RESEARCH ARTICLE

Innovation in solitary bees is driven by exploration, shyness and activity levels
Miguel Á. Collado1,2,*, Randolf Menzel3, Daniel Sol2,4 and Ignasi Bartomeus1

ABSTRACT
Behavioural innovation and problem solving are widely considered to be important mechanisms by which animals respond to novel environmental challenges, including those induced by human activities. Despite their functional and ecological relevance, much of our current understanding of these processes comes from studies in vertebrates. Understanding of these processes in invertebrates has lagged behind partly because they are not perceived to have the cognitive machinery required. This perception is, however, challenged by recent evidence demonstrating sophisticated cognitive capabilities in insects despite their small brains. Here, we studied innovation, defined as the capacity to solve a new task, of a solitary bee (Osmia cornuta) in the laboratory by exposing naive individuals to an obstacle removal task. We also studied the underlying cognitive and non-cognitive mechanisms through a battery of experimental tests designed to measure associative learning, exploration, shyness and activity levels. We found that solitary bees can innovate, with 11 of 29 individuals (38%) being able to solve a new task consisting of lifting a lid to reach a reward. However, the propensity to innovate was uncorrelated with the measured learning capacity, but increased with exploration, boldness and activity. These results provide solid evidence that non-social insects can solve new tasks, and highlight the importance of interpreting innovation in the light of non-cognitive processes.

KEY WORDS: Behaviour, Behavioural plasticity, Cognitive ability, Task solving, Learning, Osmia cornuta

INTRODUCTION
Animals exhibit an extraordinarily wide repertoire of behaviours. Bees, for example, have developed a broad range of sophisticated behaviours that facilitate foraging, nesting, navigation and communication (Roulston and Goodell, 2011; Loukola et al., 2017). Although the ecological and evolutionary importance of behaviour is widely recognized, our current understanding of how new behaviours emerge is insufficiently understood. Some simple behaviours have a clear genetic basis, and hence may have been acquired through mutation and natural selection. Studies in Drosophila show, for example, that a mutation in a single neuropeptide caused several abnormalities in their behavioural circadian rhythms (i.e. biological clocks; Renn et al., 1999). However, the accumulation of mutations seems insufficient to explain the emergence of new behaviours. Rather, the development of novel behaviours from more simple cognitive processes requires the processing of new knowledge by means of experience to guide decision making (Dukas, 2008). The emergence of new learnt behaviours is a process known as behavioural innovation (Ramsey et al., 2007; Lefebvre et al., 2004; Reader, 2003; Sol, 2003).

The concept of innovation has attracted considerable interest because of its broad implications for ecology and evolution (Ramsey et al., 2007; Lefebvre et al., 2004; Reader, 2003; Sol, 2003). Innovation suggests the possibility of constructing plastic behavioural responses to novel ecological challenges, thereby potentially enhancing the fitness of the individual animals when exposed to unusual or novel situations. For instance, evidence is accumulating that innovation ability enhances the success of animals when introduced to novel environments (Sol et al., 2005). By changing the relationship of individuals with the environment, innovative behaviours also have great potential to influence the evolutionary responses of the population to selective pressures (Lefebvre et al., 2004; Reader et al., 2016). Hence, in the context of global change, innovative behaviours are considered central to understanding how animals will respond to rapid changes induced by human activities.

While innovation is considered to be one of the main processes behind the emergence of novel behaviours in vertebrates (Reader, 2003; Ramsey et al., 2007), the relevance of innovation is currently insufficiently understood in insects. Insect behaviour has been studied for many years (e.g. Maeterlinck, 1901), and the traditional notion holds that it tends to be relatively inflexible and stereotypical. This perception partially arises because of their small brains and smaller number of neurons than in more studied taxa like mammals or birds (Dukas, 2008). Such a belief is, however, changing as evidence accumulates of unsuspected sophisticated capabilities beyond the most simple associative learning that transcend basic forms of cognition (Avargues-Weber et al., 2012), including rule learning (Gil et al., 2007), numerosity (Howard et al., 2018; Howard et al., 2019; Dacke and Srinivasan, 2008), cultural transmission (Alem et al., 2016) and exploratory learning (Menzel and Giurfa, 2001; Degen et al., 2016). Even adaptations to global change have been observed recently, such as solitary bees cutting plastic bags instead of leaves to build their nests (Allasino et al., 2019). The fact that insects exhibit sophisticated responses to new situations suggests that this new behaviours may also be commonly acquired through the process of innovation.

