resting length of ∼50 nm, consistent with a recent atomic force microscopy study of isolated talin *in vitro* [10].

One important caveat in interpreting these studies is that the technique localizes the fluorescent protein, not the target protein per se. Adding 1–2 nm (the diameter of EOS plus linker) is straightforward, but the geometry of the EOS relative to the rest of the molecule is unknown. Thus, the EOS moiety could be highly mobile or could be 'stuck' at a defined orientation relative to the amino- or carboxy-terminal domain to which it is attached. While a mobile EOS will simply broaden the Gaussian distribution for Z-axis location, a fixed orientation could introduce a small but systematic error. While the potential error is small relative to the 40 nm scale examined by Kanchanawong *et al.* [7], it may not be negligible for some applications.

This study points the way toward approaches that are likely to transform our understanding of multi-protein complexes in living or fixed cells. The ability to localize specific components in three dimensions with 10–20 nm resolution represents a major advance that will catalyze progress in many fields of cell biology. Moreover, as super-resolution acquisition times become faster, it will become possible to analyze active processes. Integrin-mediated adhesions in particular are dynamic, force-sensitive machines that respond to changes in ECM composition, topography and mechanics to determine cellular responses [6]. But the active, dynamic process by which cells read the topography and mechanical properties of the ECM are poorly understood. A detailed analysis of the location of specific protein domains in adhesions during sensing would be a huge step forward. The biology community can look forward to major advances in understanding these and other complex subcellular machines as a result of the resolution revolution.

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Animal Cognition: Concepts from Apes to Bees

New research shows that honeybees can classify arrangements of two visual patterns according to their spatial configuration. Can bees learn relational concepts of 'above' and 'below'? And are the underlying psychological processes comparable in humans and other primates facing similar tasks?

Lars Chittka and Keith Jensen

The study of concept formation in animals is notoriously contentious. On the one hand, it appears at the heart of the question of whether animals can be capable of 'abstract thinking' in a human-like sense; on the other hand, it is often difficult to rule out that what appears to be conceptual thinking cannot be explained by low-level cues. Machery [1] defines the term as follows: "A concept of x is a body of information about x that is stored in long-term memory and that is used by default in the processes underlying most, if not all, higher cognitive competences when they result in judgments about x" [1]. We invite the reader to brainstorm for a few moments as to what the concept of 'dog' entails. It is much more than a category encompassing a huge variety of distinct breeds with sizes ranging from the equivalent of a cat to a pony, with distinct colour coats and body proportions; the concept of 'dog' also includes many types of semantic information, various behaviour patterns and 'mentalities', multiple ways in which dogs can be useful to their owners, and so on [2].

Ever since Herrnstein and Loveland [3] showed that pigeons could discriminate novel stimuli (pictures with people in them as opposed to unpopulated pictures), there has been interest in whether animals have concepts. The debate centres on the question of whether what appears like concept formation can instead be explained by stimulus generalisation [4,5] and discrimination by first order perceptual features [6,7]. In one categorisation experiment, chimpanzees managed to sort a mix of various different tools and food items into separate piles — despite the fact that exemplars within each category had no obvious physical resemblance to one another [8]. Had the chimpanzees thus understood the concept of 'tools' and 'food'? It has been pointed out that subjects could have simply classified objects by whether or not they induce salivation [9]; and indeed there are many other low-level cue explanations — after all,

food and tools differ consistently in surface texture, angularity of shape, softness and other features. Nonetheless, there are convincing demonstrations of categorisation in many animal species, including bees, that may not be so easily explained by such low level processes [9,10].

To explore if animals can learn relational concepts, such as sameness and difference, subjects are often tested with 'matching-to-sample' or non-matching to sample paradigms. They are shown a stimulus, and shortly afterwards must choose between two stimuli, one similar (or identical) and one different to the one previously viewed. Many vertebrates [9], and indeed bees [10], can solve such tasks. But impressive though they may be, the level of 'understanding' of the concept of sameness or difference needed for passing such tests might be fundamentally simpler than in humans. Human subjects can appreciate, for example, the ways in which buses, ferries, and trains are 'same' (they are all means of public transport) and in which ways they are different. In typical matching-to-sample tasks, it is the *stimuli* that are either similar or not. At its basic level, this involves comparing an incoming stimulus with the contents of working memory, and the rule of whether to choose 'same' or 'different' must be stored in long term memory — but it does not necessarily require the formation of a concept with the full range of implications of the term in human psychology.

Now Avarguès-Weber *et al.* [11] have performed experiments to see if honeybees (*Apis mellifera*) can conceptualise above/below relationships. Bees were trained to collect sucrose solution in one of two arms of a Y-maze; if they chose the wrong arm, they would receive quinine solution, a strongly aversive stimulus for bees. The arm of the maze containing the reward changed unpredictably from trial to trial, and the only way bees could identify the correct arm was by choosing the correct arrangement of two visual stimuli on the back walls of the Y-maze. One of these stimuli was the 'referent', which was kept constant through all trials of each individual bee (Figure 1). The other was the target (where the reward was offered), but the visual appearance of the target changed multiple times during training. The correct arm of

Figure 1. Stimuli sequentially shown to bees on the back wall of a Y-maze.

