Mars (*14*), but those measurements were hampered by the presence of perchlorate salts. These salts, present in martian regolith, break down upon heating within the SAM instruments to temperatures of 200°C. The oxygen and chlorine hereby released react with organic molecules. Leakage of reactive agents presented another challenge. Eigenbrode *et al.* overcame both challenges by only analyzing the gases released above 400°C. They can be certain that these gases are not a result of leaking reagent or reaction with perchlorate. The authors meticulously show all data obtained on Mars by the SAM instrument since its first measurements in 2013 and have thoroughly analyzed all potential contaminants and other signals that might have influenced the actual measurements. They thereby carefully avoid any bias toward hypotheses developed over the past decades. The results convincingly show the long-awaited detection of organic compounds on Mars.

As Webster *et al.* show, methane has also been conclusively detected in the martian atmosphere (*3*). During 5 years of analysis, SAM has found not only a stable methane background, but also local seasonal peaks. It may be that the gas is released from a large subsurface reservoir, but neither the source of that methane nor the driving force of its release is understood. Although many geological processes produce methane, its possible link with biological processes warrants further study to fully understand the martian methane cycle.

The detection of organic molecules and methane on Mars has far-ranging implications in light of potential past life on Mars. Curiosity has shown that Gale crater was habitable around 3.5 billion years ago (*15*), with conditions comparable to those on the early Earth, where life evolved around that time. The question of whether life might have originated or existed on Mars is a lot more opportune now that we know that organic molecules were present on its surface at that time. ■

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ORGANISMAL BIOLOGY

Honey bees zero in on the empty set

Honey bees join a select number of animals shown to understand the concept of nothing

By **Andreas Nieder**

The number zero is central to contemporary mathematics and to our scientifically and technologically advanced culture (*1*). Yet, it is a difficult number to understand. Children grasp the symbolic number zero long after they start to understand, at around the age of 4 years, that "nothing" can be a numerical quantity—the empty set—that is smaller than one (*2*). Scientists therefore assumed that the concept of "nothing" as a numerical quantity was beyond the reach of any animal. Recent studies on cognitively advanced vertebrates challenge this view, however. Monkeys and birds can not only distinguish numerical quantities (*3*) but also grasp the empty set as the smallest quantity on the mental number line (*4*, *5*). On page 1124 of this issue, Howard *et al.* (*6*) show that the honey bee, a small insect on a branch very remote from humans on the animal tree of life, also belongs to the elite club of animals that comprehend the empty set as the conceptual precursor of the number zero.

Honey bees have a reputation as smart insects. They possess elaborate short-term memory to consider upcoming decisions (*7*), understand abstract concepts such as sameness and difference (*8*), and learn intricate skills from other bees (*9*). Bees can also estimate the number of up to four objects (*10*, *11*). But Howard *et al.* demonstrate even more astonishing number skills in these insects. The researchers report that honey bees can not only rank numerical quantities according to the rules "greater than" and "less than" but they can also extrapolate the less-than rule to place empty sets next to the number one at the lower end of the mental number line.

For their experiments, the authors lured free-flying honey bees from maintained hives to their testing apparatus (see the figure) and marked the insects with color for identification. They rewarded the bees for discriminating displays on a screen that showed different numbers (numerosities) of items. The researchers controlled for systematic changes in the appearance of the numerosity displays that occur when the number of items is changed. They thus ensured that the bees were discriminating between different numbers, rather than responding to low-level visual cues.

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First, the researchers trained the bees to rank two numerosity displays at a time. Over the course of training, they changed the numbers presented to encourage rule learning. Bees from one group were rewarded with a sugar solution whenever they flew to the display showing more items, thereby following a greater-than rule. The other group of bees was trained on the less-than rule and rewarded for landing at the display that presented fewer items. The bees learned to master this task with displays consisting of one to four items; they were able to do so not only for familiar numerosity displays but also for new displays.

Next, the researchers occasionally inserted displays containing no item. Would the bees understand that empty displays could be ranked with countable numerosities? Indeed, the bees obeying the less-than rule spontaneously landed on displays showing no item, that is, an empty set (see the figure). In doing so, bees understood that the empty set was numerically smaller than sets of one, two, or more items. Further experiments confirmed that this behavior was related to quantity estimation and not a product of the learning history.

The bees' accuracy in performance improved as the magnitude of difference between two respective numerosities increased. They found it hard to judge whether the empty set was smaller than one but were progressively better when they had to compare two, three, or larger numbers with an empty set. With this behavior, the bees demonstrated the numerical-distance effect with empty sets, a hallmark of number discrimination. The series of experiments (*6*) therefore demonstrates that bees grasp the empty set as a quantitative concept.

The findings are all the more exciting when considering the phylogenetic remoteness of insects and vertebrates. Their last common ancestor, a humble creature that barely had a brain at all, lived more than 600 million years ago (*12*), an eternity in evolutionary terms. As they evolved, separating this time, the building plans of vertebrate and insect bodies developed quite differently and independently from one another. This includes their notably different brains. For instance, a bee's brain has fewer than 1 million neurons, compared to 86,000 million neurons that make up a human brain. But such differences do not prevent bees from knowing how to understand numbers, including zero. It constitutes a fascinating case of convergent evolution of numerical competence.

