

## EVOLUTIONARY COGNITION

# Numerical ordering of zero in honey bees

Scarlett R. Howard<sup>1</sup>, Aurore Avarguès-Weber<sup>2\*</sup>, Jair E. Garcia<sup>1\*</sup>,  
Andrew D. Greentree<sup>3</sup>, Adrian G. Dyer<sup>1,4†</sup>

Some vertebrates demonstrate complex numerosity concepts—including addition, sequential ordering of numbers, or even the concept of zero—but whether an insect can develop an understanding for such concepts remains unknown. We trained individual honey bees to the numerical concepts of “greater than” or “less than” using stimuli containing one to six elemental features. Bees could subsequently extrapolate the concept of less than to order zero numerosity at the lower end of the numerical continuum. Bees demonstrated an understanding that parallels animals such as the African grey parrot, nonhuman primates, and even preschool children.

**F**our stages are used to describe the acquisition of understanding zero in human history, psychology, animal cognition, and neurophysiology (1). The first is the ability to define zero as nothing—the absence of a stimulus. The second is the categorical classification of zero as “nothing” versus “something.” The third stage is understanding zero as a quantity at the low end of the positive integer numerical continuum. The fourth, and currently designated as the most advanced stage of understanding zero, is the symbolic representation of zero, as with an Arabic number and as used in modern mathematics and calculations (2).

Several ancient human civilizations lacked the full understanding and importance of zero, leading to constraints in their numeric systems (3). Interestingly, some vertebrate animals have recently demonstrated a capacity to acquire and understand this numerical concept. Rhesus monkeys learned that empty sets of objects occupy a position on a numerical continuum (2, 3), vervet monkeys used subtraction-like reasoning to determine if food was present or absent (4), a chimpanzee reached near-perfect performance on zero-concept tasks with training (5), and an African grey parrot spontaneously labeled absent objects as “none” (6).

Honey bees have previously demonstrated the capacity to count and discriminate up to four objects (7–10) in experiments that use classic conditioning techniques. Recent advancements in conditioning protocols (11) reveal that bees can acquire rule-based relational concepts (12, 13), thus enabling remarkable plasticity to acquire

and apply seemingly advanced concepts such as size ordering (14). In this study, we tested the capacity of honey bees to extrapolate the acquired concepts of “greater than” and “less than,” as shown in primates (15, 16), and thus formally demonstrate that an invertebrate can understand the concept of zero numerosity.

We designed a set of experiments to test the extent to which honey bees may understand the concept of zero numerosity (17). In the first experiment, we trained bees to understand the concepts of less than and greater than using appetitive-aversive differential conditioning (11). Bees were trained to the respective concepts using white square stimuli containing one to four black elements (Fig. 1A, fig. S1, and table S1). After reaching a criterion of  $\geq 80\%$  accuracy, bees demonstrated in nonreinforced tests that they had learned the concept of “numerically less” [ $75.0 \pm 4.1\%$  (mean  $\pm$  SEM); logistic regression with individual as random term tested differences between observed proportion of bee choices and chance level,  $y = 0.5$ ,  $z$  score = 5.08,  $P < 0.001$ ,  $n = 10$ ] and “numerically greater” ( $75.5 \pm 3.3\%$ ;  $z$  score = 6.556,  $P < 0.001$ ,  $n = 10$ ) when presented with novel stimuli of one to four elements. Furthermore, bees were able to apply these concepts to determine that five elements were greater than two or three elements (less-than group:  $68.0 \pm 5.0\%$ ,  $z$  score = 3.411,  $P < 0.001$ ,  $n = 10$ ; greater-than group:  $75.0 \pm 3.9\%$ ,  $z$  score = 5.333,  $P < 0.001$ ,  $n = 10$ ). Interestingly, bees demonstrated an understanding that zero numerosity lies at the lower end of the numerical continuum by choosing an “empty set” stimulus containing no elements if trained to less than ( $64.0 \pm 5.4\%$ ;  $z$  score = 2.795,  $P = 0.005$ ,  $n = 10$ ; Fig. 1C) or by choosing unfamiliar stimuli containing elements if trained to greater than ( $74.5 \pm 2.6\%$ ;  $z$  score = 6.609,  $P < 0.001$ ,  $n = 10$ ; Fig. 1C).

