Bumblebees and cooperation in a laboratory environment

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Abstract

Cooperation is a diverse behavioral phenomenon prevalent in many animal species, and has been the subject matter for many studies over the past decades. In only few places in nature is cooperation more closely tied to the life of an animal than within the colonies of eusocial insects, and the details of invertebrate cooperative behaviors are still largely unstudied. In this study I aim to determine whether bumblebees can understand its partners role during a cooperative task. To this end, I trained bumblebees to push Lego blocks and bricks, both alone and in pairs, to determine their capacity for teamwork and then exposed them to testing scenarios which allowed for the behaviors of the test subjects to be monitored when their pair was delayed or completely absent. I found that the individuals trained for cooperative tasks hesitated noticeably to attempt the task if their partner was not available and that they preferred to attempt the task where the set-up resembled the state which would yield a reward the most, optimistically substituting an object for their pair. Novel behavior was also recorded during testing, which might imply greater understanding of cooperative tasks in few individuals.

Tiivistelmä

Yhteistyö on monimuotoinen käyttäytymisilmiö, joka on havaittavissa monissa eläinlajeissa ja joka on ollut monien tutkimuksien aihe viimeisten vuosikymmenien aikana. Vain harvoissa paikoissa luonnossa on yhteistyö yhtä tiiviisti sitoutunutta eläimen elämään kuin eusosiaalisten hyönteisten yhteisöissä, ja selkärangattomien yhteistyöliitännäiset käytökset ovat yhä pitkälti tutkimattomia. Tässä tutkimuksessa pyrin selvittämään, kykeneekö kimalainen ymmärtämään koekumppaninsa roolin yhteistyötä vaativissa tehtävissä. Tätä varten koulutin kimalaisia työntämään erikokoisia Legopalikoita yhdessä ja yksinään selvittääkseni niiden kapasiteetin yhteistyöhön ja altistin nämä sen jälkeen eri koeasetelmille, jotta näiden käytöstä voitaisiin seurata, kun kumppanin saapuminen joko viivästettiin tai estettiin kokonaan. Havaitsin että yksilöt, jotka koulutettiin työskentelemään pareissa epäröivät huomattavasti tehtävän ratkaisemisen yrittämistä, ja suosivat yrityksissään paikkaa, joka eniten muistutti palkinnon tuottavaa asetelmaa, korvaten parin tarjotulla esineellä. Tutkimuksen aikana havaittiin myös uudenlaista käytöstä, joka voisi viitata syvempään ymmärrykseen yhteistyöstä joissain yksilöissä.

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1.0.0 Introduction

1.1.0 Cooperation

1.1.1 Defining cooperation

Cooperation can be defined in a number of different ways (Sachs et al., 2004; West et al., 2007), but for the purposes of my thesis, cooperation is the capacity for individuals to group together in order to tackle tasks too great for a single individual, achieving greater gain for the members of the group as a whole as well as individually (West et al., 2007). It is a widespread behavioral phenomenon found throughout many species of mammals, birds and insects, coming in various forms and purposes (Dugatkin, 1997). For example, many social land carnivores hunt as groups, and coordinate in order to bring down prey heavier, stronger and faster than any the individual members (Lamprecht, 1981), various bird species call out loudly when sighting a predator to warn others (Smith, 1965), and insects have evolved to highly functional eusocial colonies (Anderson, 1984). In my thesis, I will be focusing on cooperation as it occurs in haplodiploid, eusocial insects, namely bumblebees (*Bombus terrestris*).

1.1.2 Cooperation: The problem

The emergence of cooperation is a complicated subject (Anderson, 1984; Nowak et al., 2010; Sachs et al., 2004). Even Darwin noted how potentially damning to the theory natural selection it was that what seemed like altruism was not only present, but thriving in nature (Darwin, 1859). In nature, an organism should have evolved to maximize their own capacity to pass down their own traits by breeding (Darwin, 1859), but animals like worker bees and ants were sterile and had evolved to work continually to assist the queen propagate without direct benefit to that primary goal, having even evolved behaviors and structures specialized for the purpose (Darwin, 1859). Why would an animal actively work in the benefit of another, and how could something like this evolve?