Here, we addressed the critical questions of whether insects are capable of innovation and how they achieve it. For example, innovation may be a result of trial and error, rather than a more complex cognitive process. We used a solitary common bee – Osmia cornuta (Megachilidae) – as a model system to address these questions. While our current understanding of cognition in solitary bees is limited in comparison to that of eusocial species (e.g. Chittka

1Estación Biológica de Doñana (EBD-CSIC), Avd. America Vespuccio 26, 41092 Sevilla, Spain. 2CREAF (Centre for Ecological Research and Applied Forestries), Cerdanyola del Vallès, Catalonia E-08193, Spain. 3Freie Universität Berlin, Institut für Biologie – Neurobiologie, König-Luise-Str. 28/30, 14195 Berlin, Germany. 4CSIC (Consejo Superior de Investigaciones Científicas), Cerdanyola del Vallès, Catalonia E-08193, Spain.

*Author for correspondence (xmiguelangelcolladox@gmail.com)

M.A.C., 0000-0002-4216-317X; I.B., 0000-0001-7893-4389

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and Thompson, 2009), solitary bees are easy to rear and manipulate in captivity (Jin et al., 2015). An advantage of solitary bees is that they can be tested individually for innovative propensity without having to consider the pitfalls of separating individuals from the social group. Importantly, solitary bees comprise most of the bee fauna and are suffering worldwide population declines associated with rapid human-induced environmental change (Goulson et al., 2017), posing at risk the essential pollination services that they provide for cultivated crops and wild plants (Ollerton et al., 2011). Thus, there is an urgent need to assess whether and how they are capable of innovation to cope with new environmental challenges.

The capacity to innovate is difficult to measure directly (Lefebvre et al., 2004), but one widely adopted approach is the use of problem-solving experiments motivated by a food reward (Bouchard et al., 2007; Griffin et al., 2014). In our experiments, we exposed naive O. cornuta bees to a novel task consisting of lifting a lid to reach a food reward, an assay that mimics the encounter of a new complex flower. Whether or not individuals solve the task and the latency to do so can be used as measures of innovation performance (Sol et al., 2011). Because some bees were capable of innovation, we investigated the underlying mechanisms. We first explored whether the propensity to innovate reflects a domain-general ability to learn. Hence, we related our measures of innovation performance to measures of performance in a simple associative learning test. Next, we tested the effect of a number of emotional and state-dependent intrinsic features that are suspected to either facilitate or inhibit innovation (Reader, 2003; Houston and McNamara, 1999; Sol et al., 2012), including exploration, shyness and activity levels. We finally considered whether problem-solving ability might be explained by sex, an additional intrinsic parameter (Houston and McNamara, 1999). In O. cornuta, females are more involved in parental activities (e.g. they are in charge of all nest provisioning activities) and are typically larger than males (Bosch, 1994). These fundamental differences in the biology and ecology between sexes are expected to affect how they deal with novel challenges, potentially affecting their problem-solving ability.

**MATERIALS AND METHODS**

**Study subjects**

Osmia cornuta (Latreille 1805) cocoons were bought from the company WAB-Mauerbienenzucht (Konstanz, Germany) and kept cold at 4°C. Before and during the experiments, cocoons were put in 15 ml Falcon tubes in a pitch black environment and kept in an incubator at 26°C for 24–48 h until the emergence of offspring. In total, 101 females and 42 males were born, and used in the experiments. In order to force bees to walk instead of fly, we anaesthetized them with a cold shock treatment and cut their right wings (Jin et al., 2014; Crook, 2013). Walking bees have been used in behavioural essays before, showing consistent responses (Jin et al., 2014).

