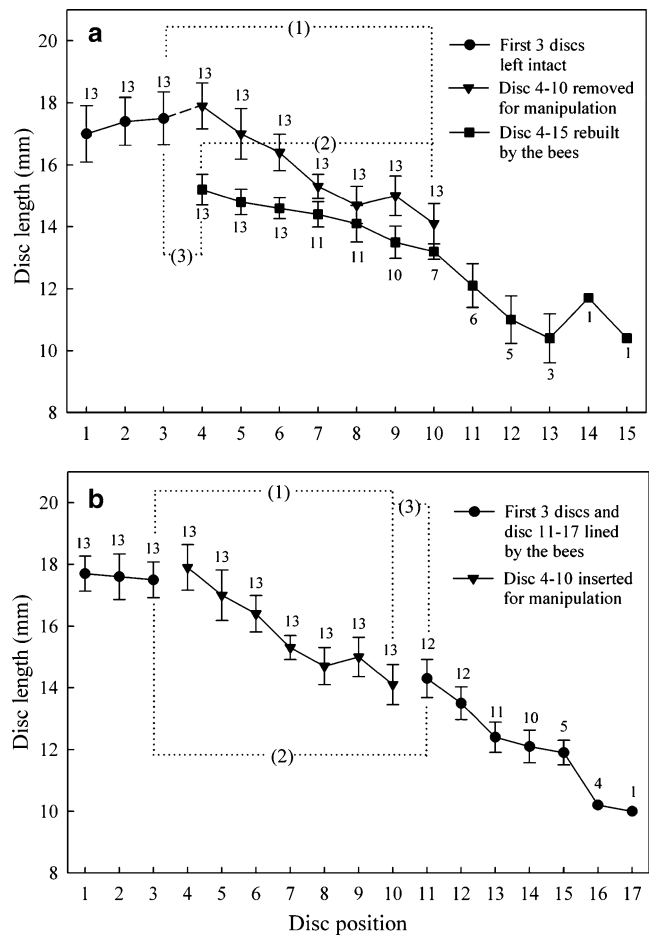


Fig. 2 Disc size (mean±SE) before and after Manipulations 1 and 3. Manipulation 1 (a): After the bee lined the tenth disc, discs 4 to 10 were removed, and the bee resumed cell building with disc 4. The number above or below each point is the sample size for the disc position. Disc size values for the same individual bee were compared between three disc positions, 10 (before manipulation), 3 (after manipulation), and 4 (bee's first response after manipulation). Manipulation 3 (b): After the bee lined the third disc, discs 4 to 10 were inserted, and the bee resumed cell building with disc 11. The number above each point is the sample size for the disc position. Disc size values for the same individual bee were compared between three disc positions, 3 (before manipulation), 10 (after manipulation), and 11 (bee's first response after manipulation)

