

action in *trans* is permitted throughout the *Drosophila* genome. Proc. Natl. Acad. Sci. USA 99, 3723–3728.

Chetverina, D., Fujioka, M., Erokhin, M., Georgiev, P., Jaynes, J.B., and Schedl, P. (2017). Boundaries of loop domains (insulators): Determinants of chromosome form and function in multicellular eukaryotes. BioEssays 39, <http://dx.doi.org/10.1002/bies.201600233>. Epub 2017 Jan 30.

Choi, O.-R.B., and Engel, J.D. (1988). Developmental regulation of β -globin gene switching. Cell 55, 17–26.

Dekker, J., and Mirny, L. (2016). The 3D genome as moderator of chromosomal communication. Cell 164, 1110–1121.

Dixon, J.R., Gorkin, D.U., and Ren, B. (2016). Chromatin domains: the unit of chromosome organization. Mol. Cell 62, 668–680.

Fujioka, M., Mistry, H., Schedl, P., and Jaynes, J.B. (2016). Determinants of chromosome architecture: insulator pairing in *cis* and in *trans*. PLoS Genet. 12, e1005889.

Gemkow, M.J., Verveer, P.J., and Arndt-Jovin, D.J. (1998). Homologous association of the Bithorax-Complex during embryogenesis: consequences for transvection in *Drosophila melanogaster*. Development 125, 4541–4552.

Geyer, P.K., Green, M.M., and Corces, V.G. (1990). Tissue-specific transcriptional enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila*. EMBO J. 9, 2247–2256.

Hartl, T.A., Smith, H.F., and Bosco, G. (2008). Chromosome alignment and transvection are antagonized by Condensin II. Science 322, 1384–1387.

Hopmann, R., Duncan, D., and Duncan, I. (1995). Transvection in the *iab-5,6,7* region of the bithorax complex of *Drosophila*: homology independent interactions in *trans*. Genetics 139, 815–833.

Kravchenko, E., Savitskaya, E., Kravchuk, O., Parshikov, A., Georgiev, P., and Savitsky, M. (2005). Pairing between *gypsy* insulators facilitates the enhancer action in *trans* throughout the *Drosophila* genome. Mol. Cell Biol. 25, 9283–9291.

Lewis, E.B. (1954). The theory and application of a new method of detecting chromosomal rearrangements in *Drosophila melanogaster*. Am. Nat. 88, 225–239.

Martínez-Laborda, A., González-Reyes, A., and Morata, G. (1992). *Trans* regulation in the *Ultrabithorax* gene of *Drosophila*: alterations in the promoter enhance transvection. EMBO J. 11, 3645–3652.

Ronshaugen, M., and Levine, M. (2004). Visualization of *trans*-homolog enhancer-promoter interactions at the *Abd-B* Hox locus in the *Drosophila* embryo. Dev. Cell 7, 925–932.

Senaratne, T.N., Joyce, E.F., Nguyen, S.C., and Wu, C.T. (2016). Investigating the Interplay between Sister Chromatid Cohesion and Homolog Pairing in *Drosophila* Nuclei. PLoS Genet. 12, e1006169.

Southworth, J.W., and Kennison, J.A. (2002). Transvection and silencing of the *Scr* homeotic gene of *Drosophila melanogaster*. Genetics 161, 733–746.

¹Lewis-Sigler Institute for Integrative Genomics, ²Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.
E-mail: tfukaya@princeton.edu (T.F.), msl2@princeton.edu (M.L.)



Primer Bee cognition

Lars Chittka

“What have we to do, some will ask, with the intelligence of the bees? ... The discovery of a sign of true intellect outside ourselves procures us something of the emotion Robinson Crusoe felt when he saw the imprint of a human foot on the sandy beach of his island. We seem less solitary than we had believed.” — Maurice Maeterlinck 1901, The Life of the Bee

Maeterlinck did not mean to suggest that honeybees rival humans in intelligence — rather he saw in the bee a qualitatively different form of intelligence, tailored to the challenges of a profoundly different kind of society and lifestyle. Insects are strange “aliens from inner space”, with sensory and cognitive worlds wholly different from our own. The 19th century discovery that ants can detect ultraviolet light triggered a golden age in the exploration of the diversity of sensory systems of insects (and indeed other animals), identifying such abilities as magnetic compasses, electrosensitivity, polarization vision, and peculiar locations for sense organs such as the infrared sensors on the abdomens of some beetles or photoreceptors on the genitalia of some butterflies. Could insect minds be equally strange and diverse?