As deoxyribonucleic acid (DNA) was discovered to be the carrier molecule of hereditary information in most animals (Dahm, 2005) and gene research progressed throughout the mid-20th century (Benzer, 1959), a paradigm shift was taking place as genes were replacing individuals as the accepted material of evolution. Armed with higher understanding of genetics which Darwin had lacked, William Hamilton introduced the concept of inclusive fitness in 1964 (Hamilton, 1964). Inclusive fitness encompasses all possible avenues through which an individual can increase its own capacity to pass its genome to the next generation (Hamilton, 1964). This means not only an individual's own capability to breed and generate offspring, but also includes the benefit to fitness gained by other means, such as through kin selection. Kin selection is the tendency of individuals to be more willing to cooperate with those they know are closely related to them, even while accumulating possible short- or long-term harm to themselves (Grafen, 1984). The more closely related two individuals are, the higher percentage of genes they should share, and the more cooperation between the two benefits the co-operator indirectly as per inclusive fitness. While kin selection alone does not explain the totality of cooperation, it does in part explain how cooperation could have developed and be sustained within the laws of natural selection (Gardner et al., 2009).

Most extreme examples of kin selection driven cooperation can be found in eusociality. While the mechanism and requirements behind eusociality are still under contention (Anderson, 1984; Nowak et al., 2010), it is safe to say that kin selection has played a key role in the development of eusocial species and the mechanics that govern their behavior (Gardner et al., 2009). Eusociality means that labor within a population is divided between castes of individuals that specialize in a specific task (brood care, foraging, etc.), showcasing behaviors relevant to that caste while lacking those specific to others (Goulson, 2010). Within a bumblebee nest, for example, only the queen produces offspring, producing sterile diploid female offspring to act as workers and only producing non-sterile diploid females and haploid males under specific circumstances (Goulson, 2010). This means that, in a majority of cases, the workers benefit more by assisting their queen propagate through inclusive fitness (Anderson, 1984). Haplodiploidy has been historically seen as beneficial to the emergence of eusociality, although being neither a precondition nor a guarantee for it (Anderson, 1984; Nowak et al., 2010)

1.1.3 Experimental approach to cooperation

While recognizing cooperation is in itself easy, determining the level of comprehension of the participants of the cooperative task have is much more challenging. To participate in cooperation, an individual needs to be neither aware of other participant's (Simões-Lopes et al., 1998) nor their role in the task (Visalberghi et al., 2000) to cooperate with them, making it hard to discern the level of comprehension that is present in the cooperation. The individuals could be copying the behavior of others (Suchak et al., 2018), or could be focusing on some change brought on by the pair's presence or activity instead of the pair themselves (Seed & Jensen, 2011). To discern the depth of individual comprehension more accurately, the cooperation must be observed in a controlled, experimental environment.

The experimental study of the cooperative abilities of animals dates back to Meredith Crawford's 1937 pioneering cooperative pulling paradigm test, with which they proved that chimpanzees could accomplish a task requiring cooperation (Crawford, 1937). Crawford's paradigm has since been reused or used as the basis for many similar experiments on a variety of different species of animals, such as with wolves (Canis lupus) (Marshall-Pescini et al., 2017), African elephants (Elephas Maximus) (Plotnik et al., 2011), keas (Nestor notabilis) (Heaney et al., 2017) and indo-pacific bottlenose dolphins (Tursiops aduncus) (Jaakkola et al., 2018a). The general structure of these tests is simple. An incentive, predominately food, is placed out of reach of the test subjects and the test subjects are made aware of it. The subjects are given access to objects (ropes, levers, heavy rocks, buttons, etc.) connected to the housing of the incentive. They are taught, through either demonstrations, coincidental interactions or other methods, to understand that when these objects are operated (pulling, pushing, etc.) simultaneously with a partner, they will be given access to the incentive reward.

But Crawford's paradigm, as is, cannot prove that the participants understand that their partner is a contributing and required factor in the task. Due to this, Crawford's paradigm has commonly been bolstered with many additions that give a clearer understanding to how the individuals perceive their partners during the tasks. This can be accomplished by introducing a delay between giving the focal subject access to the experiment set-up and the introduction of the pair (Jaakkola et al., 2018a), but alternatively by how often the subjects seek visual confirmation of the pair (Chalmeau & Gallo, 1996) or how the focal subject behaves when the pair is concealed from it (Mendres & De Waal, 2000). In certain cases, such as with chimpanzees, the pair can be wholly absent, requiring the focal subject to recruit other individuals from elsewhere to assist in the task (Hirata & Fuwa, 2007). In these cases, the choice of pair can offer further insight to the comprehension of the focal subject (Melis et al., 2006a).