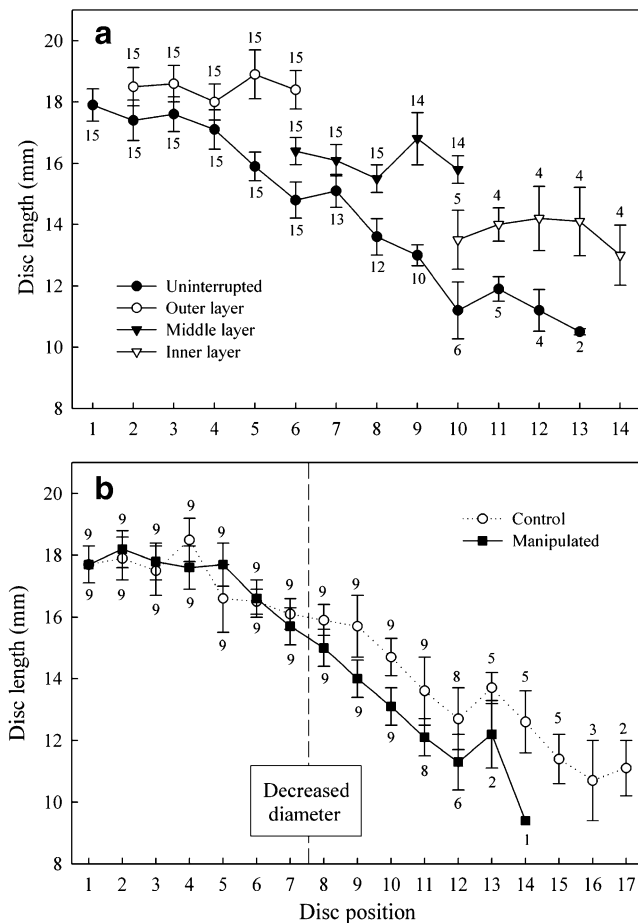


Fig. 3 Disc size (mean±SE) before and after Manipulations 2 and 4. Manipulation 2 (a): The lower connected line indicates disc size without manipulation. The upper three connected lines indicate the size of discs individually removed from three different layers; for these discs, the disc position indicates the sequence lining by the bee rather than the actual position within a cell. The second disc for the outer, sixth disc for the middle, and tenth disc for the inner layer were removed five times, respectively. The number above or below each point is the sample size for the disc position. For all of the three disc layers, the size of discs removed sequentially (upper connected lines) was significantly larger than that without manipulation (lower connected lines). Manipulation 4 (b): After the bee lined the seventh disc, a cardboard pad was inserted between the cell and the tunnel to decrease the cell’s internal diameter. The line with open circles represents disc sizes for control cells and that with filled squares those for cells that were decreased of diameter immediately after the seventh disc indicated by the vertical broken line. The number above or below each point is the sample size for the disc position

removed and uninterrupted discs for three discrete layers was significant (Fig. 3a, Wilcoxon matched-pairs test: control vs manipulated, outer layer: $N=65$ pairs, $z=4.33$, $P<0.0001$; middle layer: $N=56$ pairs, $z=5.44$, $P<0.0001$; inner layer: $N=13$ pairs, $z=3.18$, $P=0.0015$). The sequential disc removal allowed them to make a total of 15 additional discs (five discs for each layer), but the total number of discs used for the manipulated cell was similar to that of the control cell (Fig. 4, Wilcoxon matched-pairs

test: control vs manipulated, $N=11$ pairs, $z=1.53$, $P=0.1263$). The internal length of the manipulated cell was shorter than that of the control cell (Fig. 5, control vs manipulated, $N=11$ pairs, $z=2.04$, $P=0.0409$), but the internal diameter of the manipulated cell was larger than that of the control cell (Fig. 5, $z=2.45$, $P=0.0145$). Two individual bees discontinued the disc-lining activity in the middle of the experiment ($N=2$ observations) until resuming it on the following day, although no other environmental conditions seemed to hinder the activity.

Manipulation 3

The bees readily accepted a manipulated cell now having a smaller internal volume with an insert of inner disc layers. They decreased the disc size after the manipulation (Fig. 2b, one-way repeated-measures ANOVA: planned comparisons, (1) $F_{1, 11}=32.31$, $P=0.00014$, (2) $F_{1, 11}=37.60$, $P=0.00074$, (3) $F_{1, 11}=0.22$, $P=0.6508$). They placed a disc at position 11 that was comparable in size to the last disc of the insert (position 10), indicating that they adjusted the disc size to the insert. Because the bees cut a fourth disc before entering the manipulated cell and receiving no dimensional information of the cell after manipulation, I discounted it from data analysis. Thus, the disc at position 11 represents the fifth disc cut by the bees. The total number of discs used for the manipulated cell was greater than that of the control cell (Fig. 4, Wilcoxon matched-pairs test: control vs manipulated, $N=12$ pairs, $z=2.12$, $P=0.0342$). Given that the manipulated cell had received seven additional discs, the average 14.2 ± 1.9 gives the estimate of about 7.2 discs lined by the bees, which indicates that they adjusted the number of discs necessary