While biologists have long recognised the rich behavioural repertoires of social insects that orchestrate life in the colony, facilitate the elaborate construction of a communal home, secure a steady stream of appropriate food for their young, defending the colony and regulating its climate, they have often dismissed this behavioural complexity as ‘just instinct’, as if the fact that animals arrived at these solutions by evolutionary rather than individual innovation made these behaviours less impressive, or less computationally advanced. But entire books have been written about the plethora of instinctual routines that underpin just a single collective operation, such as when a swarm of honeybees moves to a new home. In terms of home construction, there simply is no vertebrate species — other than our own, and perhaps the beaver — that rivals the

behavioural complexity of the honeybee (Figure 1) and other social insects, such as ants, wasps and termites.

There are interactions between innate behaviour and intelligence at multiple levels. All healthy humans have an innate predisposition for language (an ‘instinct’) — but having the language instinct facilitates almost all cognitive abilities that we pride ourselves in, including the capacity for cultural evolution. In the evolutionary history of bees, a key event was the innovation of provisioning their young in a specially constructed nest, which required not just (instinctual) home construction skills, but also a precise spatial memory: evolution does not take kindly to a mother who forgets the location of her offspring. Add to this the (again instinctual) lifestyle of harvesting solar energy — the sugars produced by plants’ photosynthesis. This involves learning about, and making economic choices between, multiple flower species that differ in the quality and quantity of rewards, and the signals that advertise these rewards — again, the instinct that determines the lifestyle facilitates and indeed necessitates learning, and if innate predispositions of some individuals allow them to learn certain contingencies faster, this will allow fitness benefits to accrue.

Exploring and exploiting space

When a bumblebee or honeybee first leaves its nest, it engages in a tight race against time. It has only a few weeks to live and thus must minimise the time to explore its surroundings, learn about prominent landmarks to orient in its environment, and find and exploit the most profitable food sources. The life-long radar tracking of individual bumblebees has recently elucidated how bees cope with these challenges (Figure 2). The first few flights are invariably spent flying around the hive in large loops of varied directions. Once suitable foraging locations have been discovered, different individuals have profoundly different strategies: a bee might stick to single foraging locations for extended periods (Figure 2) or periodically search for more profitable patches. Bees can store multiple foraging locations, and indeed remember the times of day at which each one is profitable. When distinct flower patches have different scents, bees will activate the correct spatial memory when an



Figure 1. Simple instinct versus advanced cognition?

In social insects, many largely instinctual phenomena, such as the construction of hexagonal honeycombs, require the coordinated and cooperative activities of many dozens of individuals. Workers manufacture and manipulate wax into a highly regular hexagonal pattern (a mathematically perfect solution to honey and brood storage), and in the process have to evaluate the space available and the current state of construction, and process a diversity of communication signals from others, as well as proprioceptive input, for example to align the combs with gravity. These rich instinctual repertoires of many insects have often been thought to come at the expense of learning capacity. However, very few behavioural routines are fully hardwired and even comb construction skills have to be partially learnt by honeybees. There are interactions between instinct and learned behaviour at multiple levels, and complex instincts can facilitate advanced learning behaviour. Image by Helga Heilmann, with permission.

experimenter blows one or the other scent into the hive. Honeybees use the ‘dance language’ to inform others of the coordinates of a food source — and, curiously, some individuals will spontaneously dance in the night (when no foraging takes place), apparently retrieving the memory of a location they had visited the day before.