**Experimental device**

We conducted the experiments in a controlled environment laboratory at the Institut für Biologie–Neurobiologie (Freie Universität Berlin) from February to April 2017. Behavioural assays were conducted in an experimental device composed of two parts, the ‘arena’ (Fig. 1A) and the ‘dome’ (Fig. 1B). The arena was a 30×30×10 cm empty methacrylate rectangular prism with no roof, containing a piece of grey cardboard as the floor and placed over a wooden structure. The dome was a dark brown upside-down plastic flowerpot, illuminated homogeneously with attached LED lamps. The dome covered the arena to create a controlled environment for the experiments. We attached different geometrical figure patterns in the inside walls to facilitate the orientation of the bees during the tests (Jin et al., 2014). The dome had a hole in the roof to attach a video camera to record the tests. Citral odour was perfused evenly across the assays. In assay 1 (C), the bees started inside a refuge. The aim of the assay was to see whether the bee stayed in the refuge (as a shyness proxy), and/or explored the colour cues around it. In assays 2 and 3 (D), the bee was exposed to two drinking stalks, one rewarded and the other empty. The colour was randomly selected but maintained across the assays. In assay 4 (E), the learning test, the display was the same as in assay 2 and 3, but this time we removed the reward and both stalks were empty. In assay 5 (F), the innovation test, the display was the same as in assay 2 and 3, but this time we covered the reward with a lid, forcing the bee to innovate to lift the lid to access the reward.
and restored regularly, as it is known to stimulate bumblebees, and probably other bees, during foraging (Lunau, 1991; Shearer and Boch, 1966).

**Experimental protocol**

Over 3 days, each individual took part in a sequence of 5 behavioural assays (Fig. 1C–F) of 15 min each designed to measure five different behaviours: exploration, shyness, activity, learning and innovation (see Table 1). We waited 4 h between trials if the next trial was done the same day and around 16 h if the next trial needed to be done the next day (Fig. 1C–F). First, we defined innovation as the ability to solve a problem in a new situation, i.e. lifting a cardboard cue in a new environment. Because the mechanisms behind innovation are complex and we do not know what may be driving it, we controlled for related behaviours within and in independent tests. Activity, measured as the proportion of time in movement, not resting, was measured for every trial. Individuals did not show any correlation in their activity levels over the trials (Fig. S1) and, therefore, we did not estimate a single average activity value for each individual. Activity levels did not decrease over the trials (linear model Activity~Trial, estimate±s.e.=0.003±0.008, $P=0.718$). Note that not every bee survived to perform all the assays; only 45% of the individuals that started the experiment reached the final assay. Although individuals were not fed during the experimental process other than during the trials, the lack of correlation between the number of feeding events and activity rates during the learning test (Pearson correlation=−0.09) or the innovation test (Pearson correlation=−0.01) suggests that this high mortality is not attributable to starvation.

The first assay aimed at measuring exploration and shyness. Note that these tests represent snapshots of bee behaviour, and as such should be interpreted as transient behaviours for which the stability over time is unknown. The arena included four coloured cardboard cues (2 blue and 2 yellow; Fig. 1C). The bee was placed in a little cardboard refuge and was kept inside it for 5 min to allow habituation. Next, the refuge was opened and the individual was allowed to explore the arena. To quantify exploration, we recorded whether the bee touched all the cardboard cues during the assay and the time it took to do so. Shyness was measured as the initial time spent inside the refuge (Table 1). Re-entering the refuge was originally thought to be an indicator of shyness; however, analysis of the videos showed that bees did not re-enter the refuge to stay inside and hide, but rather did so as part of their arena exploration.

The second and third assays were the associative learning assays, where we trained bees to associate a colour with a reward (Fig. 1D). The individuals started all tests inside a black opaque box cover that was lifted at the start of the experiment. We displayed two cardboard cues with drinking stalks on them, one rewarded with 50% sucrose solution and the other empty. Blue and yellow cardboard are well discriminated by bees (Vorobyev and Menzel, 1999; Hempel de Ibarra et al., 2014). Hence, the reward for each individual was randomly assigned to one of these two colours for both trials and we let individuals explore the stalks and eat *ad libitum* over a period of 15 min. The position (left or right) of the reward was randomly assigned for each individual in each trial.