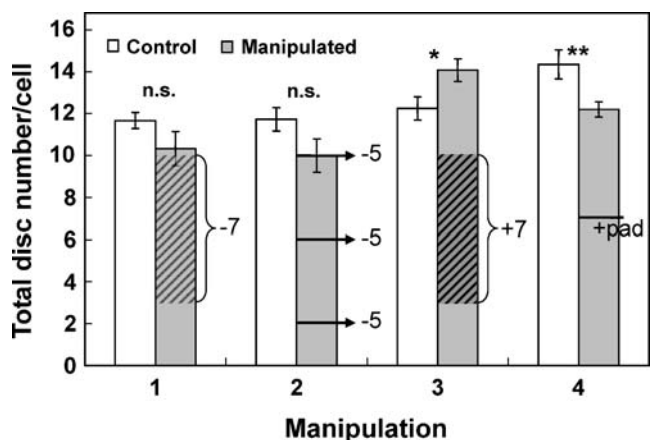
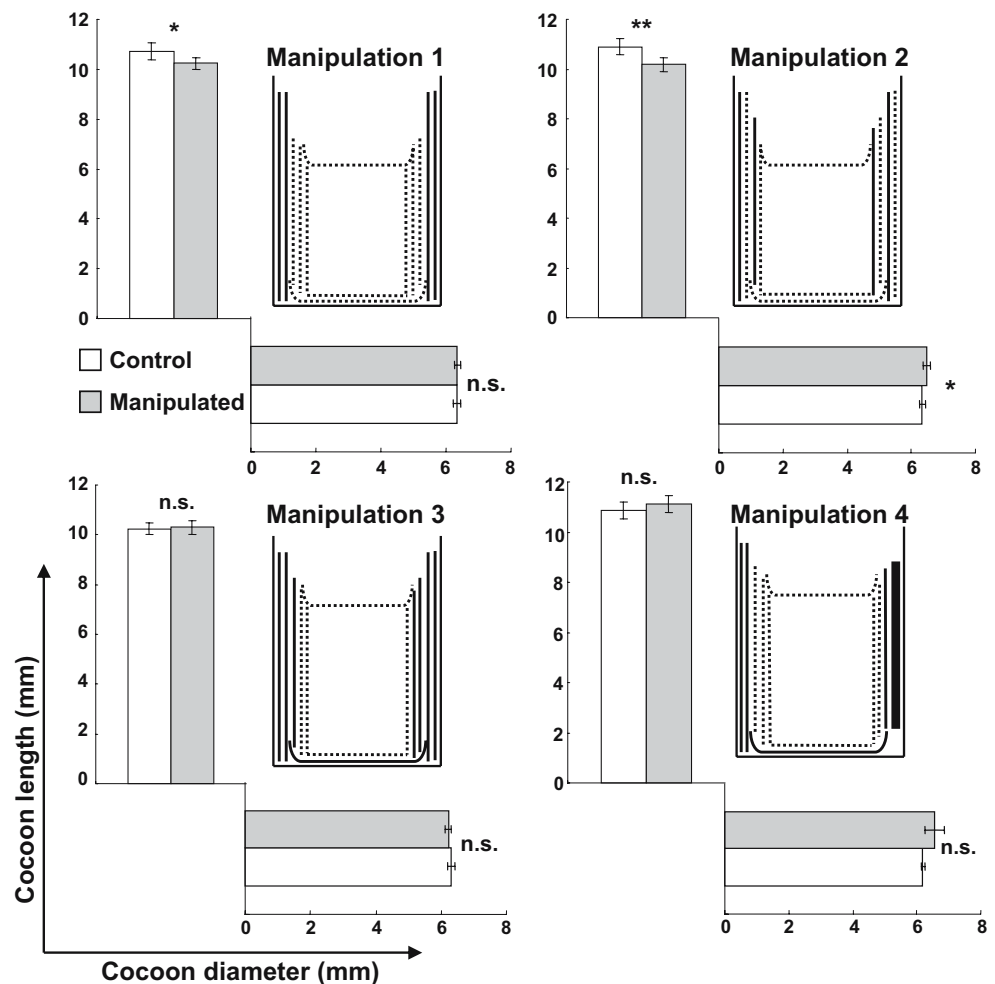