When bees move between multiple patches, they can solve the ‘travelling salesman problem’ — finding the shortest route in which each patch is visited precisely once — by trial and error exploration. As anyone who has experience with navigating natural environments over long distances (without a map or compass) will testify, this is not a trivial task and requires keen attention to landmark and details, sometimes active scanning of the scene, and efficient search strategies in the event that one has lost the path. Bees display all of these behaviours and computational studies show that even simple models of the insect brain allow the efficient storage of dozens of visual

scenes. This rich library of landmark memories needs to be integrated with celestial cues, such as the sun’s position (sometimes reconstructed from the sky’s polarization pattern when the sun is not visible), but as the sun is seen at different positions in the sky in the course of the day, bees also need to know the time of day to use the sun as a compass cue.

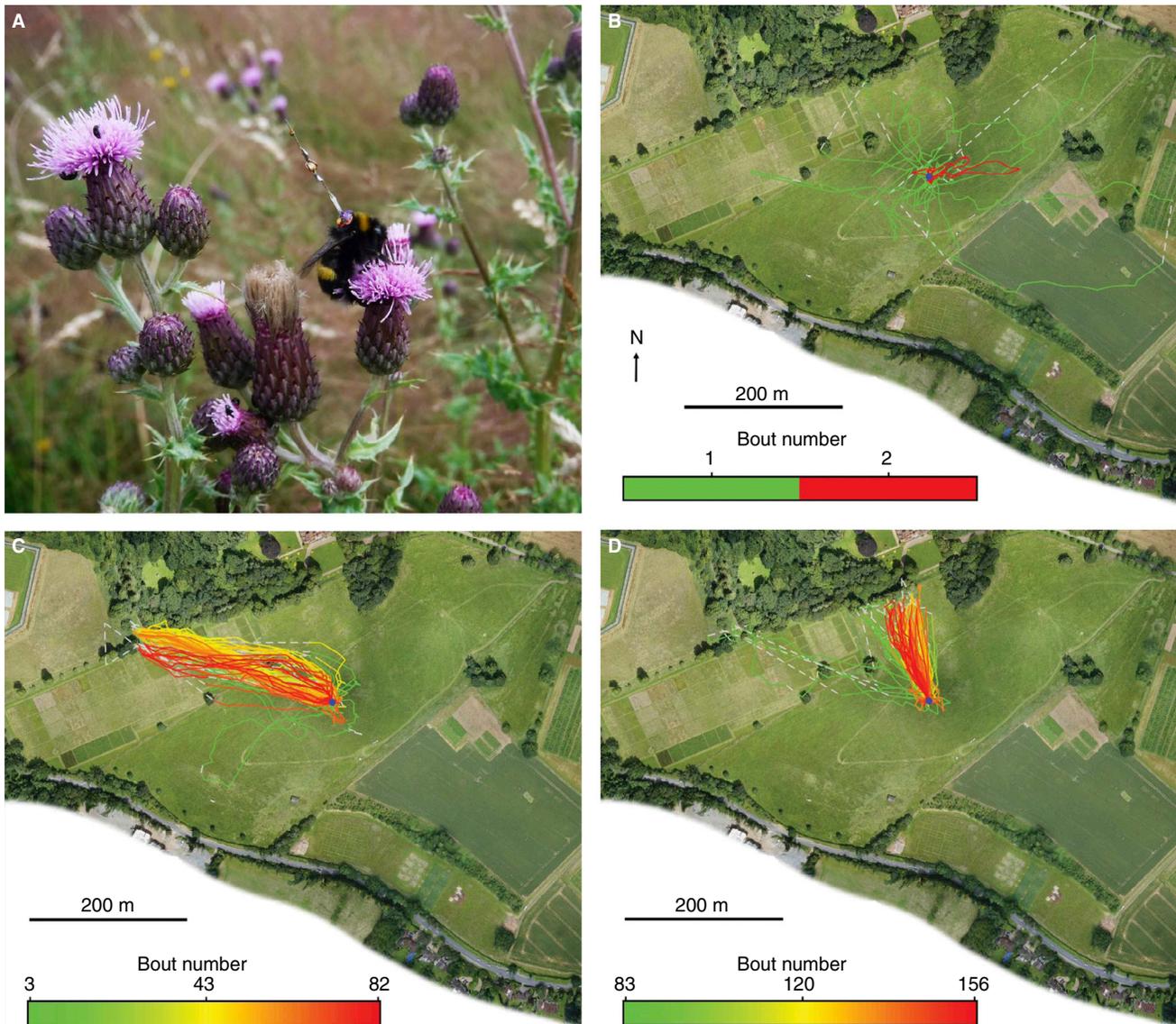
Remembering flowers

A naïve bee encountering its first meadow is faced with a bewildering variety of flower types which differ in the colours, visual patterns and odours they display. Bees have innate predispositions to tell floral from non-floral objects, and are more attracted to some flower colours than others. But these innate predispositions do not carry far: because different flower species have different profitability in terms of nectar and pollen offerings, bees need to be careful shoppers in the floral supermarket, learning to use sensory signals of flowers as predictors of rewards. Subsequently