In the second experiment, we tested the extent to which bees may understand the quantitative concept of zero in comparison with other animals. As some animals find it challenging to differentiate between the numbers zero and one

(5, 6, 18), we trained bees to less than using stimuli containing two to five elements and then tested their ability to differentiate between the unfamiliar numerosities of one and zero (Fig. 1B). After reaching a criterion of  $\geq 80\%$  accuracy, bees demonstrated the learned concept of numerically less when presented with the numbers two to five ( $73.8 \pm 1.9\%$ ;  $z$  score = 10.18,  $P < 0.001$ ). When presented with the unfamiliar numbers of one versus zero, bees chose the lower number of zero ( $63.0 \pm 2.9\%$ ;  $z$  score = 4.23,  $P < 0.001$ ; Fig. 1D), showing an understanding that an empty set is lower than one, which is challenging for some other animals (5, 6, 18).

When bees were presented with two conflicting pieces of information, two versus zero, where the two-element stimuli had always been rewarded in training and zero was the correct lower number, bees chose the empty set at a frequency level that was not significant from chance ( $56.2 \pm 3.4\%$ ;  $z$  score = 1.64,  $P = 0.101$ ; Fig. 1D); thus, bees perceived both plausible alternatives as consistent with their conditioning experience. These results demonstrate that bees were using both an associative mechanism for choosing two elements and a concept-based mechanism for choosing zero numerosity. This phenomenon was also observed in a dolphin trained to choose the numerically less option by using white dots on a black background. This result is explained in terms of an artifact of training-set conditioning causing a bias toward consistently rewarding stimuli (19).

To test if bees understood an empty set quantitatively along the numerical continuum, we evaluated numerical-distance effects, where accuracy of performance potentially improves as the difference in magnitude between two respective numbers increases (1). In the third experiment, we trained and tested bees on the less-than concept using the numbers zero to six. If bees considered zero numerosity as a number along the numerical continuum, we would expect accuracy of decisions to be the greatest with zero versus six and poorer for lower numbers versus zero numerosity (Fig. 2). After reaching a criterion of  $\geq 80\%$  accuracy during training, bees demonstrated in tests that they could discriminate an empty set from numbers one to six accurately (supplementary text S1 and Fig. 2B). Although bees could accurately discriminate all numbers from zero numerosity, there was a significant effect of numerical distance on accuracy (Fig. 2B). Bees were more accurate when numbers were numerically more distant (zero versus five and zero versus six) than when numerically closer (zero versus one), showing that bees are affected by number magnitude and thus exhibit numerical-distance effects.