Fig. 4 Number of discs in cells from each manipulation. Mean total disc numbers across the completed cells of each manipulation are given with SE. For Manipulations 1 and 3, hatched areas indicate seven discs that had been removed (-7) and added (+7), respectively. For Manipulation 2, five discs were sequentially removed from three discrete disc positions. For Manipulation 4, after the seventh disc, a cardboard pad was inserted (+pad)

Fig. 5 Internal cell dimensions represented by cocoon length and diameter of the completed cells from each manipulation. Mean cocoon length (*vertical bars*) and diameter (*horizontal bars*) are given with SE. Diagrammatic representations of the manipulations are shown with the dimensional values. *Dotted lines* represent the discs lined by the bees after the manipulations



for completing the manipulated cell. The internal dimensions of the completed cell were similar between manipulated and control cells (Fig. 5, Wilcoxon matched-pairs test: control vs manipulated, $N=12$ pairs, internal length: $z=0.08$, $P=0.9375$; internal width: $z=1.73$, $P=0.0844$).

Manipulation 4

When the cell's internal diameter was decreased by the insertion of a cardboard pad, the bees decreased the disc size with an even increment before and after the manipulation (Fig. 3b). The bees cut the eighth disc without receiving any information about the manipulated cell; therefore, they in response to the manipulation lined the ninth disc, which did not stand out from the decreasing size trend. After the manipulation point, from eighth disc on, the size reduction slope was greater for the manipulated cell than for the control cell (Regression analysis, manipulated cells: slope= -0.83 , adjusted $r^2=0.372$, $F_{1, 42}=26.5$, $P<0.00001$; control cells: slope= -0.61 , adjusted $r^2=0.309$, $F_{1, 61}=28.7$, $P<0.00001$), indicating that they speeded up the disc size reduction for the manipulated cell. The total

number of discs used for the manipulated cell was smaller than that of the control cell (Fig. 4, Wilcoxon matched-pairs test: control vs manipulated, $N=10$ pairs, $z=2.52$, $P=0.0117$), but the cell's internal dimensions were similar between the manipulated and control cells (Fig. 5, Wilcoxon matched-pairs test: control vs manipulated, $N=10$ pairs, internal length: $z=1.58$, $P=0.1141$; internal width: $z=1.78$, $P=0.0745$), indicating that the bees decreased the number of discs necessary to adjust the cell's internal diameter at a constant value.

Discussion

The leaf-cutter bee, *M. tsurugensis*, cuts leaves into discs of appropriate sizes for the internal dimensions of a cell. To characterize the behavioral mechanism as to how she regulates the size of the discs that fit in the cell, a series of manipulative experiments were performed to test a working hypothesis; that is, the bee adopts one or both of the two controlling mechanisms: (1) She uses some memory about the degree of building work accomplished

and/or (2) attends only to the immediate sensory feedback from the dimensional cues at a construction site. This hypothesis generates precise predictions. For example, in Manipulation 1 (Fig. 2a), the “memory of work accomplished” mechanism predicts that the size of the new disc placed at position 4 after the removal of discs 4 to 10 will be similar to the tenth disc, whereas the “immediate feedback” mechanism predicts that the size of the new disc will be like a fourth disc before the removal. The result of Manipulation 1 supports the “memory of work accomplished” mechanism. Contrastingly, in Manipulation 3 (Fig. 2b), the “memory of work accomplished” mechanism predicts that the size of the new disc placed at position 11 after the addition of discs 4 to 10 will be similar to the fourth disc, whereas the “immediate feedback” mechanism predicts that the size of the new disc will be similar to the tenth disc after the addition. The result supports the “immediate feedback” mechanism.