they will focus their foraging efforts on the most rewarding flower types they have encountered as individuals, and reject flowers that they know to be poorly rewarding. Sometimes the visual stimuli indicating a rewarding or unrewarding setting are similar, and in such cases bees seem to be able to assess their own level of certainty: they slow down their flight to inspect stimuli for longer periods, and indeed ‘opt out’ of a difficult task when the risk of not receiving a reward is high. Naïve bees will sometimes copy the flower choices of experienced individuals — when one has no idea about which flowers might be profitable of the multitude available, a reasonable first approach is to probe a patch that conspecifics find worth exploiting. And even seasoned foragers will tend to copy other individuals more if the foraging environment is uncertain.

The manipulation techniques required to extract nectar or pollen are sometimes complex; they can take dozens of trials to learn to perfection. An individual bumblebee can learn multiple such routines, but will be somewhat less efficient when juggling multiple tasks. The historical notion that the small brain of the bee constrains it to remember only one flower type was never tenable even when one observed their natural behaviour. It is now also no longer tenable when one considers computational neurobiological approaches, which show that insect brains have a large storage capacity.

Bees can also remember threats they have encountered on flowers. Various predators, for example crab spiders that can mimic the colours of flowers, hunt bees, but bees often manage to escape after a brief struggle. They may subsequently avoid all flowers of the type on which the attack happened, but bees sometimes continue exploiting these flowers. Such bees, however, display completely altered foraging behaviour: they fly more slowly as they approach flowers, and scan each flower before landing. Interestingly, they are also prone to ‘seeing ghosts’: false alarms, where a bee aborts the approach to a perfectly safe flower, are common. These observations show that bees do not respond inflexibly to environmental stimuli; they display emotion-like states, where multiple aspects of behaviour are affected by whether the bee is ‘optimistic’ or ‘pessimistic’, depending



Current Biology

Figure 2. Tracking a single bumblebees' foraging over its lifetime.

(A) A worker bee with a harmonic radar transponder attached to its back (photo by L. Chittka). (B) On its first day, the bee makes two excursions, looping in various directions around its hive, in the process memorizing the location of the hive, and landmarks in its vicinity. (C) On day 2, the bee begins by making another orientation loop in the (previously unexplored) Southwesterly direction, and then discovers a flower foraging patch that she visits exclusively until day 6 of her foraging career. (D) Following a few days of bad weather, the bee resumes her activity by first visiting the familiar patch on day 9, but then, on day 10 'changes her mind' halfway along a flight to this patch and flies instead to a different location she had explored only once, 9 days earlier, during an orientation flight. She then visits this location exclusively for the rest of her life until day 13 (panels B–D by Joe Woodgate, Andrew Riche and March Castle).

on previous experiences. Unexpected flower rewards can put a bee into a dopamine-dependent, optimistic state, in which it responds less adversely to a predator attack.

Behavioural strategies and neural computations underpinning cognition

Comparing the intelligence of different species makes sense only in a

quantitative framework in which the complexity of different tasks is explored in terms of the neural computations that underpin cognition. This is not a trivial challenge, given that even small brains such as a bee's are not simple — they have fewer than a million neurons, but each neuron can have a structure as widely branched as a fully grown oak tree, and make synaptic

contact with thousands of other neurons in various parts of the brain. Recent neurobiological and theoretical (computational neuroscience) studies have shown that what were once thought to be simple learning processes are not so simple in neurobiological terms, whereas what were perceived to be highly intelligent operations could be quite simple in computational terms.