1.2.0 Bumblebees as test subjects

Bumblebees being eusocial means that their internal division of labor creates a subpopulation of driven, curious, and reliable test subjects that can be readily motivated with nectar in the form of foragers. Compared to honeybees (Apis sp.), who share information regarding the positions of new feeding locations in great detail through complex waggle dances (Barron & Plath, 2017), the ability of bumblebees to communicate about new sources of food seems to be limited to sharing that such a location has been found through excitation of the hive and hormonal signaling (Dornhaus & Chittka, 1999). Beyond this, bumblebee foragers seem to work solitarily, even selectively avoiding groups of familiar flowers if they are being foraged by another bumblebee (Kawaguchi et al., 2007).

They have been shown to be able to learn through observing other bumblebees (Worden & Papaj, 2005), transfer information from one modality to another (Solvi et al., 2020), possess limited tool use capabilities (Loukola et al., 2017) and to have the rudimentary ability to count (Pahl et al., 2013). The multifaceted portfolio of cognitive skills speaks of the bumblebee's adaptability and makes them excellent subjects for experiments that feature novel problem solving and learning challenges.

1.3.0 Aim of the experiment

This experiment is a follow-up on Anna Antinoja's 2019 master's thesis, where bumblebees (Bombus terrestris) were trained to push small and large Styrofoam rectangular cuboids to gain access to rewarding sugar water. In their experiment, Antinoja provided the first evidence that an invertebrate species was capable of active cooperation with a partner. To build on Antinoja's experiment, new tests were introduced to give further insight into the level of comprehension the bees demonstrated during the experiment.

The time before the focal subject's pair was released was extended from immediately after the focal bee approached a large rectangular cuboid to 30 seconds thereafter, to gain a better understanding of the focal subject's comprehension of the prerequisites of the test.

To determine further how specific the bees are about their choice of partner, a small black, wooden ball was placed next to a brick to see how keen the bees were to attempt to solve the test with this false partner.

To determine if the bees understood their own inability to push the bricks, a test was introduced where-in a bee was given the choice of two blocks, which it could push on its own, and two bricks, which it could not. Its choice should reveal its understanding of its relationship with the objects.

2.0.0 Materials and methods

2.1.0 Subjects

Bumblebees (Bombus terrestris) were obtained from nine commercial colonies purchased from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

The experiment was conducted at the University of Oulu from December 2019 to August 2020, in temperatures of 25±5˚C between 9 am to 8 pm. The test set-ups were moved to airconditioned greenhouse-room during June, but as the higher moisture and lower temperatures seemed to demotivate the bees, and the set-ups were returned to their original space in July.

2.2.0 Materials

The colonies were housed in 11,5cm x 13,5cm x 31cm bipartite wooden nest boxes, connected to a 43cm x 25cm x 60cm wooden arena by a 25cm x 4cm x 4.5cm clear acrylic tunnel (Fig. 1). The tunnel could be divided into three subsections to control the movements of individuals and to isolate them whenever necessary. The hive comb was housed in the nest box compartment furthest from the arena, while the bottom of the second compartment was filled with cat litter to soak up bee waste. Six artificial flowers (~4 mm diameter holes with ~1.5 cm radius of blue paint around them) were made into the arena floor and used to dispense food. The bees were given free access to the arena and allowed to feed ad libitum on 30% sugar solution outside of training and testing. The bees were provided 10mg of pollen to the nest every two days.

