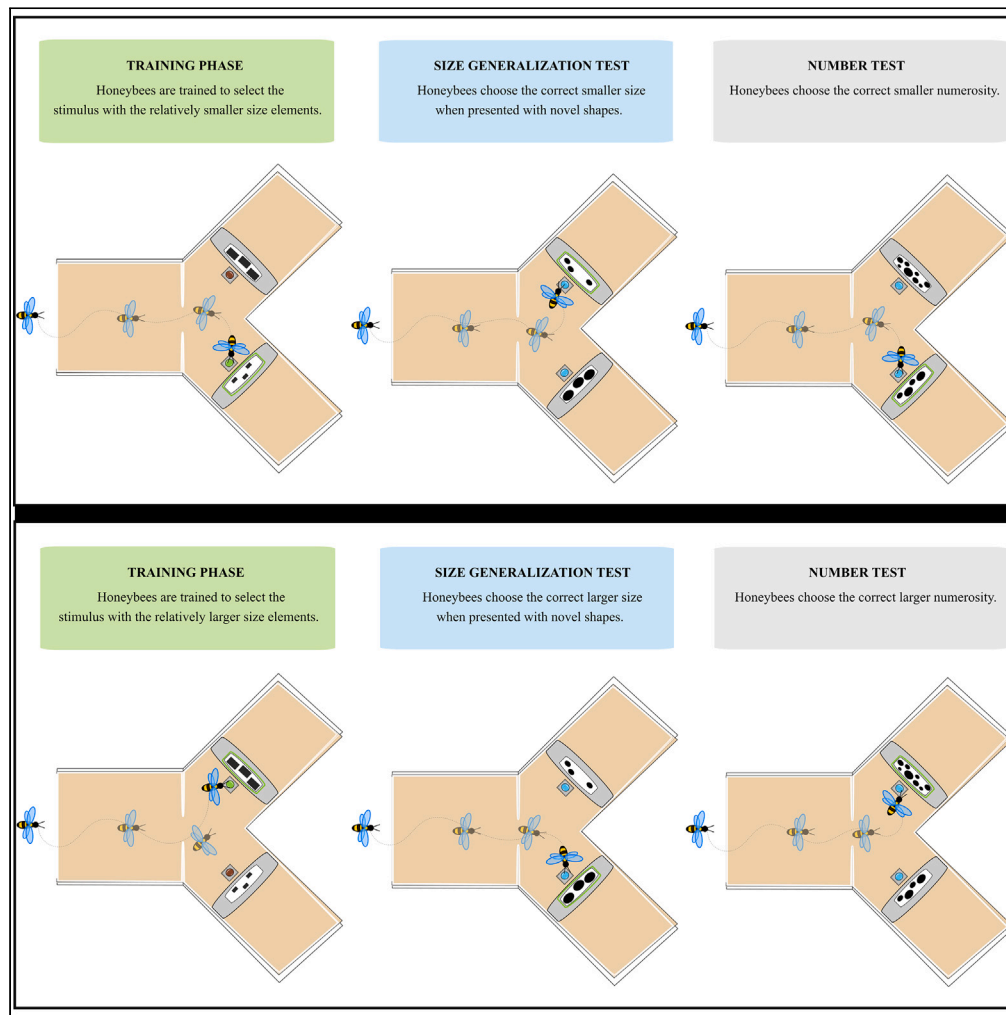


Article

Transfer from continuous to discrete quantities in honeybees



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Highlights

Bees trained to choose the larger/smaller size, chose the larger/smaller number

Bees did not rely on perceptual variables to make such a transfer

This demonstrates a bidirectional relationship between size and number

The cognitive mechanism for magnitude processing is highly adaptive and universal

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Article

Transfer from continuous to discrete quantities in honeybees

Maria Bortot^{1,2,*} and Giorgio Vallortigara^{1,*}

SUMMARY

Honeybees can estimate quantities having different dimensions: continuous and uncountable such as the relative size of visual objects in an array, or discrete and countable such as the number of objects of the array. Honeybees can transfer quantity discrimination (i.e., choosing the larger/smaller stimulus) from number to size. Here, we investigated whether honeybees could also generalize from the size (continuous) to the number (discrete) dimension. We trained free-flying foragers to discriminate between large- and small-size elements. At test, bees were presented with a comparison between larger and smaller numerosities controlled for different continuous variables covarying with numerosity such as total area, total perimeter, convex hull, and element size. Results showed that bees generalized from the size to the numerical dimension of the stimuli. This cross-dimensional transfer supports the idea of a universal mechanism for the encoding of abstract magnitudes in invertebrate species comparable to that of vertebrate species.