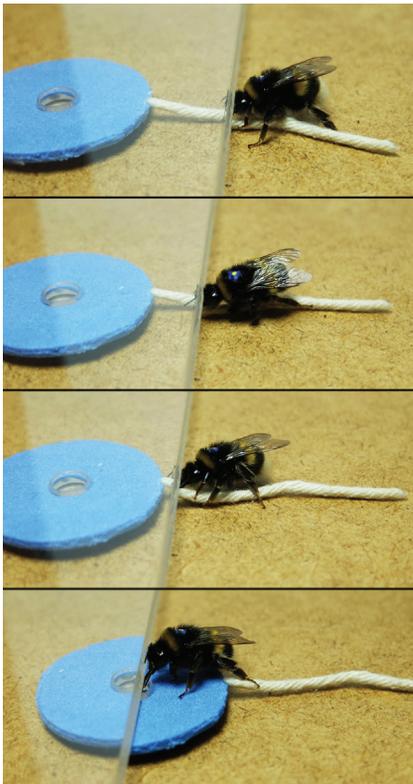


Figure 3. String-pulling by a bumblebee.

The image series shows a bumblebee forager pulling a string to gain access to a blue artificial flower under a transparent Plexiglas table; the center of the flower holds a droplet of sucrose solution (photos by S. Alem).

Associative learning — for example, linking a flower odour to a reward — is a Pavlovian process commonly regarded as simple, and could at its most basic level be mediated by altering the efficiency of a single synapse. But it turns out that learning even single associations can result in substantial changes of the density of synaptic complexes in an entire area of the insect brain where projection neurons from the olfactory sensory periphery intersect with the reward pathway, the lip region of the mushroom bodies (brain regions with multisensory inputs and a function in memory storage). On the other hand, seemingly complex operations that require the integration of multiple sensory stimuli can be performed with exactly the same circuitry that is used for ‘simple’ associative learning. A handful of feature detector neurons in the bees’ visual system (specifically the lobula) can solve a large variety of visual pattern discrimination tasks — indeed, models show that using such feature

detectors *only*, a bee might actually perform better than what has been found in empirical tests. This is despite the fact that these models are so simple as to be caricatures of the real complexity of visual processing — they model just four out of many tens of thousands of neurons of the lobula, and indeed do not assume that any form of image is stored or even perceived by the bee.

Indeed there is little direct evidence that bees actually perceive images — in the form of little virtual pictures somewhere in the bees’ brain. Recent work points to a very important role of sequential information processing in bee vision. Tethered honeybees — which cannot move their eyes — behave as if they are blind. When free-flying bees examine patterns, it appears that they cannot see them at a glance (by parallel processing); instead, sequential scanning motions are required for the bee to assemble information about pattern identity. When patterns are flashed only briefly, bees seem unable to identify them. This points to the fascinating possibility that visual patterns are not stored as images at all, but, for example, as the motor patterns that flying along the patterns’ edges induces a bee to perform. A square could be distinguished from a circle by flying along any small subsection of the edge of the shape. These considerations do not mean that bees do not see images — they just show that animals can potentially behave as if they do by using completely different strategies in comparison to humans (or other vertebrates).

Solving non-natural tasks

Scholars in comparative cognition emphasise the need for ‘intelligence tests’ on tasks that animals do not encounter in their daily lives, to probe their behavioural flexibility. For example, bees are faster than most other animals in learning to associate colours with rewards — but this is not because bees are more intelligent than for example cats, but because colours have much less meaning in the life of cats than they do in an animal that obtains all its nutrition from flowers. It turns out, however, that both honeybees and bumblebees are surprisingly good at solving some tasks that no bee has ever encountered in its evolutionary history.

For example, honeybees can count up to three items with a precision that is comparable to much larger-brained animals such as some fish.

Bumblebees can solve a string pulling puzzle, where an artificial flower is displayed under a transparent plate (Figure 3). The only way to reach the reward in the flower is to pull on a string that is attached to the flower and protrudes from under the plate. As in all cognitive tasks, there are huge inter-individual differences: most bees will require either step-wise training or the chance to observe a skilled conspecific to master the task on their own. A very small minority of individuals even solve the task by individual trial-and-error learning. When a colony is ‘seeded’ with a single knowledgeable individual, the skill spreads swiftly to the majority of foragers of the colony.