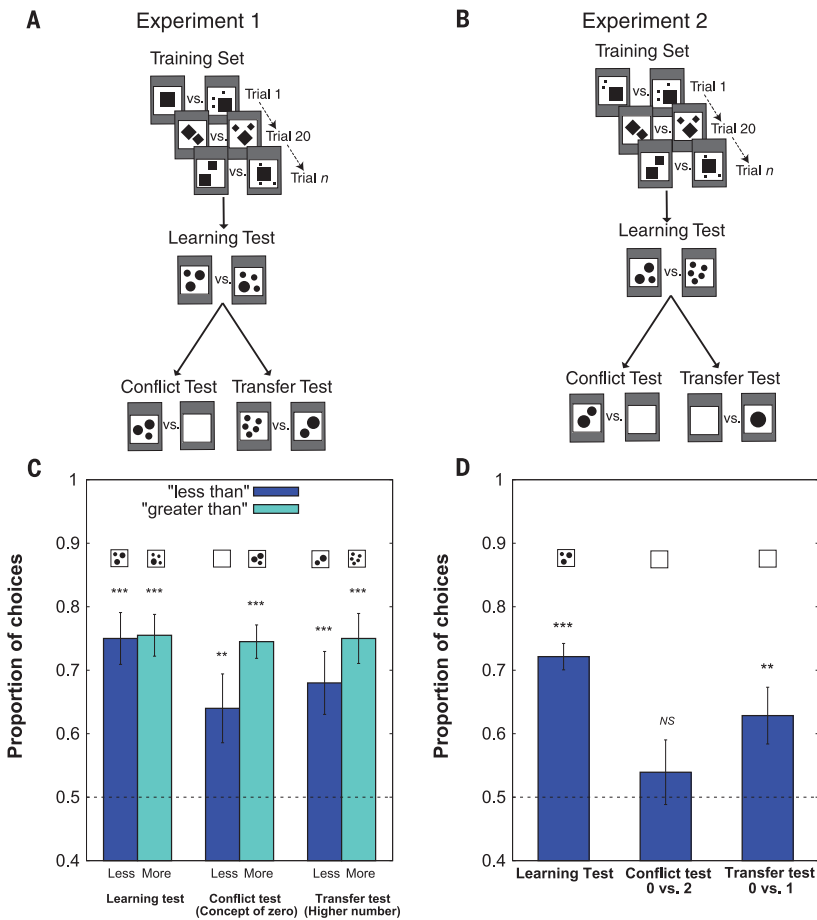
An alternative explanation for our results could be that bees have a preference for the unfamiliar presentation of an empty-set stimulus. However, control experiments showed that the bees’ understanding that zero belongs at the lower end of the numerical continuum was rule based and not driven by an unfamiliar preference (supplementary text S2 and fig. S2). The spatial

<sup>1</sup>Bio-inspired Digital Sensing (BIDS) Lab, School of Media and Communication, RMIT University, Melbourne, VIC, Australia.

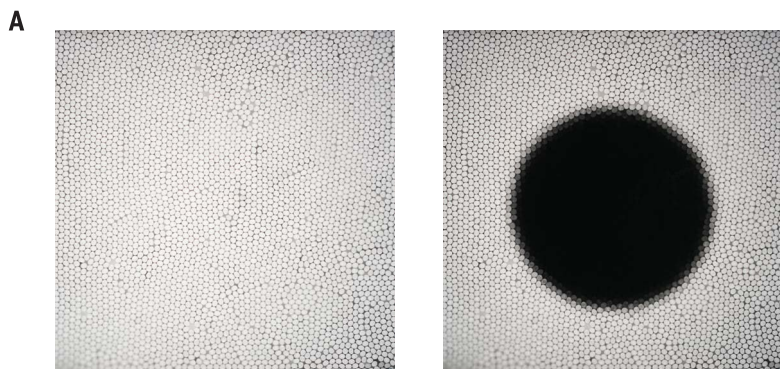
<sup>2</sup>Centre de Recherches sur la Cognition Animale, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, Toulouse, France. <sup>3</sup>ARC Centre of Excellence for Nanoscale BioPhotonics, School of Science, RMIT University, Melbourne, VIC, Australia. <sup>4</sup>Department of Physiology, Monash University, Clayton, VIC, Australia.

\*These authors contributed equally to this work.

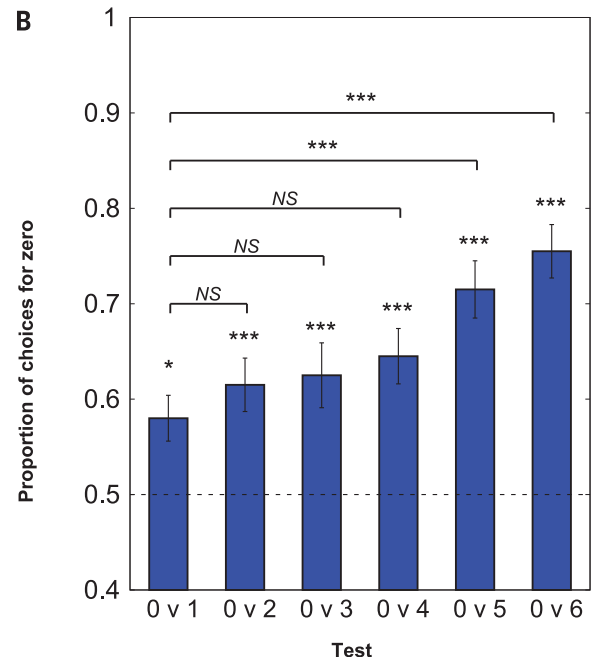
†Corresponding author. Email: adrian.dyer@rmit.edu.au



