



Do bumble bees play?

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A variety of animals have been found to interact with and manipulate inanimate objects 'just for fun', that is, to play. Most clear examples of object play come from mammals and birds. However, whether insects interact with inanimate objects as a form of play has never been systematically examined. Here, we show that rolling of wooden balls by bumble bees, *Bombus terrestris*, fulfils behavioural criteria for animal play and is akin to play in other animals. We found that ball rolling (1) did not contribute to immediate survival strategies, (2) was intrinsically rewarding, (3) differed from functional behaviour in form, (4) was repeated but not stereotyped, and (5) was initiated under stress-free conditions. Through the design of the experiment and with the support of behavioural observations, we excluded the possibilities that ball rolling was driven by exploration for food, clutter clearing or mating. Similar to vertebrate play, we also found age and sex differences for ball rolling by bumble bees: younger bees rolled more balls than older bees and male bees rolled individual balls for longer durations than females. We explicitly show that ball rolling is itself a rewarding activity. After being trained to find freely movable balls in one of two differently coloured chambers, bees showed a preference for the colour of the chamber where they had rolled balls. Our results contribute to the question of sentience in insects and lend further support for the existence of positive affective states in these animals.

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Play is not limited to humans, but is a phenomenon seen across many animal species (Groos, 1898). It is thought to contribute to the healthy development and maintenance of an animal's cognitive and motor abilities, which may, for example, benefit foraging strategies, and is considered an important aspect of animal welfare (Held & Špinka, 2011; Kaplan, 2020; Nahallage et al., 2016; Pellegrini & Smith, 2005). Most clear examples of play come from large-brained mammals and birds, whereas systematic investigations of play in other animals are very limited (Burghardt, 2005; Zylinski, 2015). This may be because it has been historically difficult to define and investigate play behaviour across different animals (Burghardt, 2005; Caro, 1988; Groos, 1898; Pellegrini & Smith, 1998; Pellis & Pellis, 2009).

Five criteria have been laid out to establish a framework for investigating the widely observed phenomenon of play across species (Burghardt, 2005). According to these criteria, play is recognized as a behaviour that is not fully functional in the

context it is expressed in and so does not result in an obvious, immediate, adaptive outcome (Criterion 1), for example the behaviour should not be done to obtain food, mates or shelter. Play is voluntary, spontaneous or rewarding in and of itself (Criterion 2), and therefore the behaviour should not require an association with another reward (e.g. food) to be performed. A play behaviour should also differ from immediately functional behaviour in form (Criterion 3). This means that the motor actions for the behaviour are different from those used, for example, when searching for food or attempting to mate. Play is repeated, but not stereotyped (Criterion 4), which separates it from one-off chance occurrences and habitual ticks. Finally, stress can prevent animals playing or temporarily pause play, although play can also reduce stress in the short term (e.g. in primates, Mustoe et al., 2014; Norscia & Palagi, 2011). Therefore, play is understood to be a pleasant phenomenon (Held & Špinka, 2011) that is initiated when an animal is in a relaxed state (Criterion 5). This is to distinguish play from other seemingly functionless behaviours that are induced by stress, such as repetitive pacing or rocking as observed in caged zoo animals.

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Play behaviour can be generally separated into three major categories: social, locomotor and object play, although multiple categories may occur simultaneously (e.g. social object play). Social play comprises playful interactions between animals, usually between juvenile conspecifics, such as play fighting (Pellis & Pellis, 2017). Locomotor play involves intense and sustained body movements, such as running and jumping, without any apparent need to do so (e.g. Miller & Byers, 1991). Object play relates to inanimate objects (e.g. Heinrich & Smolker, 1998). Previous explorations of play behaviour by insects include anecdotal evidence for social play in ants (*Formica rufa*, Huber, 1820) and a study in young wasps (*Polistes dominulus*, Dappporto et al., 2006), both on behaviours analogous to play fighting. Here we investigated object play in bumble bees.

In a previous study it was found that bees can be trained to roll balls to gain access to a reward (Loukola et al., 2017). During the execution of this experiment, the team observed that bumble bees would often roll balls for no apparent benefit. Between experiments, balls were placed in the tunnel that connected the hive to the arena that contained food. Despite there being enough space to avoid the balls, bumble bees often seemingly unnecessarily walked over and rolled balls on their way to and from food. This observation provided the motivation for the current study, where we examined whether ball rolling fulfils the major criteria for animal play and how this behaviour relates to similar object play behaviour in other animals.

Common ultimate functions for playing in other animals include improved cognitive and motor skills, while proximate mechanisms consist of positive affective states (Burgdorf & Panksepp, 2006; Held & Špinková, 2011). An important task requiring cognitive and motor skills in bumble bees is flower handling to extract nectar and pollen. Bees become faster at handling flowers with experience (Chittka & Thomson, 1997; Laverty & Plowright, 1988), meaning that they learn and improve their motor or problem-solving skills needed to access the food reward. Animals that require motor learning for food extraction, such as in nonhuman primates, are more likely to show object play behaviour during development (Bjorklund & Gardiner, 2010). If the adaptive benefits of play included practising skills for the future, bumble bees may have the potential to show object play because they require handling skills to extract food from flowers and such learning may benefit from previous practice through an unrelated, but intrinsically rewarding task. Alternatively, bumble bees may be predisposed to handle objects other than flowers because object manipulation is an important skill for their survival. Do bumble bees have the potential to show a play-like behaviour that is 'pleasurable'? Bumble bees have been shown to have positive emotion-like states (Solvi et al., 2016); therefore, although any play behaviour expressed may be rudimentary in its associated 'pleasurable' state, the activity may be somewhat rewarding for it to be engaged. If bumble bees show play-like behaviour, this has implications for our understanding of the dimensions of insect sentience.

METHODS

Ethical Note

No licences or permits were required to run these experiments. There is no legislation protecting bumble bees or official guidelines for looking after bumble bees in research. However, we followed the 3Rs principles (Russell & Burch, 1959) taking care of the bumble bees as outlined in Housing Conditions and Animal Care below.

Housing Conditions and Animal Care

Experiments were conducted inside the laboratory. Commercial bumble bee, *Bombus terrestris audax*, colonies were transferred in a

red-lit dark room to a bicompartimentalized wooden box: individual bumble bees were allowed to climb onto forceps to minimize handling stress and were transported and placed inside the wooden box. Individuals that did not climb onto forceps were gently grasped by the side of the thorax or legs with forceps during transfer. Colonies were allowed to acclimatize to the new nestbox for at least 2 days from transfer before starting the experiment. Bumble bees naturally build and maintain their nests in the ground, for example in covered abandoned rodent nests. Bumble bee nests in the laboratory were kept in wooden boxes covered to simulate a natural nesting environment, with a compartment containing the nest and a separate accessible compartment with cat litter for bees to discard waste. Each of the two compartments of the wooden nestbox had two windows covered by metal mesh providing ventilation. The compartment connecting the nestbox to the experimental arena had an additional opening leading to the tunnel attached to the experimental arena. Bees are intrinsically motivated to leave the nestbox to search for food (or mates in the case of males). Bees were handled during tagging when newly emerged before they were able to leave the nest to forage. Therefore, during experiments, bees entered the experimental arena and returned to the nest without any handling by the experimenters. During the experiments, sucrose solution and pollen were provided ad libitum. Sucrose solution was freshly made every 2–3 days using boiled water and caster sugar. Pollen was kept fresh by conserving it in the freezer. After each experimental session, honeypots in the nest were refilled to ensure the colony would be well fed until the next session. Handling was minimized and constant access to food was given to prevent any stress to the colony. The laboratory was illuminated with a 12:12 h light:dark/day:night cycle to maintain a natural circadian rhythm. Illumination was provided using Activa daylight full-spectrum fluorescent tubes (Osram Sylvania, Wilmington, NC, U.S.A.) and a black light tube (F36T8 BLB, Osram Sylvania) for additional strength in the UV. All were fitted with high-frequency ballasts (4.3 kHz; HF-B 236 TLD; Philips, Amsterdam, The Netherlands). Acrylic sheets used to cover experimental set-ups were UV-transmitting. These lighting conditions are most suited for bee vision considering their photoreceptor processing speed (Skorupski & Chittka, 2010) and spectral sensitivity (Skorupski et al., 2007).