In these experiments, the string is attached to the item that holds the reward, but bumblebees can also learn to move an unattached object (a ball) to a designated target area. This test, which is equivalent to humans using tokens in a vending machine, also reveals a remarkable social learning skill. A trick was played on a ‘demonstrator’ bee, so that only the furthest of three balls could be moved to the target area (two other balls were glued to the horizontal surface). A naïve bee was then allowed to attend the skilled bee’s performance (always moving the furthest ball) three times. But when the observer was subsequently allowed into the arena alone, it spontaneously picked the closest ball to move to the target — thus having solved the tasks in a manner inspired by the demonstrator, but clearly not ‘aping’ its performance. They even did so even if the ball was of a different colour to the one they had seen the demonstrator move, indicating that they ‘understood’ the nature of the task rather than acting according to simple sensory-motor contingencies.

There is a common perception that behavioral flexibility needs to be underpinned by large brains. However, these studies show, as does other empirical and theoretical work, that complex learning can be performed with relatively small nervous systems. Small changes in neural circuitry can generate large shifts in behavioral capacity, certainly in hard-wired routines.

The same possibly applies to motor learning in problem solving tasks, especially because in both cases existing circuits can simply be coopted. The neural circuits underlying bees' large repertoire of cognitive, social and nest-manufacturing behaviors may be pre-adaptations that allow bees to solve such complex, non-natural tasks. Why would bees excel at tasks that they are unlikely to encounter in nature? Because nature is unpredictable, and intelligent animals will be better at coping with unpredictable challenges. A video circulates on social media in which someone has plugged the nest hole of a mason bee with a nail — surely a first for any mason bee. The bee spontaneously 'knows what to do': rather than senselessly trying to squeeze past the nail, it pulls it out of the hole, using a variety of techniques in the process. Following the publication of the ball-rolling study, a member of the public emailed to report that she had observed a bumblebee rolling a small slug out of the nest entrance into which the slug had strayed, using the same technique as the bees in our study. Such rare challenges can be crucial to survival, if access to one's young or one's food storage is at stake.

Conclusions

A good Skinnerian might come up with ever-more elaborate 'simple associative' explanations for the cognitive phenomena we have described above. The attractiveness of such explanations in small-brained animals is unsurprising, given that powerful schools of thought once advocated to explain even all *human* learning in associative terms. But it turns out that we might have to revise our views on more advanced cognition even in small nervous systems. A possible alternative explanation of, for example, bees flexibly copying aspects of the 'tool use' behaviour they had seen in experienced conspecifics is that they have a basic understanding of the outcome of their own actions, and those of other bees: that is, consciousness-like phenomena or intentionality. But surely, you might say, consciousness requires a really large brain, a neocortex...? Wrong. First, one can *never* deduce the existence of any cognitive capacity from gross neuroanatomy: chimpanzees have Broca's and Wernicke's area, brain

regions that support language in humans but clearly not in chimps, so the presence (or indeed the absence) of a certain area tells us nothing about the existence of a cognitive capacity. Wholly different circuits can support similar behavioural abilities in different animals. Basic consciousness-like phenomena can be implemented with just a few thousand neurons — not a prohibitively large number for an insect brain. The study of bees has taught us that many forms of cognition might be computationally easy, whereas associative learning is not as easy as was once thought. Brains do not just support reflex loops, or else you could get by with much smaller brains in many animals. Instead, brains, even very small ones, are *wired for cognition*, for extracting rules from the environment, for predicting the future and for efficient information storage and retrieval.