INTRODUCTION

The abilities to process the numerical, spatial, and temporal properties of an object have been the focus of separated research lines for decades. Gallistel¹ first proposed the existence of a common mental currency to process different magnitudes (i.e., dimensions that can be perceived as *smaller* or *larger*). These magnitudes are either continuous and uncountable, such as time, space, length, or luminance, or discrete and countable, as number.² This hypothesis was then expanded into a broader theory (a theory of magnitude, ATOM³), suggesting the presence of a mechanism that would allow a symmetrical interaction between stimulus dimensions.⁴ Several studies in both humans⁵ and other vertebrate species^{6–10} support this hypothesis, providing evidence for this mechanism being widespread in the vertebrate subphylum.

Honeybees (*Apis mellifera*) are an excellent model for studying the presence of a similar magnitude encoding system in invertebrates. Previous research has demonstrated the ability of honeybees to process different dimensions separately. For instance, bees can process the number of stimuli, and successfully discriminate between sets of numerically different visual elements.^{11,12} Moreover, honeybees perform arithmetic operations (i.e., addition and subtraction)¹³ and consider an empty set as a conceptualization of zero numerosity.¹⁴ Finally, they proved able to discriminate the relative size of visual stimuli and generalize such learning to novel shapes.^{15,16}

We recently showed that bees could perform a transfer from the numerical to the size dimension of a stimulus, suggesting the presence of a cross-dimensional transfer in an insect species.¹⁷ However, whether the transfer between number and size would be symmetrical was not investigated. We hypothesized that if the coding of different dimensions, such as size and numerosity, originates from a common metric in the honeybee brain, we should observe a cross-dimensional transfer from continuous to discrete magnitudes as well, suggesting a bidirectional relationship between those dimensions.

Here, we aimed to investigate the ability of bees to transfer from a continuous (i.e., size) to a discrete (i.e., number) dimension. Specifically, bees were trained to associate an array with either three larger or three smaller visual stimuli with a reward, presented in a Y-maze (Figure 1). The relative size dimension of the stimuli changed during the training, albeit maintaining the same 0.5 ratio difference. At test, bees were presented with numerical comparisons involving *small* and *large numbers* of visual stimuli controlled for continuous variables that covary with numerosity. A generalization test for size discrimination ability to transfer over a different shape was also performed.

RESULTS

During the training phase, bees had to learn to approach the relatively larger ($N = 10$) or smaller ($N = 10$) elements in order to get a food reward. Honeybees trained to select the array containing the relatively larger stimuli reached the 80% accuracy criterion in 25.6 ± 4.18 (mean \pm SEM) trials, whereas bees trained to choose the relatively smaller stimuli reached the 80% accuracy criterion in 38.8 ± 6.40 (mean \pm SEM) trials. An analysis of variance revealed no significant heterogeneity associated with the group (larger vs. smaller trained: $F_{(1, 72)} = 0.17$, $p = 0.679$), the type of shape presented at training (diamond vs. square: $F_{(1, 72)} = 0.27$, $p = 0.605$), the position of the correct

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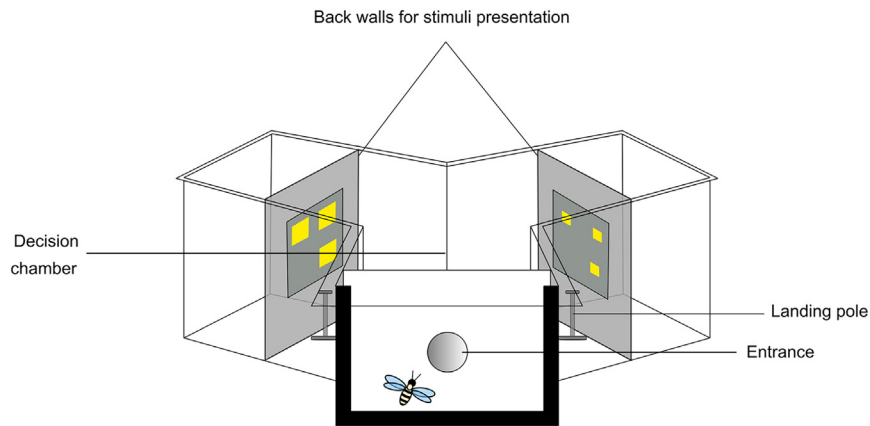