Bees were trained to collect sucrose solution in one of the arms of the maze. Each pair of patterns simultaneously presented in the Y-maze contained the same visual stimuli, only distinguished by whether the 'target' occurred below or above the 'referent', which was always the same. Bees encountered a series of different targets during training, and were rewarded only if they flew into the arm that contained the target below the referent (another group of bees was rewarded if they correctly picked the arm where targets consistently appeared above the referent). The exact positions of targets and referents on the back walls of the arms of the Y maze were varied to ensure that bees could not learn a fixed configuration [11].

the maze could be located by a rule: if the target was presented below the referent, there would a sweet reward for the bee; if the target occurred above, a bitter punishment was in store. (Another group of bees was trained in the reverse way.) The bees swiftly learnt this task, and were even able to pass a transfer test when presented with a completely unfamiliar target, which, however, appeared again in the correct location relative to referent.

Thus, even though the same pairs of stimuli were always presented in the right and left arm, the bees were able to choose the correct arrangement,

so that they could not solve the task based on the presence or absence of the two items in each arm of the maze. Avarguès-Weber *et al.* [11] also altered the exact positions of the two items to discourage subjects from learning a fixed compound of both. A trivial explanation for the findings might be that bees simply could not distinguish the various targets, and therefore treated them identically. However, the authors ensured that the targets presented sequentially were of different colours and shapes, and indeed they demonstrated empirically that bees could discriminate them when trained to do so. These elegant

Figure 2. A natural analogue of the above/below-ness concept for bees.
Bees might be using the shape of otherwise near-indistinguishable hillocks as a landmark (the referent) and then pick the familiar one according to whether the area beneath (a flower meadow) contains targets, or not. Other bees might have trained themselves to forage from targets above the reference — in this case, trees presenting their flowers above the horizon of a different hillock.

experiments clearly show that bees can do much more than just associate the visual properties of a target with a reward.

To understand why animals are good at certain laboratory tasks, it often helps to explore what the nearest natural analogue to the experimental setup is. Bees outperform many species of vertebrates at colour learning [9] presumably because associating colours with rewards is a fundamental part of a forager bees' lifestyle [12]. Their remarkable ability to solve sameness/difference tasks in a matching-to-sample paradigm might also be related to natural foraging behaviour, where a strategy to win-stay or lose-shift is employed in deciding which flower species to visit, depending on recent rewards [13]. In the new experiment, let us replace the 'referent' with a natural landmark — say the horizon profile of a hillock, where there are several similar hillocks nearby. The task for a bee to pick the hillock with a rich flower meadow beneath — i.e., pick the landmark, if it has a target beneath — *any* target, no matter the flower species or the exact location (Figure 2). In nature, as in the laboratory experiments by Avarguès-Weber *et al.* [11], the exact location of the landmark relative to the potential target will vary depending on the approach angle, distance to target, and so on. Thus, we need to consider the possibility that bees might solve the task by a relatively simple algorithm: fly to the referent (the landmark) if there is something — *anything* — in the ventral visual field as you're approaching the referent (or the dorsal visual field if trained to pick stimuli above the referent). When viewed in a naturalistic setting, the findings of the new study by Avarguès-Weber *et al.* [11] make beautiful biological sense.

This is one possible explanation; another is that bees had indeed conceptualised the relationship of above-ness and below-ness. To explore this possibility, it us useful to compare the experimental procedures that have been used in primates to test for the existence of such concepts. An important difference between the new study on bees and those conducted on baboons (*Papio papio*) [14] and capuchin monkeys (*Cebus apella*) [15] is that primates were asked to further discriminate above and below relationships when the referent also changed. Both capuchins and baboons succeeded (excluding the possibility that the referent is simply used as a 'landmark'), but bees remain to be tested. A striking difference observed in those tests that were more directly comparable, though, was that it took the primates several hundreds or indeed thousands of trials to master similar tasks, whereas the bees took only a few dozen. Human infants at three months of age fail altogether to show a concept of above and below unless the targets remain the same [16], though by the time they are 6 months old, infants perform comparably to bees and monkeys [17]. But while the tasks are seemingly similar, are these performances indeed comparable? Bees were allowed to move freely to and from the apparatus and could thus solve the tasks in a manner equivalent to a spatial navigation task, and motivation remains high in forager bees since they forage for the colony and thus never satiate. Primates were tested in highly non-biological settings — the baboons,

for example, had to manipulate a cursor onto a fixation point using a joystick, no doubt a challenging task in itself. For infants, there was no reward or punishment whatsoever; instead performance was assessed via looking times depending on the apparent novelty in the stimuli. Thus, while the tasks themselves might be roughly comparable, there were profound differences in how subjects could go about solving them, and presumably in motivation. And even if all these differences were eliminated, it would be hard to determine the exact strategy by which subjects solved the task, and indeed there might even be inter-individual differences with respect to the underlying strategy: even if performance is the same, some individuals might solve a task using relatively simple heuristics while others might use a more abstract reasoning.