Experiment 1: Do Bumble Bees Engage in Play-like Object Manipulation?

Study animals

Bumble bees from one colony (Koppert Biological Systems, Berkel, The Netherlands) were individually tagged ($N = 45$) using number tags (Opalith Zeichenplättchen Leuchtfarben, Bienen-Voigt & Warnholz, Ellerau, Germany). To monitor the age of the bees during the experiment, individuals were tagged when callow, within 24 h of emerging from the larval cell, identifiable by the silvery fur which later turns black and yellow (Goulson, 2010). Ages of bees ranged from 1 to 23 days from emergence during the experiment (females: 1–23 days; males: 1–17 days). The sex of individual bees was also identified: 28 female and 17 male bees were observed in the experiment. Subjects were allowed to forage for food during the experiment. To ensure that experience outside the nest was always monitored, bees were not allowed to leave the nestbox unless the experiment was running. Therefore, after an experimental session each day, remaining empty honeypots were manually filled with a pipette daily and 7 g of pollen dropped onto the nest every 2 days to ensure bees were well supplied with food between experimental sessions. This was done to prevent any stress to the colony that could potentially arise from a lack of food supplies in the hive while waiting for the next experimental session the following day.

Objects

Eighteen wooden balls (diameter = 15 mm) were used in the experiment. Twelve balls were spray painted (Plasti-kote, Valspar, Minneapolis, MN, U.S.A.) yellow or purple. The remaining six balls were left with their original wooden colour. All 18 balls were also plastic coated (Plasti-kote) to enable cleaning with water and 70% ethanol, to remove any scent cues that may have been left by bees each day.

Set-up

The hive was connected to an arena via an acrylic tunnel (25.5 × 3.5 cm and 3.5 cm high; Fig. 1). The arena (47 × 25 cm and 12 cm high) was partitioned by a wall to form an area containing objects and a feeding area providing ad libitum sucrose solution (30%) and pulverized pollen (Koppert Biological Systems). The acrylic tunnel led to an unobstructed path (17 × 4 cm) between the immobile object area on the left (as a bee left the hive) and the mobile object area on the right (17 × 7 cm each). The boundaries of the object areas were marked by ca. 3 mm high barriers made of solidified hot glue to stop objects from entering the clear path. When the bees entered the arena, they had the options of walking through the unobstructed path to reach the feeders or deviating from this path into the areas with immobile and mobile balls. A 2.2 cm high ceiling covered the object area, and a higher 12 cm ceiling covered the foraging area where bees could also fly. The dimensions of the arena were selected so that we could videorecord all the activities by the bees during foraging and object manipulation simultaneously, keeping bee number tags detectable.

Experimental procedure

The experiment was run for 3 h every day for 18 days. During this time, bees were given access to the arena containing both objects and feeders. Before starting each experimental day, all objects and the experimental arena were cleaned with water first and 70% ethanol to remove any odour cues left by subjects following object manipulation. Nine balls (three of each colour) were pseudorandomly placed in both the mobile and immobile areas, where the objects in the immobile object area were made stationary by fastening them to the floor with a small amount of adhesive material (Blu Tack, Bostik, Leicester, U.K.) for easy removal and cleaning at the

end of the experiment. Food was provided in a separate feeding area in feeders where the bees fed at ground level. A clear rubber tube was used to refill sucrose solution (30% by weight) into the feeder from outside the experimental set-up to minimize disturbance to bees during the experiment. The relative position of sucrose and pollen in the foraging area (left or right of the entrance to the feeding area) were swapped every experimental day. The activity of bees throughout the experiment was recorded with an iPhone 5 (Apple Inc, Cupertino, CA, U.S.A.). Videos were recorded to include the object area and foraging area simultaneously.

Video analysis

Videos were analysed for the following information: number of foraging bouts, number of entries into each object area, number of action types performed in each object area and the duration, distance and colour of each ball rolled. Durations of ball-rolling actions were recorded using Solomon coder beta 17.03.22 software (2019, by András Péter, Hungary). Distances of balls rolled were extracted by drawing tracks of each ball's movement when being rolled by a bee using the 'pencil' tool on Solomon coder beta 17.03.22 software. The track was started at the centre of the ball when the bee made contact with the ball. The track was drawn manually at frames every 0.2 s, while keeping the pencil tool at the centre of the ball. Mating attempts between males and females in the object arena were recorded as indicators of motivation to mate in males to be compared with object manipulation. The image of each track was then processed in MatLab (Mathworks, Natick, MA, U.S.A.) to extract the length in pixels and then converted to millimetres. The scale from pixels to millimetres was extracted by drawing the diameter of a ball, of known size, using the Solomon 'pencil' tool. The number of foraging bouts was estimated by sampling the video material: the number tag of each bee drinking at the sucrose feeder was recorded for 2 min (1 min before and after) every sixth minute mark. For this experiment, observer 1 (H.S.G.D.) extracted the number of ball-rolling actions and their durations. Observer 2 (A.K.) watched all videos again and extracted the ball-rolling tracks resulting in 100% of the video material being checked by a second observer. To monitor interobserver reliability, observer 1 extracted a proportion of data collected by observer 2 and vice versa. Intra-class correlation coefficients (ICC) to compare behavioural

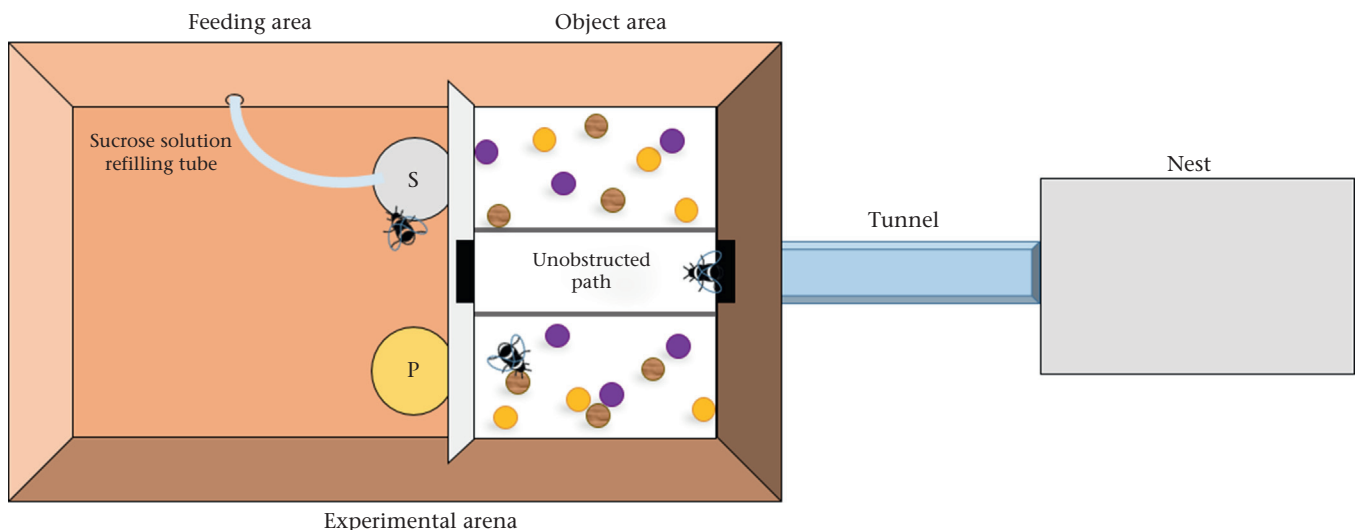


Figure 1. Experimental set-up. Aerial view of the section of the experimental arena containing the foraging area and object area. A nestbox containing a bumble bee colony was connected to an arena via a plastic tunnel. The tunnel led to an unobstructed path in the object area with coloured objects on the sides of the path: nine mobile balls on the right and nine immobile balls on the left. The path led to the foraging area which contained ad libitum 30% sucrose (S) and ground pollen (P). Sucrose was refilled externally through a tube. The location of the sucrose and pollen were alternated every day to avoid bees developing a side bias.

observations between experimenters were calculated using a two-way mixed model with absolute agreement (Landers, 2015) on IBM SPSS statistics 27 for Windows (2020, IBM Corp, Armonk, NY, U.S.A.). Observer 1 drew a random proportion of 10% of the tracks (92/910 tracks) drawn by observer 2 ($\text{ICC}(2,2) = 0.998$). For experiment 2, observer 1 extracted the number of ball-rolling actions from videos. Observer 2 extracted 20% of the data from videos under blind conditions, unaware of which tagged bees belonged to a given age group ($\text{ICC}(2,2) = 0.85$).