Figure 1. Schematic representation of the Y-maze

stimulus (right vs. left: $F_{(1, 72)} = 1.26$, $p = 0.266$), and their interactions (shape x position: $F_{(1, 72)} = 0.19$, $p = 0.666$; shape x group: $F_{(1, 72)} = 2.1$, $p = 0.152$; position x group: $F_{(1, 72)} = 0.21$, $p = 0.648$; shape x position x group: $F_{(1, 72)} = 0.39$, $p = 0.533$).

During the following test phase, we investigated the ability of bees to generalize their response toward smaller and larger elements having a novel shape (i.e., circle; *size generalization test*). Moreover, in order to test honeybees' ability to make a cross-dimensional transfer from the size to the numerical dimension, we presented three versions of a 4 vs. 8 numerical comparison separately controlled for various perceptual variables usually covarying with visual non-verbal numerosity, such as total area, total contour length, convex hull, and element size (i.e., *number total area test*, *number total perimeter test*, *number same size test*; renamed also *number tests*). For the analysis, we first computed the proportion of choices for the correct relative size at the *size generalization test* (i.e., larger/smaller elements for bees previously trained to select the larger/smaller elements, respectively) and then for the congruent number at the three *number tests* (i.e., 4 or 8 elements for bees previously trained to choose the smaller/larger elements, respectively).

An analysis of variance revealed no significant differences between groups (larger vs. smaller trained: $F_{(1, 72)} = 0.224$, $p = 0.64$), type of test ($F_{(3, 72)} = 2.44$, $p = 0.072$; [Figure 2](#) for visualization of the performance at *number tests*) and their interaction (group x type of test: $F_{(3, 72)} = 0.52$,

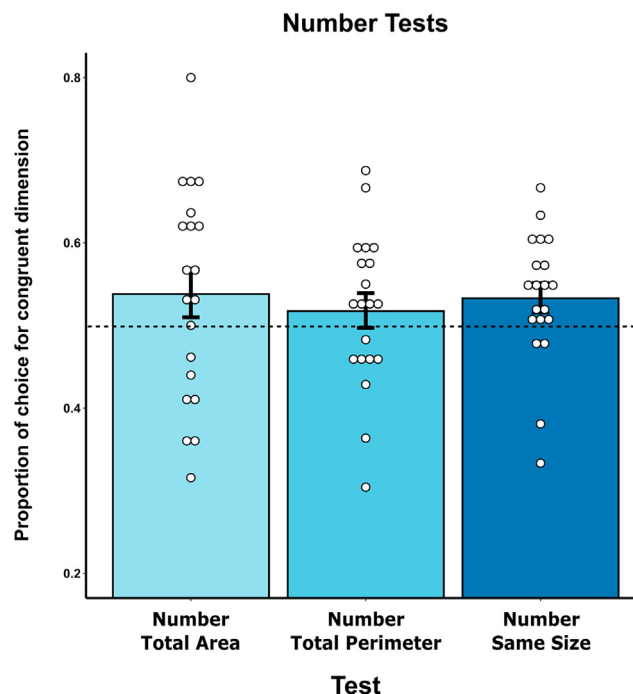


Figure 2. Qualitative overview of the performance at the number tests

Results of the *number tests* (group means with SEM are shown); white dot represents single subject performance; dashed line indicates 50% chance level. No significant effect of the type of test was found (Analysis of variance (ANOVA): type of test ($F_{(3, 72)} = 2.44$, $p = 0.072$); no asterisks indicate no significance).