We also have to revise the perceived contrast between innate behavioural traits (often regarded as 'primitive' even in the case of highly complex behaviours such as home construction in social insects or the agriculture practised in leafcutter ants) and cognition (viewed as more 'advanced'). If you can evolve ability X in a small-brained species, there is no reason to assume that you cannot also 'invent' (learn to perform) X in the small brain of an individual, and indeed the neural adjustments might in part take place in the same circuits. The absence of a particular behavioral capacity in wild animals is not evidence that the ability is 'hard to evolve', or for the lack of adequate levels of intelligence, but might in many cases simply reflect the absence of relevant natural challenges. For example, the reason that social bees do not recognise each other individually is not that it is not technically feasible with a small brain, rather their individuals are too similar and too numerous for face recognition to be useful. Conversely, some open nesting wasps with small colonies have an innate ability for individual face recognition — and honeybees can be trained to recognise images of human faces. It is likely that in both cases the same circuits for visual pattern recognition are employed, and adjusted either over evolutionary time (wasps) or on an individual's lifetime (bees). A latent preparedness for complex problem solving might

exist in many species whose lifestyles require advanced learning abilities, and might relatively easily be refined over evolutionary time, should the relevant selection pressures arise.

FURTHER READING

- Alem, S., Perry, C.J., Zhu, X., Loukola, O.J., Inghram, T., Sovik, E. and Chittka, L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. *PLoS Biol.* *14*, e1002564.
- Ardin, P., Peng, F., Mangan, M., Lagogiannis, K., and Webb, B. (2016). Using an insect mushroom body circuit to encode route memory in complex natural environments. *PLoS Comp. Biol.* *12*, e1004683.
- Bateson, M., Desire, S., Gartside, S.E., and Wright, G.A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Curr. Biol.* *21*, 1070–1073.
- Giurfa, M., Zhang, S., Jenett, A., Menzel, R. and Srinivasan, M.V. (2001). The concepts of 'sameness' and 'difference' in an insect. *Nature* *410*, 930–933.
- Katz, P.S. (2011). Neural mechanisms underlying the evolvability of behaviour. *Philos. Trans. R. Soc. Lond. B* *366*, 2086–2099.
- Loukola, O.J., Perry, C.J., Coscos, L., and Chittka, L. (2017). Bumblebees show cognitive flexibility by improving on an observed complex behavior. *Science* *355*, 833–836.
- Maeterlinck, M. (1901). *La Vie des Abeilles*. (Editions Frasnelle, Paris.)
- Nityananda, V., Skorupski, P., Chittka, L. (2014). Can bees see at a glance? *J. Exp. Biol.* *217*, 1933–1939.
- Peng, F., and Chittka, L. (2017). A simple computational model of the bee mushroom body can explain seemingly complex forms of olfactory learning and memory. *Curr. Biol.* *27*, 224–230.
- Perry, C.J., Baciadonna, L., and Chittka, L. (2016). Unexpected rewards induce dopamine-dependent positive emotion-like state changes in bumblebees. *Science* *353*, 1529–1531.
- Perry, C.J., Barron, A.B. (2013). Honey bees selectively avoid difficult choices. *Proc. Natl. Acad. Sci. USA* *110*, 19155–19159.
- Robinson, G.E., and Barron, A.B. (2017). Epigenetics and the evolution of instincts *Science* *356*, 26–27.
- Roper, M., Fernando, C., and Chittka, L. (2017). Insect bio-inspired neural network provides new evidence on how simple feature detectors can enable complex visual generalization and stimulus location invariance in the miniature brain of honeybees. *PLoS Comput. Biol.* *13*, e1005333.
- Seeley, T.D. (2010). *Honeybee Democracy*. (Princeton University Press, Princeton.)
- Shanahan, M. (2006). A cognitive architecture that combines internal simulation with a global workspace. *Consciousness Cognition* *15*, 433–449.
- Sheehan, M.J., and Tibbetts, E.A. (2011). Specialized face learning is associated with individual recognition in paper wasps. *Science* *334*, 1272–1275.
- Tautz, J. (2008). *The Buzz about Bees* (Springer Verlag, Berlin).
- Webb, B. (2004). Neural mechanisms for prediction: do insects have forward models? *Trends Neurosci.* *27*, 278–282.
- Woodgate, J.L., Makinson, J.C., Lim, K.S., Reynolds, A.M., and Chittka L. (2016). Life-long radar tracking of bumblebees. *PLoS ONE* *11*, e0160333.

Queen Mary University of London, Biological and Experimental Psychology, School of Biological and Chemical Sciences, Mile End Road, London E1 4NS, UK.
E-mail: l.chittka@qmul.ac.uk