Behaviours recorded

Entries into the areas with mobile and immobile balls were recorded. An entry was defined as a bee crossing the barrier from the clear path into an object area. Object interactions were actions where bees touched a ball with their antennae and/or front legs. Ball rolling was defined as when an individual bee was on top of a ball and, while maintaining contact with the ball, rotated it along the floor (Fig. 2, Supplementary Video S1). In addition, a bee's head and body axis had to be in line with a ball during their approach and for the ball-rolling action to be at least 0.4 s. This was to help ensure the ball-rolling action was 'intentional', that is, not an accidental contact with a ball during the bee's regular locomotion.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.anbehav.2022.08.013>.

Experiment 2: Does Age Influence Engagement in Ball-rolling Activity?

A second experiment was run to test the effect of age versus novelty of balls on the number of balls rolled by bees. Bees (46 workers) were tagged within 1 day of emergence from the larval cell (eclosion) to monitor individuals' age (days from eclosion) during the experiment. Bees were tagged daily as they emerged

from the pupae so that a sufficient number of bees were at least 10 days old by the start of the experiment. We had two groups of bees in the experiment: young bees (≤ 3 days old, $N = 13$) and older bees (> 10 days old, $N = 33$). The age groups were chosen based on the ball-rolling activity as a function of age found in experiment 1 (see Results), so that the young group's age was earlier than the peak of ball-rolling activity (3 days old) and the older group's age was after the peak (9 days old). The experimental set-up was similar to that used in experiment 1 (Fig. 1), where bees had access to an arena containing a clear path leading to the foraging area and ad libitum sucrose and pollen. On either side of the clear path were two mobile object areas, each one containing nine movable balls. Bees were given access to the arena with the feeders and object areas for 3 h per day, and the experiment ran for 10 days. The number of ball-rolling actions was recorded for all number-tagged, age-identified bees.

Experiment 3: Can Ball-rolling Behaviour Act as an Unconditioned Stimulus?

Set-up

The nestbox was connected to a chamber (17×13 cm and 2 cm high; Fig. 3) via an acrylic tunnel (25.5×3.5 cm and 3.5 cm high). Two different chambers were used in the experiment, each with its internal walls and floors coloured either yellow or blue. The entrance of a chamber contained two baffles (2×2 cm) so that bees could not see from the acrylic tunnel whether a chamber contained balls. The chamber was connected, via another acrylic tunnel (9×3.5 cm and 3.5 cm high), to an arena (40×34 cm and 15 cm high) containing ad libitum 30% sucrose solution and ground pollen (Koppert Biological Systems). The arena and chambers were covered in clear, UV-transmitting acrylic sheets. Blue and yellow were chosen because bees can easily distinguish these colours from



Figure 2. Ball-rolling action. The nine panels show the sequence of a ball-rolling action over time lasting, in this instance, approximately 4 s (time stamps in red at top left). The bee (a) approaches the wooden coloured ball while facing it, (b) touches the ball with her forelegs, (c) holds onto the ball using all of her legs, (d–h) rolls the ball past the yellow ball and (i) finally detaches from and leaves the ball. See Supplementary Video S1 (speed $\times 0.5$).

one another (Gumbert et al., 1999). All internal walls were laminated to enable cleaning with water and 70% ethanol, to remove any scent cues left by bees each day.

Experimental procedure

Training. Female bees ($N = 42$) of known age (range on test day 4–19 days from emergence), from four colonies, were used in the experiment. A conditioned place preference paradigm was used. Tagged bees were given access to the feeding arena through a coloured chamber (blue or yellow) for 4 h per day on 2 consecutive days. Chambers were cleaned with 70% ethanol at the start of the experiment. One coloured chamber was presented and swapped with the other coloured chamber every 20 min. This was done to expose bees to both coloured chambers for equal durations. Only one of the coloured chambers contained balls throughout training. One group of bees ($N = 21$) experienced balls in the yellow chamber and a second group of bees ($N = 21$) experienced balls in the blue chamber. The number of balls rolled by individual bees was recorded.

Testing. At the end of the second day of training each bee was given a choice between both chambers at the same time, as shown in Fig. 4. Prior to this preference test, an individual bee was sequestered between two sliding barriers in the tunnel until the chambers were put in place. A choice for a chamber was defined as the first time the bee passed the first baffle. Chambers were cleaned with 70% ethanol between tests. Balls were never presented in either chamber in the test condition, but the baffles at the entrance of the chambers prevented bees from seeing whether the chambers contained objects. Both training and testing sessions were video-recorded. Half of the bees were tested with yellow on the left and blue on the right and half of the bees with the opposite colour side contingency.

Statistical Analysis

For experiments 1 and 2, data handling, calculations and statistics were performed with MatLab R2018a. A Wilcoxon signed-rank test was used for pairwise comparisons to test object

mobility preference. Where samples were independent and sizes differed between groups being compared, a Wilcoxon rank sum test was used; this was used to test for age group (younger and older) and sex differences. A Friedman test was performed to assess ball colour preference. To assess whether ball-rolling activity was associated with chamber colour in experiment 3, we used generalized linear mixed models (GLMM) and generalized linear models (GLM) with a binomial distribution and logit function, using R 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria) with the 'lme4' package. A full GLMM model included the following fixed factors: colour associated with ball rolling during conditioning (blue, yellow), age (days from eclosion), last colour experienced before testing (blue, yellow) and chosen chamber side during the test (left, right). Colony was included as a random factor. Factors other than the colour associated with ball rolling during training had no effect on the colour chosen in the test. Removing these factors from the GLMM improved the Akaike's information criterion (Akaike, 1974) resulting in a better ranking model.

RESULTS

In three different experiments, we examined whether ball-rolling behaviour by bumble bees (Fig. 2, Supplementary Video S1) fulfils the established criteria for play (Burghardt, 2005) and how ball rolling resembles object play in other animals.

Experiment 1: Do Bumble Bees Engage in Play-like Object Manipulation?

A total of 910 ball-rolling actions by 45 bumble bees were recorded. Individual bees rolled balls between 1 and 44 times on an experimental day, and between 1 and 117 times across the whole duration of the experiment. Most bees (37/45) rolled balls for at least an additional day after feeding in the foraging area and 29 bees for at least 2 additional days after feeding.