In the light of these contrasting results, the bee’s disc size regulation can be roughly explained by a concept of stigmergy (Grassé 1959). Thus, the bee may regulate the disc size according to the immediate sensory information coming from the internal cell dimensions that have just been altered by the preceding step of her building activities. The concept of stigmergy has been originally developed to explain indirect task coordination and regulation in nest reconstruction in the termite genus *Macrotermes*. Grassé 1959 showed that the coordination and regulation of building activities do not depend on the workers themselves but are mainly achieved by the stigmergic stimulus–response sequences interplayed between the workers and the nest structure (Theraulaz and Bonabeau 1999); that is, workers respond to a stimulus from the nest structure changed by an immediately preceding step of their building activities. To explain the stigmergic stimulus–response sequences at the individual level, funnel entrance construction by a solitary wasp (Smith 1978) has been presented as an example of behavioral preadaptation to sociality, although the funnel structure is not an adequate choice in the first place because of its unique physical and functional constraints. Some additional studies in solitary builders seem to support the stigmergy theory (Freeman and Johnston 1978; Brockmann 1980).

The application of stigmergy to the explanation of complex building behaviors of social colonies has an advantage of analyzing the process of information cuing between the numerous builders and their shared nest structure. However, more detailed explanation for the solitary bee’s behavioral response to Manipulation 1 requires one to consider additional behavioral elements such as reinforcement of building activities and processing of dimensional information. Because these two typically take place within the individual builders, they need to be examined before stigmergy is used as a sole

framework to explain the behavioral mechanisms of solitary bees and wasps, although these insects have numerous eusocial relatives.

Firstly, “reinforcement of building activities” as an additional behavioral element needs to be considered along with the stigmergic mechanisms. Stigmergic mechanisms observed in the building behavior of social species may be traced back to some behavioral repertoires of extant solitary species but do not represent an entire set of preadaptive traits exhibited by modern-day solitary builders. This is largely due to the decreasing complexity of individual behavior and adult workers’ reproductive potential along with their increasing colony size (Karsai and Wenzel 1998; Bourke 1999). In solitary aculeate hymenopterans, a single totipotent maternal individual allocates brood cells and food materials to her offspring according to a sequential investment schedule that is potentially influenced by her egg production (Kim 1997,1999; Kim and Thorp 2001; Rosenheim et al. 1996). In fact, ovulation cycles in *Megachile flavipes* give substantial constraints to her cell-building behavior (Sihag 1985,1986). Because she must finish each of the complex cell building steps to fulfill the goal, completion of a cell, she is likely to be under the influence of reinforcement. Hughes et al. (1989), by providing preformed artificial nests to domestic hens, found that performance of their building behavior itself was reinforcing, which is consistent with Herrnstein’s (1977) hypothesis that behavior itself is a potential source of reinforcing stimuli in the presence of appropriate cues. However, when a hen was given a choice between premolded nests and moldable nests, she preferred and accepted the former. Duncan and Kite 1989 concluded that she does not have to build a nest herself as long as the premolded nest allows the performance of building behavior. Interestingly, these findings in the hens parallel the bee’s response to Manipulation 3 (Fig. 2b), although exact proximate cues involved remain undefined for the two taxonomically different builders.

In the light of the hypothesis, the bee’s response to Manipulation 1 (Fig. 2a) may reflect a carryover effect after a sudden cessation of a continuous positive reinforcement, while in Manipulation 2 (Fig. 3a), a proper reinforcer was absent because the internal dimensions of a cell were constantly set back to the original state at each disc removal. The end result of either manipulation was the reduced internal cell length (Fig. 5), indicating that these manipulations influenced the bee’s disc size regulation beyond the degree of normal dimensional ranges. Her response to Manipulation 3 (Fig. 2b) is within a normal range of an adaptive behavior. Under natural conditions, a leaf-cutter bee often encounters usurpation of her cell by conspecifics, which bring in additional discs while she is outside the nest. This leads to an abrupt decrease in the cell’s internal volume.

The owner upon return usually does not remove the foreign materials but continues bringing discs into the altered cell (Barthell and Thorp 1995; Kim 1997).