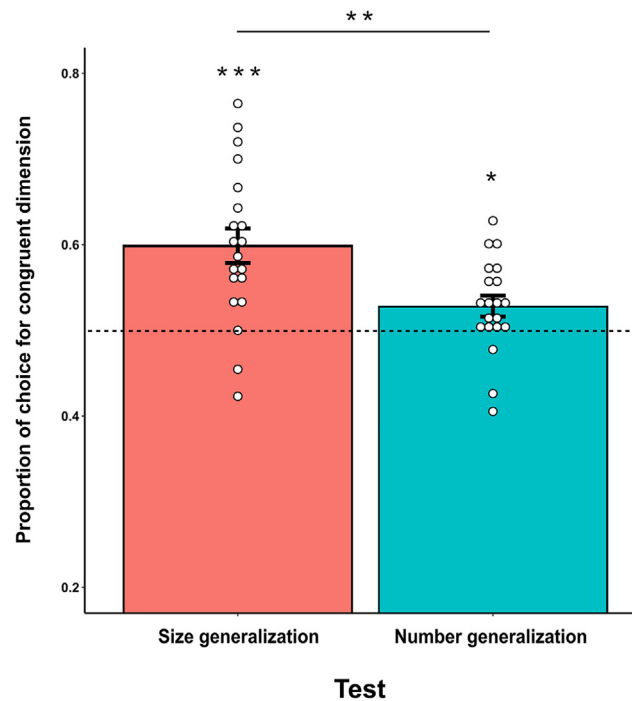


Figure 3. Results of the size and number generalization tests

Results of the *size* and *number generalization* (overall) tests (group means with SEM are shown; white dot represents single subject performance; dashed line indicates 50% chance level). In the *size generalization* test, honeybees chose the larger or smaller relative size according to their previous training (*size generalization* test: $59.89\% \pm 2.01\%$, mean% \pm SEM%; two-tailed one-sample t test: $t_{(19)} = 4.91$, $p < 0.001$). In the *number generalization* test bees chose the congruent stimulus according to their previous training (i.e., 4 elements if they were trained to choose the smaller size stimulus, and 8 elements if they were trained to choose the larger size stimulus during the training) (*number generalization* test: $52.84\% \pm 1.23\%$, mean% \pm SEM%; two-tailed one-sample t test: $t_{(19)} = 2.31$, $p = 0.032$) (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

$p = 0.67$). Since no difference between tests was found, we merged the three *number* tests (i.e., *number total area*, *number total perimeter*, *number same size*; renamed as *number generalization* test) as they all contained the numerical information, allowing us to investigate the ability of bees to make a transfer from a previously trained size dimension to the numerical one. The analysis of variance performed on this dataset revealed a significant effect of the type of test (*size generalization* test vs. *number generalization* test: $F_{(1, 36)} = 8.71$, $p = 0.0055$). Again, no significant effect of group (larger vs. smaller trained: $F_{(1, 36)} = 0.07$, $p = 0.794$) and interaction between the experimental group and type of test was found ($F_{(1, 36)} = 0.96$, $p = 0.333$).

Honeybees presented with a size comparison of elements having a novel circular shape successfully choose the larger or smaller relative size according to their previous training (*size generalization* test: $59.89\% \pm 2.01\%$, mean% \pm SEM%; two-tailed one-sample t test: $t_{(19)} = 4.91$, $p < 0.001$; Figure 3). Similarly, in the *number generalization* test, bees selected significantly the congruent stimulus according to their previous training (i.e., 4 elements if they were trained to choose the smaller size stimulus, or 8 elements if they were trained to choose the larger size stimulus during the training; $52.84\% \pm 1.23\%$, mean% \pm SEM%; two-tailed one-sample t test: $t_{(19)} = 2.31$, $p = 0.032$; Figure 3).

Spatial frequency (i.e., number of light/dark cycles or oscillations of a pattern occurring per unit of distance in a given space) was proposed to play a role in visual discrimination of honeybees.^{18,19} Thus, we analyzed whether spatial frequency could have been used as a perceptual feature to solve our cross-transfer task.

In our experiment, spatial frequency covaried with the size of elements, as the arrays containing larger stimuli also had averaged higher spatial frequency both in stimulus presented during the training (*smaller* size total power = 28878.9 ± 70 , mean \pm SEM; *larger* size total power = 31594.8 ± 296.8 , mean \pm SEM; Wilcoxon rank-sum test: total power of *smaller* vs. *larger* size: $W = 256$, p value < 0.001 ; Figure 4) and the *size generalization* test (*smaller* size total power = 27725.4 ; *larger* size total power = 29760.3 ; Figure 4), and with numerosity only when the stimulus size was controlled (i.e., *number same size* test; 4 elements total power = 27954.02 ; 8 elements total power = 28851.5 ; Figure 4). Conversely, when the total area was equated between arrays (i.e., *number total area* test), spatial frequency was almost identical between arrays (4 elements total power = 29716.9 ; 8 elements total power = 29715.0 ; Figure 4). In the *number total perimeter* test, spatial frequency was inversely related with numerosity, as the higher total power value was associated with the smaller numerosity (4 elements total power = 33944.7 ; 8 elements total power = 31020.3 ; Figure 4). The performance that we observed in our experiment is thus not consistent with a spatial frequency-based strategy, suggesting a cross-dimensional transfer from the size to the numerical dimension not supported by low-level perceptual cues.