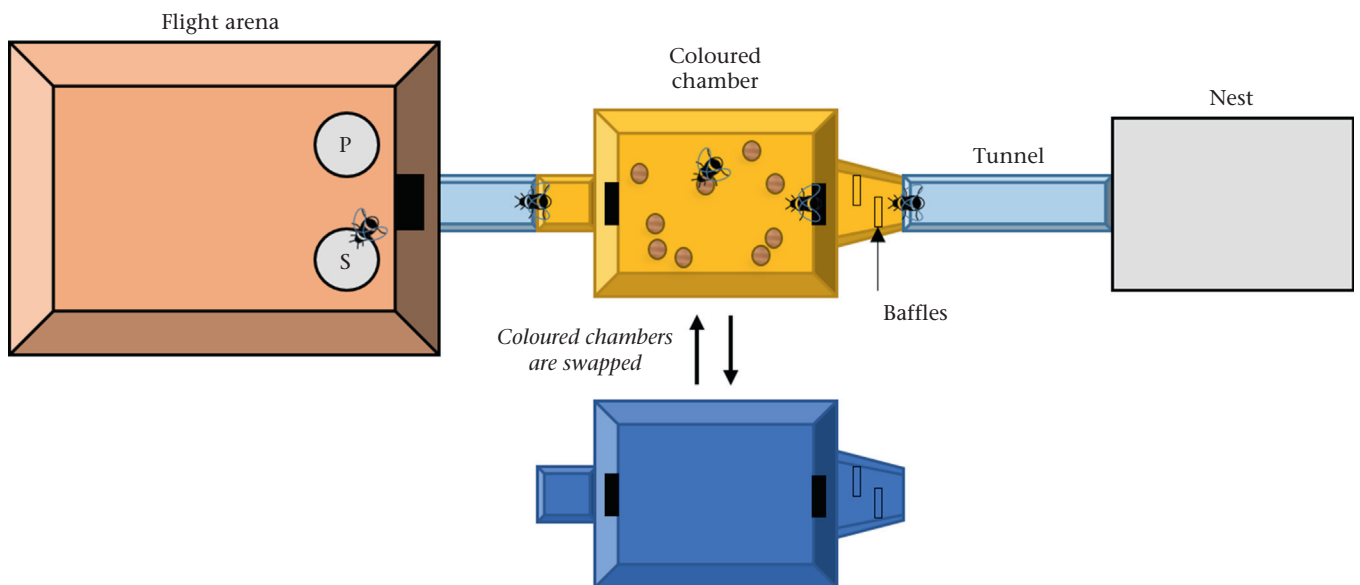


Figure 3. Experimental set-up for the training stage (aerial view). A nest was connected to a coloured chamber via a tunnel. The chamber was connected to a flight arena with feeders providing ad libitum sucrose (S) or pollen (P); their positions were swapped each experimental day. The coloured training chamber was either yellow or blue. One of the coloured chambers contained movable balls and the other was empty. Baffles at the entrance of the coloured chamber prevented bees seeing the presence/absence of objects. Only one coloured chamber was presented at a time and they were alternated every 20 min (six times each) for a total of 2 h exposure for each colour. One group of bees was trained with the yellow chamber containing balls and the other group with the blue chamber containing balls. This experimental stage was carried out on 2 consecutive days for each bee.

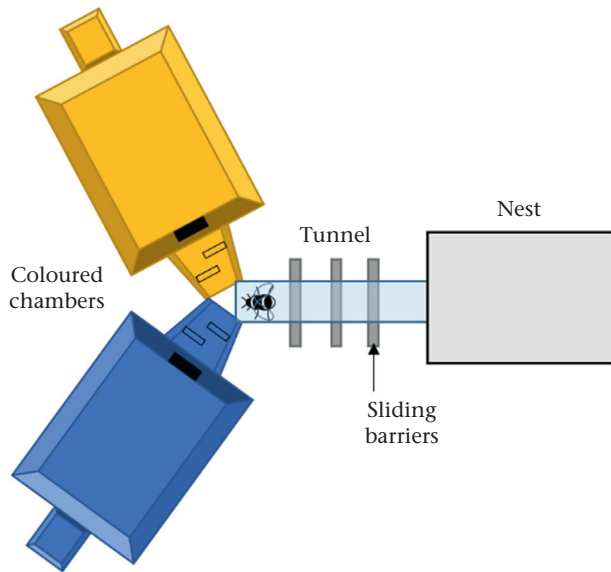


Figure 4. Experimental set-up for the testing stage (aerial view). Bees were tested on the second day of training, after the second training session. A single bee was allowed to exit the tunnel from the nest on the second day with the use of sliding barriers to limit access. At the exit, bees were presented with the two empty coloured chambers simultaneously. Baffles at the entrance of each chamber prevented bees from seeing whether chambers contained objects or not.

Fig. 5 shows the number of entries into each object area (total $N = 2636$) before and after a bee's first ball-rolling experience, with mobile balls or immobile balls ($N = 45$ bees). Before a ball-rolling experience, there was no significant difference between entries into the immobile object area (total entries 99, mean \pm SE = 2.2 ± 0.4 entries, $N = 45$) and the mobile object area (total

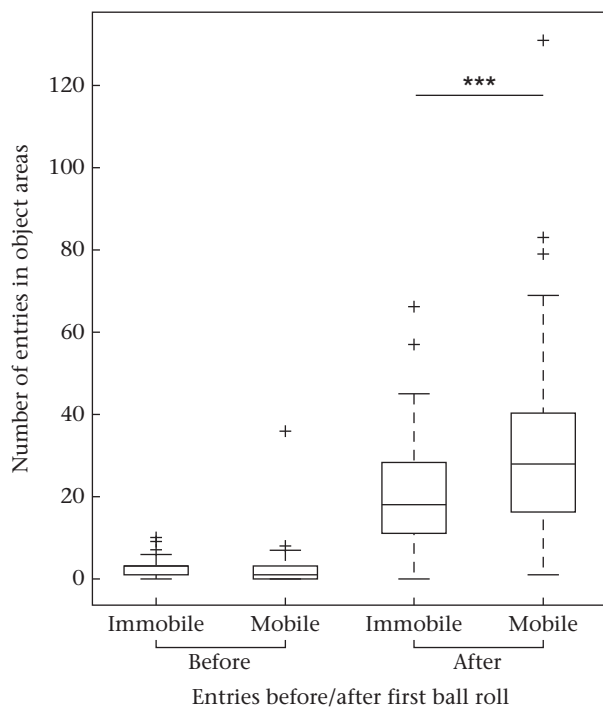


Figure 5. Object area preference before and after the first ball-rolling experience. The total number of entries into the immobile object area and mobile object area for each bee is shown, before and after their first ball-rolling experience. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the crosses are outliers. *** $P < 0.001$.

entries 118, mean \pm SE = 2.6 ± 0.8 entries, $N = 45$; Wilcoxon signed-rank test: $Z = -0.47$, $P = 0.63$). However, after their first instance of ball rolling, bees were more likely to enter the mobile object area (total entries 1467, mean \pm SE = 32.6 ± 3.7 entries, $N = 45$) than the immobile object area (total entries 952, mean \pm SE = 21.1 ± 2.2 entries, $N = 45$; Wilcoxon signed-rank test: $Z = 5.12$, $P < 0.001$). After rolling at least one ball and subsequently leaving the mobile area, a bee was more likely to re-enter and interact with a ball (any direct contact with the antennae or legs to the ball, including ball rolling) in the mobile area than to enter and interact with a ball in the immobile area (mean \pm SE = $62.1 \pm 3.7\%$ entries, $N = 45$; Wilcoxon signed-rank test: $Z = 3.17$, $P = 0.001$). Individuals showed no preference for any particular ball colour presented (Friedman test: $\chi^2 = 3.47$, $P = 0.18$).

The durations of each instance of ball rolling and the distances over which the balls were rolled were also recorded. Individual ball-rolling actions lasted from 0.4 to 31 s (Fig. 6a) and for distances from 2 to 601 mm. These measures were used to assess the variability of the actions performed within and between individuals. Changes in duration corresponded to changes in distance over the course of repeated ball-rolling actions, resulting in no improvement in speed with experience (Fig. 6b; Pearson correlation: $r = -0.39$, $N = 22$, $P = 0.06$). Samples of ball-rolling tracks are shown in Fig. 6c.