In the fourth assay, the learning test, we tested whether individuals had learned to associate colours with rewards as trained. The test consisted of both cues displayed as in the second and third assays, but this time with both stalks empty (Fig. 1E). We measured whether the individuals approached the formerly rewarded coloured cue and quantified the time it took to do so. Otherwise, if the bee first checked the wrong feeder, we considered this as a failure in the learning test. To ensure that bees had learned to associate colour and reward, we switched the colour of the rewarded stalk between the two learning assays in 36 randomly selected individuals (hereafter, ‘control’ group). While more sophisticated general learning abilities could not be measured, associative learning is a widespread elemental skill in insects which can be easily measured and serves as a first-order test for the importance of simple learning processes.

In the final assay, we measured the propensity for innovation by using the same coloured cue and reward combination as in assays 2 and 3, but this time the stalk containing the reward was covered with a cardboard lid (Fig. 1F). Bees had thus to innovate, i.e. lift the cardboard, to reach the reward. Innovation propensity was measured in terms of innovation success and latency to innovate (Table 1).

Control bees used in the learning assays were not tested for innovation.

**Data analysis**

We modelled problem-solving performance in the innovation assay as a function of associative learning, shyness, exploration and activity (see Table 1 for definitions). We first modelled the success or failure in solving the task using a Bayesian generalized linear model with a Bernoulli family and a logit link (Package *brms*; Bürkner, 2017). Second, to model the latency to solve the task, we instead used survival analyses based on Cox proportional hazards regressions for continuous predictors (Cox and Oakes, 2018; Table 2). Survival analysis allowed us to account for censored data, as some individuals did not pass the test within the stipulated time frame. Hence, for individuals not solving a particular task (e.g.

### Table 1. All variables measured during the tests

<table>
<thead>
<tr>
<th>Behavioural component</th>
<th>Behavioural variable</th>
<th>Assay</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shyness</td>
<td>Latency to exit the refuge</td>
<td>1</td>
<td>Initial time spent inside the cardboard refuge once the assay started</td>
</tr>
<tr>
<td>Exploration</td>
<td>Exploration success</td>
<td>1</td>
<td>Touching the four cardboard pieces during the 15 min assay</td>
</tr>
<tr>
<td></td>
<td>Latency to explore the full arena</td>
<td>1</td>
<td>Latency to touch all four cardboard pieces in assay 1. Bees that did not explore the four cardboard pieces were assigned the maximum time possible (15 min).</td>
</tr>
<tr>
<td>Activity</td>
<td>Activity time</td>
<td>1–5</td>
<td>Time spent moving, measured as the proportion of time active (from 0 to 1)</td>
</tr>
<tr>
<td>Learning</td>
<td>Learning success</td>
<td>4</td>
<td>Choice of the correct cue (yes/no).</td>
</tr>
<tr>
<td></td>
<td>Latency to learn</td>
<td>4</td>
<td>Latency to make the correct choice. Bees that failed the test were assigned the maximum time possible (15 min).</td>
</tr>
<tr>
<td>Innovation</td>
<td>Innovation success</td>
<td>5</td>
<td>Success in lifting the lid and reaching the reward</td>
</tr>
<tr>
<td></td>
<td>Latency to innovate</td>
<td>5</td>
<td>Latency to open the lid and reach the reward. Bees that did not solve the problem were assigned the maximum time possible (15 min).</td>
</tr>
</tbody>
</table>

Bold indicates variables selected as predictors for the innovation analyses.
Innovation-like behaviours have previously been observed in wild solitary bees. These include the use of new materials for nesting (Allasino et al., 2019) and anecdotal examples of bees nesting in new places, such as cardboard, wooden blocks (Bosch and Kemp, 2001) or Styrofoam blocks (MacIvor and Moore, 2013). However, innovation in the context of problem solving had never been demonstrated before in controlled laboratory experiments with solitary bees. Here, we show that 38% of the tested individuals were able to lift the lid, and this was mainly achieved by using their mandibles or head, and not by accidentally walking around the spur.