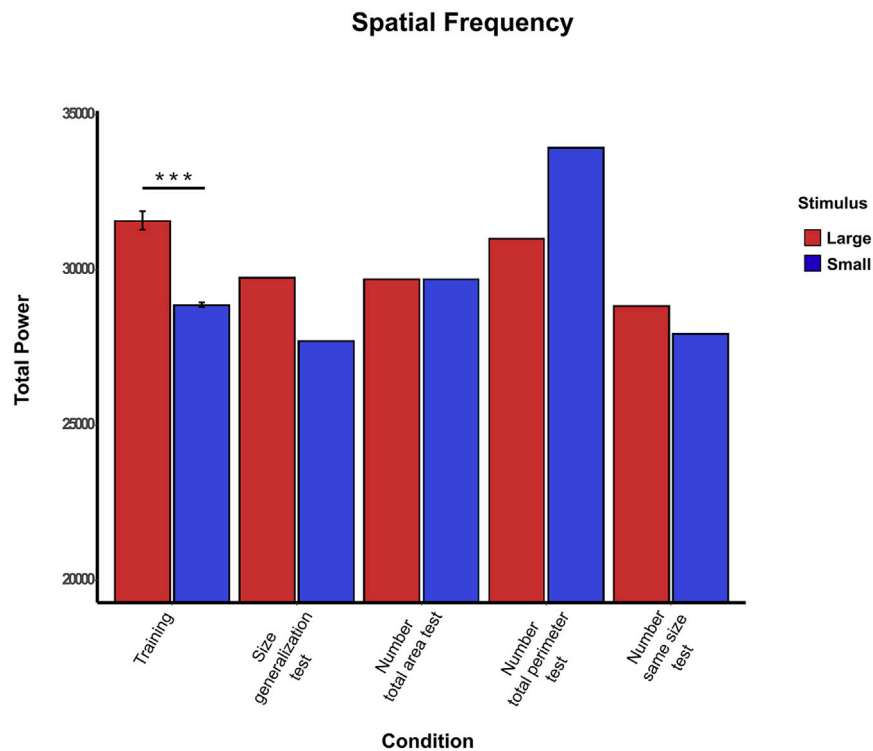


Figure 4. Spatial frequency analysis of the training and test stimuli

Spatial frequency of the stimuli presented during the training and the test phase. Spatial frequency varied directly along the stimulus dimension (i.e., either size or numerosity) during training (smaller size total power = $28878,9 \pm 70$, mean \pm SEM; larger size total power = $31594,8 \pm 296,8$, mean \pm SEM; Wilcoxon rank-sum test: $W = 256$, p value < 0.001), the size generalization test (smaller size total power = $27725,4$; larger size total power = $29760,3$; Figure 4), and when the stimulus size was controlled (i.e., number same size test; 4 elements total power = $27954,02$; 8 elements total power = $28851,5$; Figure 4). Spatial frequency was similar when the total area was equated between arrays (i.e., number total area test; 4 elements total power = $29715,0$; 8 elements total power = $29716,9$), while it was inversely related with numerosity when the total contour length was controlled (4 elements total power = $33944,7$; 8 elements total power = $31020,3$) (** $p < 0.001$).

DISCUSSION

The results of the size generalization test confirmed that bees can discriminate between different relative sizes¹⁶ and can generalize such learning to novel shapes.¹⁵

The results of the number tests (i.e., number total area, number total perimeter, and number same size test) revealed an overall spontaneous preference for congruent numerosity. Bees showed a lower tendency to choose the congruent numerosity in comparison to choosing the correct size, highlighting that transferring the learned “choosing the smaller/larger dimension” rule from a continuous to a discrete dimension could be a more difficult task than transferring within a single dimension. Nonetheless, bees appear well equipped with the capacity to make a transfer from a continuous (size) to a discrete (number) dimension.