Bees rolled balls more when younger, particularly when aged 3–7 days from eclosion (Fig. 7a and b). We compared the ball-rolling activity over age with the foraging activity to determine whether the pattern of object manipulation was a result of a general increase in activity related to foraging. We found that foraging activity followed a different pattern to ball-rolling activity (Fig. 7). Overall, foraging activity seemed to increase over age for females, whereas ball-rolling activity peaked earlier and then decreased (Fig. 7a). Males showed similar patterns to females for ball rolling, but much less foraging activity (Fig. 7b). These results suggest that bees had a different motivation to move objects compared to that of foraging.

Experiment 2: Does Age Influence Engagement in Ball-rolling Activity?

Experiment 1 did not allow us to separate the effect of age from a possible effect of novelty of balls. In other words, it may be that no matter what age a bee is, the first time they come in contact with balls they will show the same pattern of ball rolling over a few days. In experiment 2 we tested whether the observed increase and decrease in ball rolling was due to age or novelty. Over the experiment, younger bees (at most 3 days old at the start of the experiment; 11/13 bees rolled balls) displayed significantly more ball-rolling actions (mean \pm SE = 11 ± 3.7) than older bees (at least 10 days old; 14/33 bees rolled balls; mean \pm SE = 4 ± 1.4 ; Wilcoxon rank sum test: $Z = 2.30$, $P = 0.02$; Fig. 8). Therefore, we can be more confident that the ball-rolling activity was seen more in younger bees and engagement in the behaviour was age dependent, similar to many other animals that play.

The age at which ball-rolling activities peaked differed between males (at age 5, mean \pm SE = 4.2 ± 1.6 balls rolled) and females (at age 3, mean \pm SE = 4.1 ± 1.9 balls rolled), but there was no difference in the peak number of balls rolled (Wilcoxon rank sum test: $Z = -1.14$, $P = 0.25$; Fig. 7). Male bees performed individual ball-rolling actions for longer (mean \pm SE = 4.8 ± 0.7 s; Fig. 9a) than female bees (mean \pm SE = 1.7 ± 0.1 s; Wilcoxon rank sum test: $Z = -4.67$, $P < 0.0001$). A similar pattern was observed for the distances of balls rolled, where males rolled for greater distances (mean \pm SE = 106 ± 15 mm) than females (mean \pm SE = 40 ± 4 mm; Fig. 9b; Wilcoxon rank sum test: $Z = -4.48$, $P < 0.0001$).

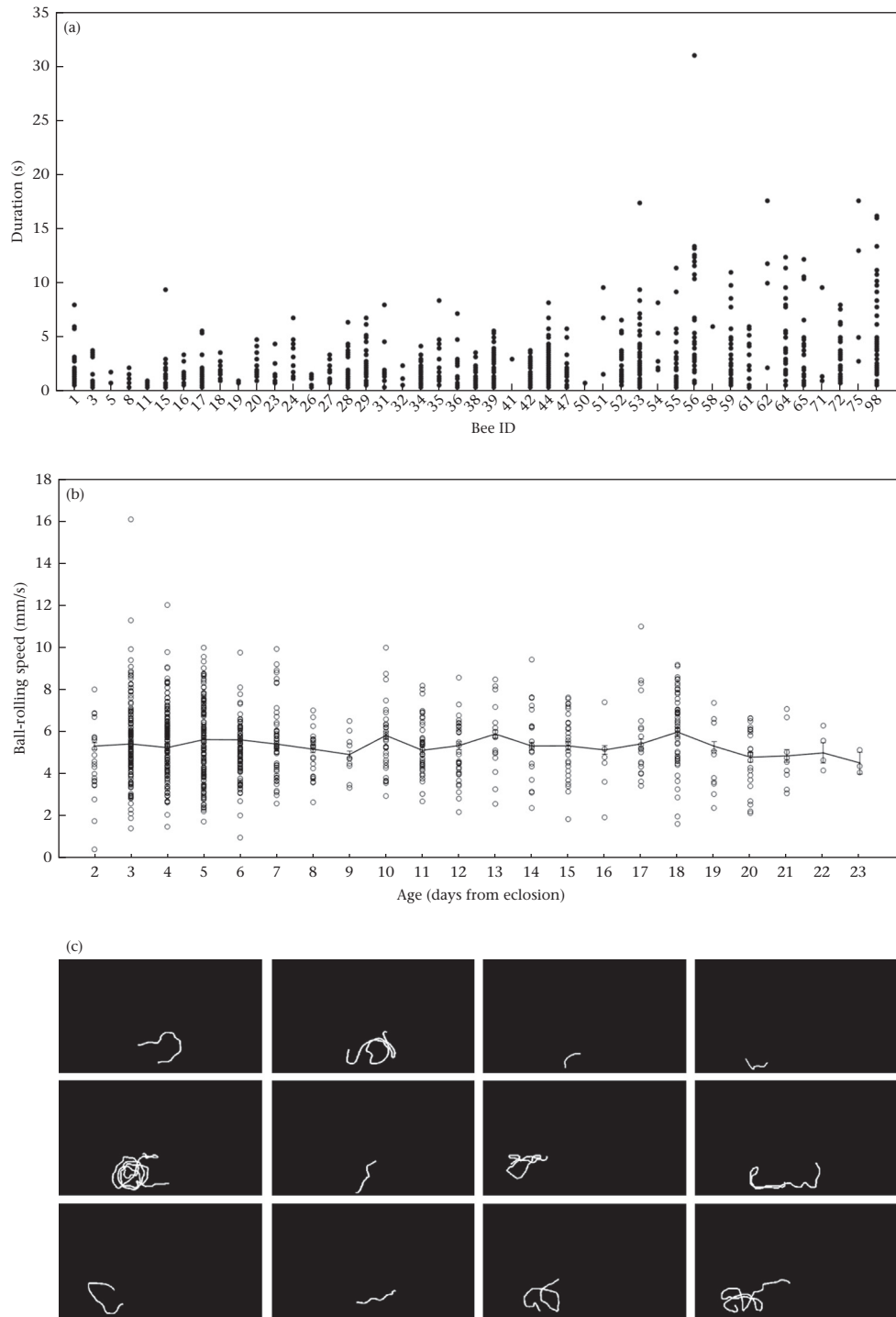


Figure 6. Durations, speeds and track samples of ball-rolling activity indicative of variation in ball rolling within and between bees. (a) Durations of individual ball-rolling actions for each bee. Each filled circle shows the rolling of one ball by a given bee. (b) Average speeds of ball-rolling actions over experience. An average speed was calculated for each bee for each day of age. The average across bees is shown by the curve. Each circle shows the speed of individual ball-rolling actions by all bees. (c) Sample tracks of ball-rolling activity by bee 56 (male). Each panel contains an individual track showing where the bee travelled while rolling a single ball. The perimeter of each panel is indicative of the object arena.

Experiment 3: Can Ball-rolling Behaviour Act as an Unconditioned Stimulus?

To further examine whether ball rolling is inherently rewarding, we asked whether it could act as an unconditioned stimulus. Bees were given access to two coloured chambers, one always containing moving balls and one without any objects (see [Methods](#)). In a

subsequent choice test with both chambers available, bees showed a preference for the colour of the chamber previously associated with balls (GLM: estimate = 1.696, SE = 0.761, $Z = 2.228$, $P = 0.026$). Despite an obvious overall bias for yellow ([Fig. 10](#)), which chamber was associated with ball rolling had a clear effect on bees' choices during the test. These results again suggest that bumble bees find ball rolling rewarding.