**Fig. 1. Graphic representation of the method and results of experiments 1 and 2.** (A and B) Examples of possible stimuli combinations during trials and tests in experiments 1 and 2. (C and D) Performance during the unreinforced testing phases during experiments 1 and 2. Data shown are means  $\pm$  SEM for both treatment groups. Bees trained to less than are shown in dark blue; bees trained to greater than are shown in turquoise. Stimuli above the columns represent the choices for those stimuli in the data. In experiment 1, in the conflict test evaluating the bees' concept of zero, data shown for the less-than group ( $n = 10$ ) are choices for zero, and data shown for the greater-than group ( $n = 10$ ) are choices for stimuli containing elements. In the transfer test to a higher number, data shown for bees trained to less than are choices for a lower number, and data shown for bees trained to greater than are choices for the higher number of five. In experiment 2 ( $n = 25$ ), the conflict and transfer tests show the bees' choices for zero. Dashed black line at 0.5 indicates chance-level performance. Significance from chance-level performance is indicated by \*\*  $P \geq 0.01$  and \*\*\*  $P \geq 0.001$ . NS, not significant.



**Fig. 2. Photographic representation of stimuli and results from experiment 3.** (A) Representation of honey bee spatial vision when viewing stimuli of either zero or one (22). (B) Honey bee performance during experiment 3, testing the behavioral effects of numerical distance of numerosity zero. Data shown are means  $\pm$  SEM for the choice of the zero stimuli. Dashed black line at 0.5 indicates chance-level performance. Significance from chance-level performance and from other tests is indicated by \* $P \geq 0.05$  and \*\*\* $P \geq 0.001$ . NS, not significant.



frequencies of stimuli are also ruled out as a potential explanation for results (supplementary text S1 and table S1). We additionally conducted further control experiments to exclude the possibility that bees learn to match pairs of num-

bers during training (supplementary text S3 and fig. S2).

Our findings show that honey bees can learn and apply the concepts of greater than and less than to interpret a blank stimulus as represent-

ing the conceptual number of zero and place zero in relation to other numerical values. Bees thus perform at a level consistent with that of non-human primates by understanding that zero is lower than one (5).

An open question remains as to whether such advanced numerical understandings may be widespread across many animals that deal with complexity in their environments or if our findings are the result of independent evolution in honey bees. Recent comparative studies of primate and crow brains found that similar levels of numeric processing are facilitated by very different brain structures, suggesting independent evolution of numeric processing (20, 21). Because it can be demonstrated that an insect, with a different brain structure from primates and birds, can understand the concept of zero, it would be of high value to consider such capacities in other animals.

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#### SUPPLEMENTARY MATERIALS

[www.sciencemag.org/content/360/6393/1124/suppl/DC1](http://www.sciencemag.org/content/360/6393/1124/suppl/DC1)  
 Materials and Methods  
 Supplementary Text  
 Figs. S1 to S4  
 Table S1  
 References (23–26)  
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### Understanding zero

It has been said that the development of an understanding of zero by society initiated a major intellectual advance in humans, and we have been thought to be unique in this understanding. Although recent research has shown that some other vertebrates understand the concept of the "empty set," Howard *et al.* now show that an understanding of this concept is present in untrained honey bees (see the Perspective by Nieder). This finding suggests that such an understanding has evolved independently in distantly related species that deal with complexity in their environments, and that it may be more widespread than previously appreciated.

*Science*, this issue p. 1124; see also p. 1069

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