Despite the lack of any significant difference among the three number tests, a qualitative analysis of the data may suggest that the best performances were observed in the number total area and number same size tests (Figure 2). It could be that honeybees mainly used contour length as the primary perceptual feature in the size discrimination during training. This could have favored the choice toward the congruent numerosities when the total area and the stimuli size were controlled, since here contour length varied in a congruent way with numerosity (i.e., a larger numerosity also has a larger overall perimeter, and vice versa). The use of contour length as a primary cue could also explain the results of the only study that showed evidence of transfer from length to number in non-human vertebrates (angelfish¹⁰). Fish trained to select the shorter line (with the smaller perimeter) chose the smaller numerosity in a 10 vs. 20 elements comparison whose area was matched between arrays. The smaller quantity contained a smaller amount of contour length: thus, angelfish could have relied on this cue to solve the task.

However, although contour length has been argued to be used during numerosity discrimination in human infants,²⁰ recent studies demonstrated that humans and non-human species, including honeybees, can perform numerosity discrimination when the perimeter is equated between sets of stimuli (human infants;^{5,21} archerfish;²² chicks;^{23–25} honeybees;¹²). We believe therefore that the slight tendency for a worse performance in the number total perimeter test could be explained in another way. The geometrical constraints associated with this condition led to the creation of the numerically smaller array with three larger elements and one smaller element, and the numerically larger array with only one larger element and seven smaller ones (Figure S3). These configurations could have been perceived by the bees as

more similar to the size comparison experienced during the training. Thus, a response toward the more perceptually familiar configuration (i.e., larger elements contained in the numerically smaller group for bees previously trained to select the larger size, and smaller elements contained in the numerically larger group for subjects previously trained to choose the smaller size) could explain the tendency.

Our data potentially show the presence of individual differences in solving our visual task. The occurrence of different strategies to solve cognitively demanding tasks could indeed benefit the colony as more diversification in individuals' behavior would allow a more efficient resource acquisition.^{26,27} Interestingly, those individual differences are maintained within a sensory modality, despite the changing of the perceptual discrimination or increasing level of abstraction of the task.²⁶ Conversely, the observed individual differences between bee foragers are not conserved between different sensory modalities, as a higher performance in the visual domain does not implicate a subsequent higher performance in an olfactory discrimination task.²⁶

Interactions between different magnitudes, such as space, time, and quantity have been reported in different species (time, space, and quantity:⁵; time and quantity:^{6,7} space and time:^{8,9}; space and quantity:¹⁰); in humans and non-human primates,^{2,28} they seem to be associated with a common activation of the parietal cortex.³ Recent evidence suggested the columnar neurons of *Drosophila melanogaster* lobula as a probable neural substrate for number perception in this insect species.²⁹

There are ecological reasons to posit that time, space, and number³⁰ must be represented by a common magnitude system in organisms as different as vertebrates and invertebrates. As stressed by Gallistel,¹ discrete quantity (i.e., numerosness) and continuous quantity (i.e., space or time) must be represented by some sort of common mental currency, which he refers to as "magnitude", to enable biological organisms to perform arithmetic operations across domains. For instance, when a bee calculates the rate of return to a flower, it has to compute number and time in a single currency. In foraging situations, it has been hypothesized that bees use a strategy involving the computation of both the rate of return and the encounter of rewarding flowers to maximize their nectar collection rate.²⁷ Our previous work in honeybees demonstrated the existence of a cross-dimensional transfer from the discrete (number) to a continuous (size) dimension.¹⁷ The present results demonstrated that bees could make a transfer from the size to the numerical dimension of visual stimuli as well, suggesting a symmetrical and bidirectional interaction between those dimensions. This strongly advocates for the presence of a universal magnitude processing mechanism in invertebrate species comparable to vertebrate species.

Limitations of the study

This study presents an investigation into the ability of bees to make a transfer from the size to the numerical dimension in honeybees. However, further studies should investigate the presence of other bidirectional relationships between magnitudes to further support the existence of a general magnitude mechanism in invertebrate species. Moreover, the data presented in this study do not completely rule out the possibility that bees could have relied on perceptual variables (e.g., perimeter) to solve the transfer task.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.108035>.