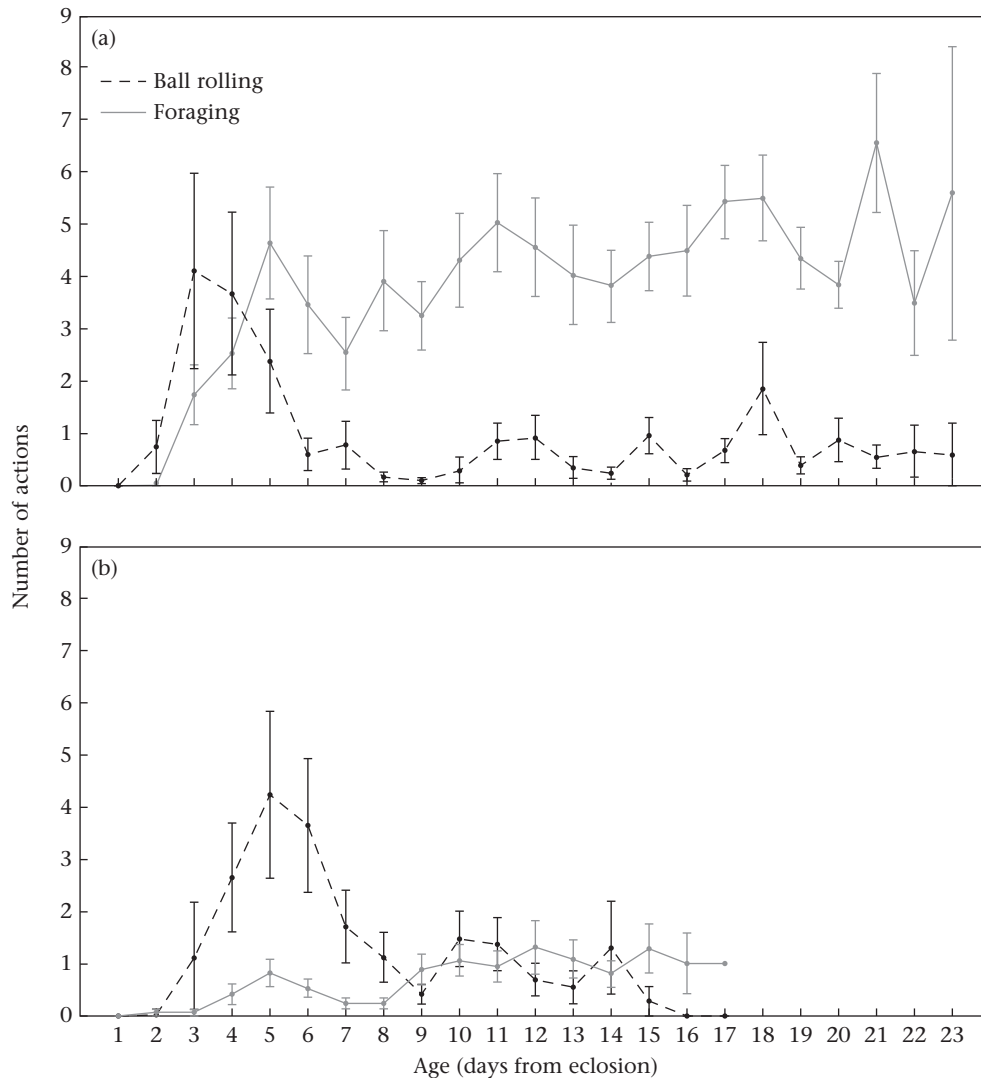


Figure 7. Ball-rolling activity and foraging activity over age for (a) female and (b) male bees. The number of actions (\pm SE) for each day since the emergence from the larval cell is shown. The average is based on the total number of balls rolled or total estimated number of foraging bouts per bee on that day of age divided by the number of bees that reached that age in the experiment.

DISCUSSION

Animal play is a widely observed phenomenon; however, most examples come from juvenile mammals and birds. Play actions are repeated by individuals but lack an apparent immediate outcome in the context in which it is expressed. Instead, play appears to be performed and repeated due to its enjoyable/rewarding nature. Play-like behaviour in invertebrates has rarely been reported. Here we have shown that a seemingly functionless behaviour, ball rolling, by an insect fulfils the criteria of animal play. In addition, we found age and sex differences similar to those in mammals (further discussed below), for the first time in an invertebrate. We begin our discussion by examining how bees' behaviour in this study compares with the criteria for play as established by Burghardt (2005).

Ball Rolling is Not Immediately Functional (Criterion 1)

It is in the nature of play to co-opt motor actions from existing functional behaviours within an animal's behavioural repertoire (Burghardt, 2005; Lorenz, 1956). However, in assessing whether a

particular behaviour is done for play, we must determine whether that behaviour helps with immediate survival needs.

Could ball rolling in bumble bees be explained by a searching behaviour for food? Where reward is constant, it is advantageous for bees to visit the same food source rather than visit other flower types (Keasar et al., 2013; Oster & Heinrich, 1976). In fact, once bumble bees locate a nectar source, they usually fly straight to it and rarely explore other areas (Woodgate et al., 2016). Furthermore, bees quickly decrease their visits to flowers that do not provide nectar/pollen when they can find other flowers that do (Evans & Raine, 2014; Heinrich et al., 1977).

Ball rolling in our experiments never resulted in the provision of food, the balls were not visible from where bees collected pollen or nectar; the bees had a clear path between the nest and food area, and bees were not required to come in contact with the balls while foraging (see Methods and Fig. 1). However, bees rolled balls repeatedly; even after finding the continuous supply of nectar and pollen in the separate foraging area, they continued to enter the ball areas and roll balls by diverging from a direct path to food. If ball rolling was an act related to foraging, we should expect to see the temporal patterns of foraging and ball rolling over bees' age and

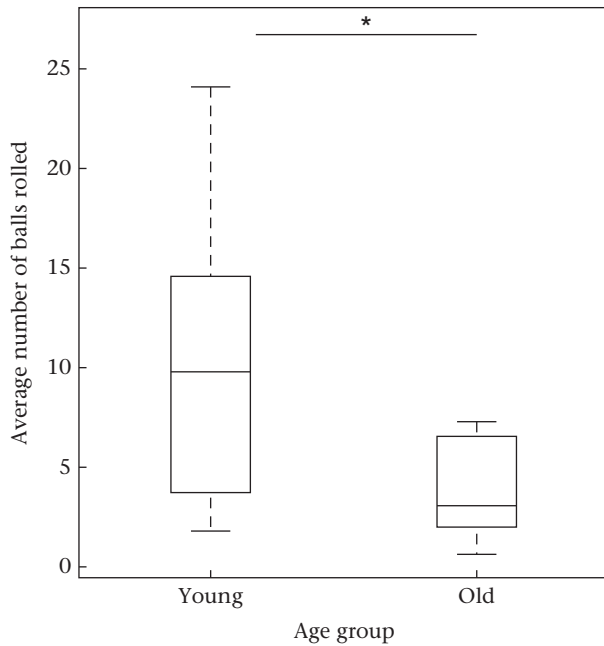


Figure 8. Number of ball-rolling actions by young and older bees. The young group of bees was 3 days old or less from emergence and older bees were over 10 days old from emergence at the start of the experiment. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range. * $P < 0.05$.

experience (Fig. 7) to overlap closely. In contrast, for females (the foragers of the hive) ball rolling increased prior to foraging; then, after foraging rose sharply, on average, ball rolling decreased as foraging continued to rise gently with age. For males (which do not forage for the colony), ball-rolling activity was decisively higher than foraging for the first 8 days of age.

Additionally, if bees were searching for food, we should have seen evidence of behaviours indicative of flower handling and searching for nectar/pollen at the balls (Lavery, 1980; Leadbeater & Chittka, 2008). However, we observed no instances of bees extending their proboscis while in contact with balls, or biting them, suggesting they did not view them as potential food sources. If ball rolling was simply a result of exploring for food, ball-rolling frequency should have decreased quickly after the initial exploration. In contrast, bumble bees' ball-rolling activity actually increased over a few days and then decreased, a temporal pattern that is more similar to play than exploration in mammals (Hutt, 1966; Vanderschuren et al., 1995). Together, these results suggest a search for food was not driving ball-rolling behaviour.