Although innovation is generally believed to be a dimension of domain-general cognition (Lefebvre et al., 2004), we did not find
evidence that individuals that were better at associative learning solved the innovation task faster. The failure to relate innovation and associative learning does not simply reflect that we studied associative learning over shorter training periods, as success in the learning test was comparable with that found in previous similar experiments using more training days (e.g. Jin et al., 2014; Jin et al., 2015). However, associative learning is a simple elemental skill available to most animal species, even with the simplest nervous systems (Zhang et al., 2005), and perhaps more sophisticated general learning abilities (Bouchard et al., 2007) show a different connection with innovation capacity.

Regardless, a more likely explanation is that other factors are more relevant for innovation and may have masked the effect of associative learning. Indeed, we found consistent differences between fast and slow innovators in their tendency to approach and explore the experimental apparatus. Specifically, individuals that were able to lift the lid to access the food reward tended to be bolder and to explore more slowly than those that failed to solve the task. As suggested for other taxa, there may be a trade-off between exploration speed and accuracy, which could translate into how information is processed. For example, in great tits (*Parus major*), fast explorers return more quickly to previously experienced foraging patches whereas slow explorers prefer to seek new information or update old information close to the feeders (Matthysen et al., 2010). Boldness and exploration have previously been identified as important determinants of innovation propensity in vertebrates and highlight that innovation propensity may largely reflect particular motivational states or emotional responses of individuals to novel situations rather than cognitive differences (Sol et al., 2013). To what degree our measured single values of boldness and exploration are stable in time or more transient behaviours remains to be tested. In line with this conclusion, successful innovators also exhibited higher activity levels. Activity may reflect motivation to feed, which in other animals has been found to be a major determinant of innovation propensity (e.g. Sol et al., 2013). However, it may also increase the chance of solving the task accidentally by trial and error. However, closed environmental spaces can also be stressful and what we defined as ‘fast exploring’ could be a by-product of stereotyped stress behaviours. In any case, we found large variation in how bees approach and/or solve the task, meaning that there are probably individual differences in task solving.

The lack of support for the importance of associative learning does not mean that innovation does not require learning. Learning is...
not only necessary to fix the new behaviour in the individual repertoire (Ramsey et al., 2007; Lefebvre et al., 2004; Reader, 2003; Sol, 2003) but also important to solve the task itself. Indeed, we found that bees that succeeded in the innovation test went directly towards the lid covering the reward, probably reflecting that they had learnt the rewarding colour during training assays. In our assays, several individuals tested were able to associate colour and reward – after only two training trials – regardless of their differences in shyness, exploration and activity. Thus, the lack of effect of learning ability on innovation might reflect that most individuals were similarly proficient in associative learning. Testing other more sophisticated learning domains would be important to advance our knowledge in this direction.

Learning is widely held to have important advantages in the wild. In bees, learning is critically important for vital tasks such as foraging, identification of high-quality foraging sites, finding the right mixture of nectar and pollen, and navigating back to the nest for brood provisioning (Roulston and Goodell, 2011; Minckley et al., 2013). Surprisingly, we found intriguing sex-related differences in learning. Males showed a tendency to perform better in the associative learning test than females. This is unexpected because females have to deal with more tasks during their lifetime, including foraging and nest provisioning, and may perhaps indicate that the cognitive demands for males to locate females are higher than suspected. However, it is known that males and females can vary in problem solving in other taxa (Hopper et al., 2014; Lucon-Xiccato et al., 2020).

Our results suggest that solitary bees can accommodate their behaviour to novel contexts through innovative behaviours. In the context of global change, the ability to rapidly adapt behaviour to novel contexts seems highly relevant. In novel environments, bees must, for instance, solve how to forage on new plant species, which sometimes presents complex flowers with which bees have not co-evolved (Bartomeus et al., 2010). Therefore, we should abandon the notion that insect behaviour is inflexible and stereotypical, and better appreciate that insects can readily adjust their behaviour to changing conditions through innovation and learning.

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Competing interests

The authors declare no competing or financial interests.

Author contributions


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Supplementary information

Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.232058.supplemental

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