A broader question is whether the performance of bees and primates (including infants) qualifies for concept formation in a stricter sense. While learnt rules of same/different, above/below, more/less relationships are crucial ingredients of concepts, are such rules themselves concepts? As pointed out above, a sameness/difference judgment can take on very different levels of complexity, and the same is true for above/below relationships. Once humans have understood the concept of this relationship, they can appreciate the ways in which the sky is above them, the king is above the knight, the way in which 'above/below' relates to 'better/worse', and so on. Transfer of relational rules across domains or to novel situations is rarely explored in animal studies (but see [10]) — for example, would bees transfer their learnt preference for target above a reference to the higher of two flowering species in their natural three-dimensional environment? Because of the proximity of the term 'concept' to 'abstract thinking', we must be careful not to conclude too swiftly that fulfilling basic prerequisites of concept formation is indicative of the full range of cognitive phenomena that come with conceptual thinking in humans [5]. Similar behavioural performances by different animals, and the fact that current definitions permit application to a range of species' abilities, don't necessarily mean that the underlying processes are similar (or similarly complex).

There might be differences in how apes (including humans) and monkeys form concepts [18], and other animals, including bees, could use yet other solutions towards similar behavioural problems.

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Cytokinesis: Thinking Outside the Cell

How might the extracellular matrix contribute to cytokinesis? In a recent report, evidence is presented that the conserved extracellular matrix protein hemicentin[HIM-4] is required for cytokinesis in worms and mice.

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The extracellular matrix (ECM) functions akin to a cellular exoskeleton, providing structural support and sites of attachment for the cells it surrounds. However, the ECM is much more than just a scaffold. With established roles in cell migration, tissue separation, and cell signal transduction [1], the ECM is clearly a dynamic player in many cellular functions.

Cytokinesis is the physical division of one cell into two daughter cells that occurs at the end of the cell cycle. Cytokinesis is accomplished by constriction of a contractile ring composed of filamentous actin and the motor myosin-II (together actomyosin) [2]. In order to divide, a cell must recruit and coordinate a host of regulatory and structural proteins to the division plane, but existing models of cytokinesis do not consider a contribution from secreted extracellular proteins.

The secreted ECM protein hemicentin (HMCN1 or fibulin-6 in *Homo sapiens*, HIM-4 in *Caenorhabditis elegans*) is of particular interest as it may have an evolutionarily conserved role in cytokinesis. Hemicentin is a relative of a multigene family of proteins called fibulins, secreted proteins that assimilate into the ECM and form higher order structures, such as elastic fibers [3]. The hemicentin protein contains a single, highly conserved von Willebrand A domain, a long stretch of immunoglobulin repeats, epidermal growth factor domains, and a fibulin-like carboxy-terminal module [4]. In the roundworm *C. elegans*, hemicentin[HIM-4] has been found in cell-matrix adhesion sites known as hemidesmosomes and at multiple connecting junctions throughout the body [5]. Cells surrounding the hermaphroditic worm gonad also secrete hemicentin[HIM-4], and functional disruption of the *him-4* locus in *C. elegans* leads to a high incidence of males due to defects in segregation of the X chromosome, resulting in XO male progeny [6]. The *him-4* mutants also display pleiotropic defects in motility, adhesion, behavior, and gonad morphology [5].

In a paper published in a recent issue of *Current Biology*, Xu and Vogel [7] took a closer look at the gonad morphology defects in *him-4* mutants. In the *C. elegans* gonad, germ cells form as part of a syncytium, with incomplete cytokinetic furrows partitioning single nuclei that share a common cytoplasm [8]. These nuclei are eventually segregated completely as the cell matures into an oocyte and physically separates from the syncytium [8]. Hemicentin[HIM-4] labeled with green fluorescent protein localized in a ring structure at the base of these incomplete furrows in the gonad. In the absence of hemicentin[HIM-4], the gonad in aged worms became disorganized due to the formation of multinucleated germ cells. Temporal analysis revealed that while nascent membrane partitions appeared to have a normal structure, they soon became destabilized and eventually collapsed.

The recruitment of hemicentin[HIM-4] to the tips of the membrane partitions in the *C. elegans* gonad was dependent on the highly conserved Rho family guanine nucleotide exchange factor (GEF) ECT-2. During cytokinesis, ECT-2 activates Rho and initiates assembly of the actomyosin ring at the constriction site [2]. In an ECT-2-deficient background, the majority of hemicentin[HIM-4] remained in the pseudo-coelomic fluid outside of the gonad. Taken together, these results suggest a role for hemicentin[HIM-4] in maintaining the cytokinetic membrane partitions in older worms, and that its