Could ball rolling be the result of bees clearing clutter? Object relocation, such as the removal of dead adults and larvae from the nest or movement of debris within the nest, is naturally performed by bumble bees (Munday & Brown, 2018). Therefore, it may be that ball rolling was a result of bees attempting to remove objects from what they considered their hive space. However, balls were located well outside the nest and in sequestered areas which provided a direct decluttered path from the nest to the food sources. Furthermore, previous work has shown that bees can control the direction of movable balls a specific location (Loukola et al., 2017). Individual tracks of the ball-rolling activity from experiment 1 show that the balls were taken in various directions, including towards the path, and bees often continued to roll the balls even when they had reached the outer walls. These observations suggest that bumble bees were not rolling balls in an attempt to clear clutter.

Could ball rolling by male bees be an attempt to mate? When attempting to mate, male bumble bees position themselves on top of a female and, while holding onto her with their legs, try to insert their genitalia (Amin et al., 2012; Djegham et al., 1994) which are otherwise contained inside the abdomen. Therefore, if males were attempting to mate with the balls, we should have seen instances of these actions. However, we never observed male bees everting their genitalia while in contact with the balls. This perhaps should not be surprising, as male *Bombus pratorum* have been shown to be more likely to interact with queens than with workers and far less likely to attempt to mate with wooden objects shaped like bees (Free, 1971). Further, in experiment 1, a total of only 16 mating attempts were observed, all with female bumble bees. Of these, two occurred in the mobile ball area, four in the immobile ball area and 10 in the pathway to the feeders). Comparing these to the males' 528 interactions (any direct physical contact) with balls, including 330 ball-rolling actions, in the mobile ball area, suggests that the motivation to interact with balls was distinct from attempting to mate.

The seemingly functionless ball-rolling activity is analogous to well-studied cases of solitary object play in mammals such as stone handling in macaques (*Macaca* spp.; Nahallage et al., 2016; Pelletier et al., 2017) and 'rock juggling' in various species of otters (Allison et al., 2020; Bandini, 2021). In both examples, stones are held and repeatedly manipulated and do not result in individuals gaining any immediate material reward similar to what we observed when bumble bees rolled balls.

Ball Rolling is Voluntary, Spontaneous and Inherently Rewarding (Criterion 2)

In experiment 1, bees were given a clear path from the nest to the pollen and nectar food sources and consequently could easily avoid contact with any balls throughout the experiment, suggesting the act of ball rolling resulted from some type of intentionality. As discussed above, balls never provided any food for the bees and there was no evidence that bees viewed the balls as potential food sources or mates, yet they rolled balls repeatedly. In addition, in experiment 1, bees developed a preference for the area with mobile balls over immobile balls (Fig. 5), supporting the idea that ball rolling is inherently attractive above and beyond other physical characteristics of the balls. Social play was shown to be rewarding for rats, *Rattus norvegicus*, by training and testing them in a conditioned place preference paradigm (Calcagnetti & Schechter, 1992). In experiment 3, using a similar paradigm, we showed that bees' preference between two differently coloured chambers was shifted towards the chamber where they had experienced ball rolling (Fig. 10). Together, these results support the notion that ball rolling is rewarding in and of itself.

Ball Rolling Differs from More Adaptive Behaviour in Form (Criterion 3)

Activities that include some type of object manipulation for bees include flower handling during foraging, mating and defensive behaviours. Ball rolling differs in form from foraging on flowers in that rotating an object is not part of the flower-handling repertoire. Moreover, as noted above, we observed no instances of proboscis extension or biting (actions involved in feeding from flowers) while bees were in contact with the balls. While ball rolling, bees partially enveloped the ball, which may resemble positions taken when attempting to mate or sting when defending their nest or in an agitated state. However, we observed no instances of genitalia eversion by males (Amin et al., 2012; Djegham et al., 1994), defensive buzzing (Pritchard & Vallejo-Marín, 2020) or stinger

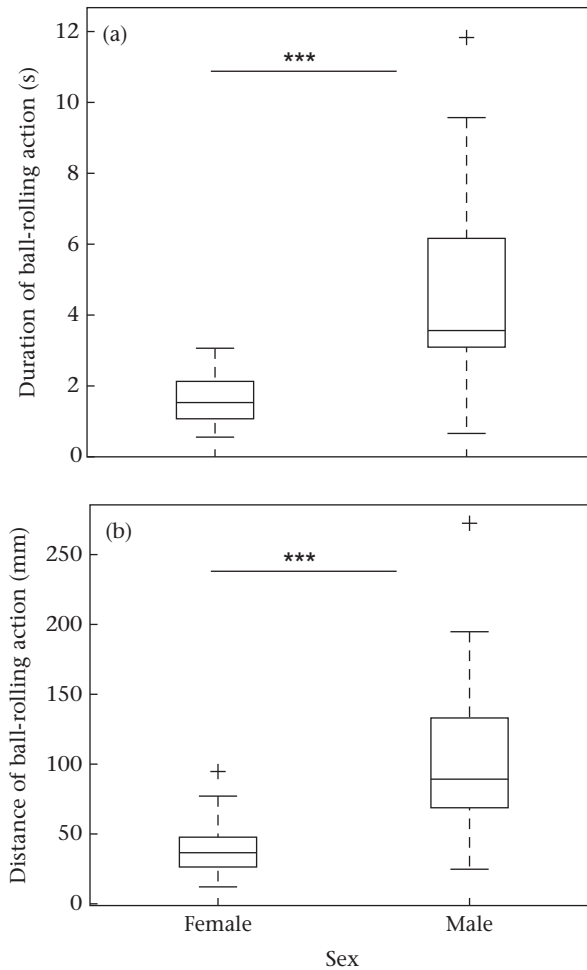


Figure 9. Sex differences in ball-rolling (a) duration and (b) distance. The average rolling duration or distance of all ball-rolling actions was calculated for each bee in the female ($N = 28$) and male ($N = 17$) groups. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range and the crosses are outliers. *** $P < 0.0001$.

extension by females while in contact with the balls. Therefore, the motor patterns involved in ball rolling are structurally different from more adaptive activities in bumble bees' normal repertoire.

With goal-oriented behaviour, such as flower handling, bees tend to become better with experience (Chittka & Thomson, 1997; Laverty & Plowright, 1988). If bees were interacting with the balls in a way that related to a more adaptive functional behaviour, then we should expect that the overall action should become more efficient. A reduction in the time taken to complete a behaviour with increased experience can be used as a measure of improvement of that behaviour (Thorndike, 1898). However, we found no improvement in the speed at which balls were rolled for any bee, additionally suggesting that ball rolling is not a goal-directed behaviour (Fig. 6b), but more indicative of play.

Ball Rolling is Repeated but not Stereotyped (Criterion 4)

Stereotypy is behaviour that seems functionless and is repeated but, unlike play, is unchanging and often observed in animals housed in suboptimal living conditions (Mason & Latham, 2004). For example, a common stereotypy indicative of stress which is seen in captive bears is pacing (Vickery & Mason, 2004). The pacing follows a predictable unvaried pattern at every repetition (such as

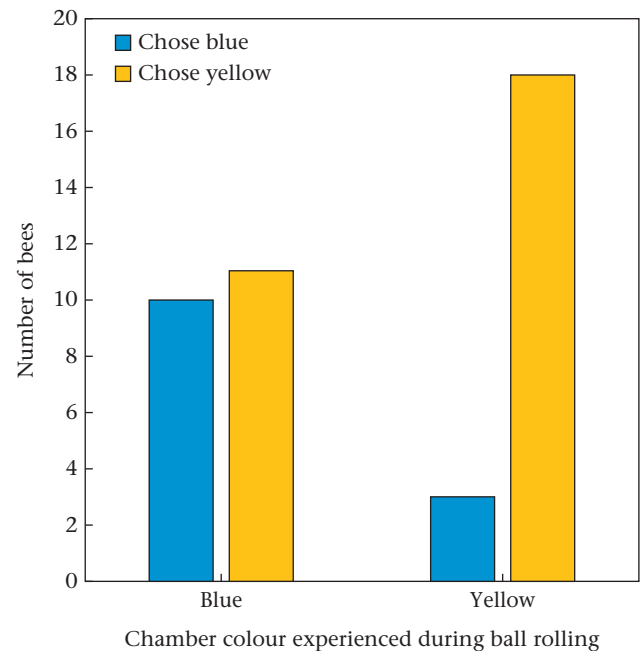


Figure 10. First chamber colour chosen by bees during the test. One group of bees experienced ball-rolling activity only in the yellow chamber and no objects in the blue chamber ($N = 21$). The second group of bees experienced the reversed conditions ($N = 21$).