What may have been the selective pressures that gave rise to numerical competence in such diverse and independently evolved animal groups? Studies examining animals in their ecological environments suggest that numerical competence is beneficial for animals by enhancing their ability to reproduce, navigate, exploit food sources, hunt prey, avoid predation, and engage in social interactions (*3*). Numerical competence is of adaptive value because it helps animals survive and pass on genes to the next generation. This can explain why numerical competence is so widespread in the animal kingdom.

The advanced numerical skills of bees and other animals raise the question of how their brains transform "nothing" into an abstract concept of zero. The neurophysiological basis of number competence is still unknown in insects. Studies on nonhuman primates and corvid songbirds, however, show that "number neurons" in associative endbrain areas give rise to numerical cognition (*13*). Such number neurons respond preferentially to a specific number of elements in a set, irrespective of stimulus appearance. The representation of abstract numerical quantities is demanding, but conceiving of "nothing" as a quantity is even more challenging for the brain. After all, brains have evolved to process stimuli, which represent "something." Without light, a visual neuron does not signal optic information; without sound, an auditory neuron carries no acoustic information.

But this is only part of the story, as "nothing" can be informative. A study in trained monkeys showed that, beyond the sensory input at higher processing stages of the brain, cortical neurons actively represent empty sets as conveying a quantitative null value (*14*, *15*). Such neurons could arise from reinforcement learning if "nothing" constitutes a behaviorally relevant category. It stands to reason that such neurons also emerge in children that learn to understand numerical symbols and the number zero. We have only just begun to zoom in on "nothing" as a relevant quantitative concept for the brain. ■

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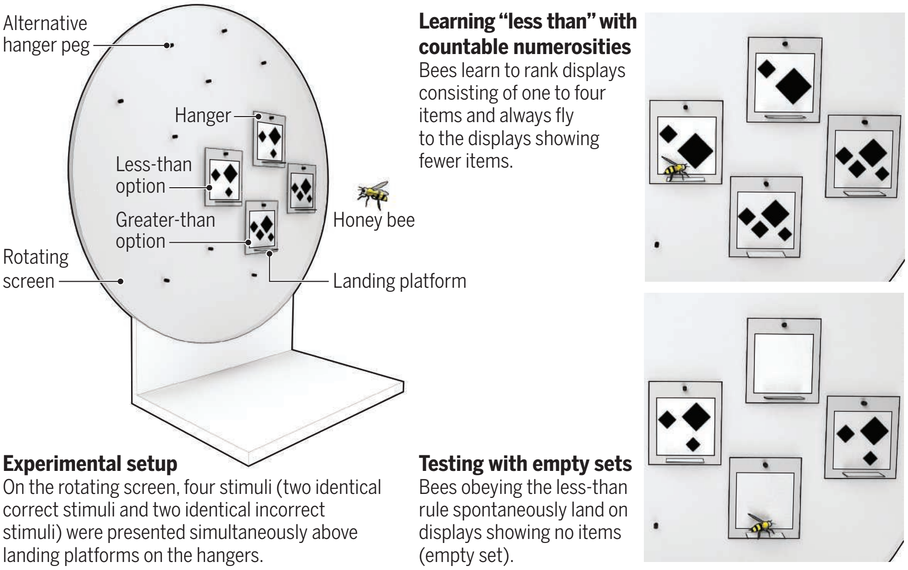
Testing bees on number discrimination

One group of bees was rewarded with sugar solution whenever they flew to the display showing more items (greater-than rule); the other group was rewarded whenever they chose fewer items (less-than rule).



Experimental setup
On the rotating screen, four stimuli (two identical correct stimuli and two identical incorrect stimuli) were presented simultaneously above landing platforms on the hangers.

Learning "less than" with countable numerosities
Bees learn to rank displays consisting of one to four items and always fly to the displays showing fewer items.

Testing with empty sets
Bees obeying the less-than rule spontaneously land on displays showing no items (empty set).

GRAPHIC: A. KITTERMAN/*SCIENCE*

PLANT IMMUNOLOGY

Targeting microbial pathogens

Plants secrete extracellular vesicles to prevent fungal infection

By **Bart P. H. J. Thomma**[1] *and* **David E. Cook**[2]

One of the most important challenges in agricultural production is to safeguard crops from pathogen infection. Uncovering the molecular mechanisms governing plant-microbe interactions can provide new strategies by which to sustainably intensify agriculture and can additionally contribute to our broader understanding of interspecies interactions (*1*). On page 1126 of this issue, Cai *et al.* (*2*) report that plant hosts secrete extracellular vesicles containing small RNA (sRNA), which are taken up by, and lead to silencing of, fungal virulence–related genes during infection (see the figure). These findings address the previously unknown phenomenon by which host sRNA can alter gene expression in other organisms, highlighting the role of extracellular vesicle–mediated transport as a key element of cross-kingdom RNA interference (RNAi). This could be exploited in the development of RNAi-based pathogen control strategies to protect crops.

Since Harold Flor's gene-for-gene theory in the 1940s describing host-pathogen interactions, which postulated that a matching gene pair in the host and pathogen dictate disease development, it is now apparent that hosts and pathogens are involved in an ongoing coevolutionary arms race in which the pathogen aims to continue the symbiosis that the host tries to interrupt (*1*). Considerable attention has been paid to proteinaceous molecules used by both interaction partners, including pathogen-secreted effectors that perturb host immunity and host-secreted antimicrobial proteins. Recently, cross-kingdom RNAi was identified as an additional pathogen-host interaction mechanism in which sRNAs are used in an attempt

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