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AUTHOR CONTRIBUTIONS

M.B. and G.V. designed the research; M.B. conducted the experiments; M.B. analyzed the data and produced the figures; M.B. wrote the original draft of the manuscript; M.B. and G.V. revised and edited the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Raw data	This study	https://doi.org/10.17632/zyjjngtdwv.1
Data analysis code	This study	https://doi.org/10.17632/zyjjngtdwv.1
Experimental models: Organisms/strains		
European honeybee foragers (<i>Apis mellifera</i>)	Colonies managed by Sperimentarea, Civic Museum of Rovereto, Italy	N/A
Software and algorithms		
R-Studio (R, version 4.1.3)	www.r-project.org/	N/A

RESOURCE AVAILABILITY

Lead contact

Additional information and requests for resources should be directed to and will be fulfilled by the lead contact: Maria Bortot, maria.bortot@unitn.it.

Materials availability

This study did not generate new materials.

Data and code availability

- Raw data have been deposited at Mendeley Data and are publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- All original code has been deposited at Mendeley Data and is publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

A total of 28 naïve free-flying honeybee foragers (*Apis mellifera*) were involved in this study. We discarded 8 subjects for inability to complete the training. The remaining 20 bees were analyzed, and the results are reported in this study. Honeybees were randomly allocated to one of the two experimental groups.

METHOD DETAILS

The experiment was performed from June to October 2023 at SperimentArea, a field station run by the local Natural History Museum, in Rovereto (North of Italy).

Subjects

Free-flying honeybee foragers (*Apis mellifera*; N = 20) were individually trained to enter a wooden Y-maze located in a wooden house in the park. The entrance of the maze was connected to a single window that allowed the control of the entrance of single subjects. The room was illuminated by natural light and by two white light stripes attached to the ceiling (Ledpoint light solution, Honglitrionic, 1340 lumen/meter, CCT 2900-3000K white). The artificial lights were positioned above each arm of the maze. Half of the bees (N = 10) were trained to associate the larger elements with an appetitive reward, and the other half (N = 10) with the smaller elements. Honeybees were marked to allow individual recognition and at the end of the experiment, they were marked with a red dot on the thorax to prevent double testing and then released.

Apparatus and stimuli

The apparatus was a wooden Y-maze (¹⁷; [Figure 1](#)). We used landing poles to provide the reinforcement and the punishment to the bees. They consisted of grey poles with an attached open, transparent capsule where a drop of solution was placed. In this way, we prevented bees could discriminate between the positive (i.e., sucrose) and negative (i.e., quinine) solutions by directly touching them with their antennae since bees

were forced to land on the pole to be able to taste the solution. Capsules were changed between individuals and cleaned with 30% ethanol at every trial.

All stimuli consisted of yellow elements (RGB: 255, 237, 0;¹⁵) on a grey background (10 × 10 cm; RGB: 144, 150, 146;¹⁵) shown at a 10 cm distance from the decision chamber of the maze. Stimuli could have either a square, diamond, or circular shape (Figures S1–S3). The first two shapes were used for training purposes, whereas the third one was only presented during the test phase. The training stimuli consisted of comparisons between three larger and three smaller elements having a 0.5 ratio difference. Using GeNEsIS software,³¹ we created 16 pairs of stimuli (i.e., N = 8 squares, N = 8 diamond) having four different side dimensions (i.e., 1 vs. 2 cm; 1.15 vs. 2.3 cm; 1.35 vs. 2.7 cm; 1.5 vs. 3 cm). Stimuli were controlled for element disposition, that could be arranged in a line (N = 7) or a triangular (N = 9) configuration. The inter-distance between elements (i.e., the distance computed from the center of the elements) was controlled so that larger and smaller elements of specific comparisons had the same position in the array (Figures S1 and S2). During the training, each stimulus was rotated by 90 degrees in the following trials to prevent bees to learn a particular spatial configuration.

The *size generalization* test was composed of a comparison between three large and three small elements having a novel shape (i.e., circular) and a novel relative size (i.e., 1.25 vs. 2.5 cm). This test aimed to confirm the learning of the task (i.e., choose the smaller/larger size) irrespective of the element shape and in the absence of any reward (Figure S3).

During the *numerical generalization* test, a 4 vs. 8 elements comparison was presented under different stimuli control conditions. We maintained the magnitude ratio difference experienced in the training phase (i.e., 0.5). To check for the role of perceptual variables covarying with numerosity, we created a total of three couples of stimuli separately controlled for total perimeter, total area, and element size. In all three pairs of stimuli, the convex hull was controlled for. Moreover, the smaller and larger elements were identically present in both numerical arrays (Figure S3). These tests aimed to investigate whether bees were able to make a transfer from the size to the numerical dimension irrespective of the availability of perceptual features, such as area, contour length, or element dimensions.