one cage length), for example a straight line, circle or figure-of-eight, repeating the same number of steps, placing the feet at the same locations during the walk (Vickery & Mason, 2004). To assess whether ball rolling by bees differs from stereotypy, we looked at the characteristics of each ball roll. We found a large variation in the durations and distances of ball rolls within and across individuals (Fig. 6), and that ball rolling occurred irregularly across days. These results suggest that ball rolling lacks the characteristics of stereotyped behaviour.

Ball Rolling Occurs when Bees are Not Stressed (Criterion 5)

In rodents, stressful factors such as food deprivation (Baldwin & Baldwin, 2010), restraint by handling (Romeo et al., 2006) or predator odour (Siviy et al., 2006) have been shown to reduce or temporarily pause play activities. In a troop of captive Japanese macaques, *Macaca fuscata*, a medical examination by the researchers, low environmental temperatures and experiencing aggression by fellow troop members also temporarily paused or reduced engagement with stone handling (Nahallage & Huffman, 2008). These factors disrupted the animals' typical behaviour, supporting the view that stress can affect play. Although mild stress may initiate play that in turn reduces stress, severe stress can induce nonplay-related stereotypy as described above (under criterion 4). To ensure that any object manipulation was not stress-induced, we minimized factors that may disrupt typical bee behaviour, including food deprivation (Couvillon & Dornhaus, 2010), handling (grasping, shaking) that would mimic a predator threat (Bateson et al., 2011; Ings & Chittka, 2008) and social isolation (Maleszka et al., 2009). To remove any potential stress, without disturbing any of the bees, we provided constant access to food via ad libitum feeders of nectar and pollen during experiments and refilled honeypots outside of experiments (Methods). Bees were not handled during the experiments, were given free access to all the areas during the experiments and were not isolated. Furthermore, bees did not display lethargic movement, a symptom of

starvation, or defensive buzzing resulting from a perceived threat (Pritchard & Vallejo-Marín, 2020). As a result of these precautions, as outlined in Housing Conditions and Animal Care (Methods), we believe it is unlikely that ball rolling was a stress-induced behaviour. We cannot exclude that object play in bumble bees occurs following a lack of stimulation that would otherwise be provided by the natural environment. Captive animals are provided with play opportunities as enrichment to maintain cognitive and physical stimulation (Tarou & Bashaw, 2007). Further research is needed to identify motivations driving ball rolling in bumble bees. However, irrespective of what causes bumble bees to roll balls, we report that they show the capacity for solitary object play behaviour and that reward acts as a proximate mechanism.

Temporal Pattern of Ball Rolling as a Function of Age

Although play behaviour can continue to be expressed in adulthood (Behncke, 2015; Hall, 1998; Leca et al., 2007; Nahallage & Huffman, 2007), play tends to be displayed more by juveniles in species previously studied. For example, nonhuman primates such as Japanese macaques handle stones when young and continue to do so when adult (Nahallage & Huffman, 2007). However, in many cases where mammalian play is predominantly a juvenile activity, the temporal pattern shows an increase and then a decrease during the juvenile stage of development (Byers, 1998; Byers & Walker, 1995). The results of experiment 1 show that bees were more likely to roll balls when younger, particularly when aged 3–7 days from eclosion (Fig. 7), independent of their experience with balls (Fig. 8). The pattern of ball-rolling activity over age in bees (an increase then decrease) has been found for play in many young mammals (Bekoff, 1974; Byers, 1998; Byers & Walker, 1995; Caro, 1995; Kahlenberg & Wrangham, 2010; Maestriperieri & Ross, 2004; Sharpe, 2005). This is the first evidence of age-dependent play behaviour in invertebrates. A possible ultimate cause associated with juvenile play was presented by Byers and Walker (1995) who proposed that the age distribution and peak of play activity in young rodents and cats coincides with the time frame in which the brain, particularly the cerebellum, is most plastic. This means that play may provide the experience necessary to fine-tune synapses in the brain and develop motor skills necessary for later in life during a developmentally sensitive phase (Berghänel et al., 2015). Interestingly, there is evidence that bumble bee mushroom bodies (areas of the insect brain responsible for sensory information processing and memory formation; Menzel, 2014) show most plasticity during the first 7 days after eclosion (Jones et al., 2013; Kraft et al., 2019). Whether the temporal overlap of play and neural plasticity in any animal are causally linked will require further research; however, bumble bees may provide a tractable model for such enquiries.

Differences in Ball Rolling Between the Sexes

Sex differences in play can be found in some mammals (Berghänel et al., 2015; Jamieson & Armitage, 2010; Nahallage et al., 2016; Pellis et al., 1997), for example female Kanyawara chimpanzees, *Pan troglodytes*, of Uganda have been observed to cradle sticks until they have young of their own, resembling children's doll play. This occurs more commonly in females than males (Kahlenberg & Wrangham, 2010). In our study, male bumble bees were found to roll individual balls longer than females. Unlike female bumble bees, males do not supply the colony with food, that is, they forage entirely for themselves (Goulson, 2010). Shortly after emergence, bumble bee males tend to leave the nest and do not typically return, instead searching for queens with which to mate (Kraus et al., 2009). In our experiments, no virgin queens were present. In

contrast, females had constant access to pollen and nectar, providing ample opportunity to carry out their prime objective for the colony. The motivation for carrying out functional activities may limit how long individuals can engage with a play activity at any one time (Burghardt, 2005). Therefore, perhaps the differences observed in ball rolling between male and female bumble bees were because males had more 'free time' in laboratory conditions, that is, had less motivation to stop ball rolling. Future research should examine whether play-like actions occur in the wild, and whether there are any benefits of play-like behaviour to performance in later activities, such as flower handling or mating behaviour (Berghänel et al., 2015; Brown & Baer, 2005; Chittka & Thomson, 1997; Pellis & Pellis, 2009).

Conclusions

In this study, we systematically described a behavioural phenomenon in bumble bees resembling object play. Bees rolled inedible coloured balls repeatedly. This activity did not result in an apparent immediate function, such as gaining food; however, bees' repeated interactions with balls suggest that the behaviour was rewarding. This rewarding aspect of ball rolling was further supported by bees' ability to form a positive association between a neutral-coloured stimulus and ball rolling. The amount of ball-rolling activity varied within and between individuals, showing that the behaviour was not stereotyped over repetitions. Similar to vertebrate play, age and sex differences were found where younger workers and male bees rolled balls more often and for longer, respectively. We suggest that the behaviour observed here has actual hedonic value for bumble bees, which adds to the growing body of evidence of a form of sentience in these insects (Bateson, 2014; Birch, 2020; Held & Špinková, 2011; Solvi et al., 2016). Further work should explore the possible ultimate advantages of such behaviour, and the ways in which play behaviour might benefit early brain development.

Author Contributions

H. S. Galpayage Dona: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Visualization. **C. Solvi:** Conceptualization, Methodology, Formal Analysis, Writing – Review and Editing, Supervision. **A. Kowalewska:** Investigation. **K. Mäkelä:** Investigation. **H. MaBouDi:** Conceptualization, Methodology. **L. Chittka:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding Acquisition.

Data Availability

Data will be made available on request.

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