Secondly, “processing of dimensional information” as another behavioral element needs to be considered. In eusocial bees, evidence has been accumulated for the abilities to process complex spatial information (Seeley 2002; Saleh and Chittka 2007). Although the exact method including walking to measure a nest cavity space is still under investigation (Seeley 1977; Franks and Dornhaus 2003), that for measuring a cell cavity size by queen honey bees is known to be touching it with their forelegs (Koeniger 1970).

The leaf-cutter bee in the present study performed stereotypical forward and backward walks and antennating within a cell under construction before taking off to cut a disc. This suggests her direct measuring of the cell’s internal dimensions, and the dimensional information is presumably stored in a form of haptic memory (Henriques et al. 2004). A trichogrammatid wasp, a bees’ distant relative, is known to directly measure a host egg surface area and curvature by transit walks and antennal angles, and this parasitic wasp translates the dimensional information to the number of her eggs to oviposit in the host (Schmidt and Smith 1986, 1987a,b,c). In the leaf-cutter bee, the internal cell dimensions are translated to disc dimensions, an exact process of which is unknown but probably a certain haptic reproduction (Lanca and Bryant 1995; Chan 1996; Vogels et al. 2001). Importantly, these two dimensional parameters not only correlate but match in values. In other words, the bee’s disc size regulation is accomplished by the absolute measurement (Downing and Jeanne 1988a) of internal cell dimensions and cutting of a disc based on that size, while the disc dimensions and perhaps their haptic memory may work as stigmergic cues for the measurement and determination of subsequent discs.

The absolute measurement of disc dimensions is a functional requirement because the internal length and diameter of a completed cell are two of the critical dimensions. This is apparent from the bee’s responses that she adjusted the internal diameters at a fairly constant value regardless of the drastic manipulations performed (Fig. 5). To fulfill this task, she invested exhaustive efforts in bringing discs one after another at every removal of a previous disc and eventually lining about twice the number of discs necessary for normal cell building in Manipulation 2 (Figs. 3a and 4). Contrastingly, she reduced the total number of discs when the diameter was artificially decreased in Manipulation 4 (Fig. 4). It needs to be noted that as the diameter was decreased, the bee speeded up the disc size reduction (Fig. 3b) to decrease the internal cell length (Fig. 5). Interestingly, these two dimensional elements, cell diameter and length, are correlated, and this correlation reflects the bee’s separate determination of disc

width and length in response to the tunnels of the large vs small diameters, respectively.

How are these critical dimensions determined? Grassé’s stigmergy theory was proposed partly because he found “no evidence” for a blueprint of a species-specific nest. There may be no “internal image” of a cell, but at least a builder’s body parts are known to function as measuring devices such as the honey bee’s forelegs (Koeniger 1970) and the oriental hornet’s wings (Ishay 1976). Similarly, a megachilid bee *Osmia* is observed to measure a size of a cell by making a reference point in a nesting tunnel by using her body length as a ruler (Frolich 1983). She builds a threshold at the point where she places a cell partition (Torchio 1989). *Megachile* seems to use a few last discs to determine the final length of a cell, which is equivalent in function to determine the cell length in *Osmia* (Kim 1992).

The leaf-cutting behavior for obtaining a disc of a specific size is far more specialized in *Megachile* than other megachilid genera such as *Hoplitis* and *Trachusa* (Stephen et al. 1969; Westrich 1989) that also cut leaves. The latter two genera clearly lack beveled edges of mandibular dentation (Mitchell 1962) for efficiently cutting leaves. Not only the morphological features but neuroethological hardwares and perhaps a software, “an action program,” are also required for the highly tuned swing and gait control of appendages that *Megachile* uses for cutting a smooth trajectory into thin leaves to obtain discs of appropriate size specifications (Hasenkamp 1974). Presently, the exact neuroethological mechanisms are unknown as to how the information about cell dimensions is acquired, stored, and processed to select specific action programs for a cutting trajectory to acquire a disc of appropriate size. However, it is apparent that the bee uses this information processing as a certain quantitative rule, which, importantly, does not exist in the bee’s artifact but does in her behavior itself.

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