Training procedure

The experimental procedure comprised habituation, training, and test phases. All phases could be completed in 1 or 2 consecutive days. During the habituation phase, each bee was collected from a gravity feeder placed in the apiary and trained to gradually fly inside the apparatus to collect food by landing on the two landing poles placed in both arms, in the absence of visual stimuli. Each subject was individually marked to allow bee recognition. In the training phase, a comparison between relatively larger and smaller elements was presented. Half of the subjects learned to associate the relatively larger stimuli with positive reinforcement, whereas the other half of the bees learned to choose the relatively smaller stimuli in the comparison. During this phase, an appetitive-aversive conditioning paradigm was used where the correct size was always associated with food (i.e., sucrose solution 50% w/w), whereas the incorrect size was always associated with punishment (i.e., 60 mM quinine solution; see³²). Each bee had to reach a criterion of $\geq 80\%$ accuracy over 10 consecutive trials. The stimuli were presented in a pseudo-random sequence (i.e., the correct stimulus was never presented more than two consecutive times on the same side). For the overall duration of this phase, the position of the experimenter was always at the end of the Y-maze, in a symmetrical position with respect to both arms to avoid any side cue.

Test procedure

Once reaching the accuracy criterion, honeybees entered the test phase where four tests were given (i.e., *size generalization* test, *number total area* test, *number total perimeter* test, *number same size* test). Each test was presented twice to counterbalance the position of the correct array and avoid side preferences. The tests lasted one minute during which the behavior of the experimental bee was video recorded (LifeCam Studio, 30 fps). During the test phase, the experimenter moved away from the apparatus and was thus not visible to the bee to avoid any side bias.

The videos were analyzed by the researcher in blind condition (i.e., no information about the specific training undergone by the subject was provided in the videos that were analyzed six months after the conclusion of the experiment; 0.33x velocity). The number of choices computed by the subjects (i.e., landing behavior or direct contact made with antennae or legs, on one of the two capsules on the landing poles places in front of each stimulus) was scored. All tests were conducted in probe conditions (i.e., in the absence of reward and punishment), and a drop of water was placed in the two capsules positioned in front of each stimulus.

The percentage of choices for the correct size and congruent numerosity (i.e., congruent respect to the specific training: 8 elements for bees trained to choose the larger elements at training, 4 elements for bees previously trained to select the smaller elements at training) was calculated for each subject and analyzed, giving rise one single value per bee to exclude pseudo-replication.

QUANTIFICATION AND STATISTICAL ANALYSIS

The training data were checked for normality (Shapiro-Wilk normality test: $W = 0.97$, $p > 0.05$) and homoscedasticity (Levene's test: $p > 0.05$) and then analyzed with parametric statistical tests. An analysis of variance was performed with group (larger vs. smaller size as positive), type of stimulus (square and diamond), and position of the reinforced stimulus (left vs. right) as factors.

We performed an offline blind scoring of the videos of the test phase, and we calculated the proportion of choices for the correct stimulus size during the *size generalization* test and the proportion of congruent numerosity during the *number total area* test, the *number total perimeter* test and the *number same size* test. The data were checked for normality (Shapiro-Wilk normality test: $W = 0.97$, $p > 0.05$) and

homoscedasticity (Levene's test: $p > 0.05$) and analyzed with parametric statistical tests. An analysis of variance was conducted with group (larger vs. smaller size as positive) and type of test (*size generalization*, *number total area*, *number total perimeter*, *number same size*). The proportion of choices for the correct and congruent stimuli was analyzed with a two-tailed one-sample t-test (Figure 3; see also the Results section).

We calculated the spatial frequency of our images to estimate the contribution of this parameter to the behavioral outcome. Spatial frequency was computed as the fast Fourier transformation of images, followed by the calculation of the radial average of the signal amplitude in the frequency domain and the final sum of the frequency contributions of its power spectrum, as reported in a previous study.²² All the frequency analyses were performed with a custom MATLAB script of the GeNEsIS program;³¹ GitHub platform: <https://github.com/MirkoZanon/GeNEsIS>. Significant differences between the spatial frequency of training stimuli were analyzed with a non-parametric Wilcoxon rank sum test (Figure 4; see also the Results section). In all the analyses, an α -value of 0.05 was specified. All the analyses were conducted with R-Studio (R, 4.1.3 version).