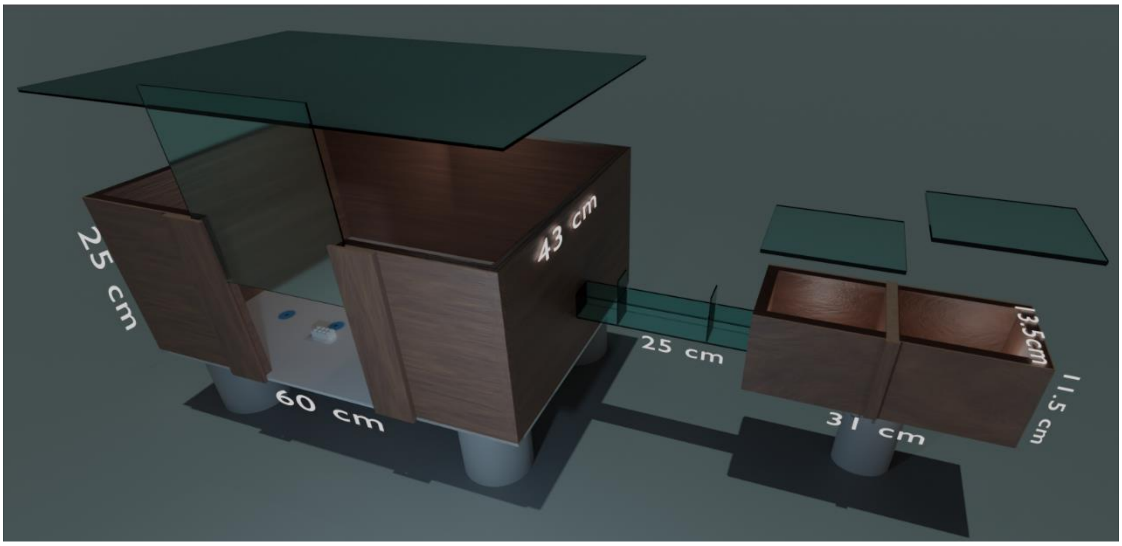

Figure 1: To-scale model of the training arena, corridor, and the bipartite nest-box

To mark the bees, individuals were trapped into a plastic tube flaubert and marked with either Posca markers by gently pressing them to the bees' thorax or abdomen, or with numbered labels that were affixed to the thorax with quick drying gel glue. For temporary markings, a feeding bee could be marked with a Posca marker without the need to capture the bee.

Hollowed out Lego (Billund, Denmark) pieces ("Blocks": 15.8 mm x 15.8 mm x 11.4 mm and "Bricks": 31.6 mm x 15.8mm x 11.4 mm) were used throughout the experiments as obstacles for bees to push to gain access to the flowers and sugar solution, during training and testing. Blocks were sanded slightly to create an indent at the bottom, allowing bees to assist pushing by allowing them to lift the block with their proboscis to minimize friction. Magnets were fastened within bricks with blue tack to allow them to be moved by the experimenter via another magnet underneath the arena floor. The magnets became necessary when it became clear during pilot experiments that due to individual variation, some bees could push the bricks alone while some pairs together could not.

Small white, acrylic discs were used to cover up artificial flowers when they were not in use.

CO2 was used to anesthetize bees for a short amount of time when clearing the arena before training, testing, or cleaning, but its usage was otherwise limited due to concerns for bumblebee welfare and testing performance. It was also used when new nests were moved from their packaging into the bipartite housing, but the practice was discontinued due to uncertainty over its effects on the queen's health.

The arena was cleaned regularly with 70% ethanol, particularly before, between and after tests, to remove cumulated waste and scent markings to avoid chemical cues affecting the choices of the bees.

As the temperatures rose in the greenhouse, the solid plastic covers on top of the nest-box roofs were replaced by ones with mesh covered openings to better ventilate the nest. Shade was provided to the nest with modified cardboard roofs that also funneled airflow into the nest. Electric fans were placed to blow air into the nest-box as temperatures began to reach 30°C.

A small, wooden, black-painted bead was used in the Black ball test.

Step 2 of paired and control training and testing was recorded with a Sony Xperia XZ Premium phone, and the gathered video footage was analyzed with the help of Behavioral

Observation Research Interactive Software (BORIS; boris.unito.it). Statistical analyses were ran and figures were plotted in RStudio version 1.4.1106 (Boston, MA, USA). Generalized linear mixed-effects models (GLMMs), were generated using the 'glmmTMB' function in package lme4, Fischer tests were conducted with the matrix function of exact 2x2 package and the image plots were created with ggplot function in the gglplot 2 package as well as the plot and plot(all effects()) function of the effects package. The image used to portray the nest-arena set-up was modeled and rendered using Blender (Community, B. O. (2018). Blender - a 3D modelling and rendering package. Stichting Blender Foundation, Amsterdam. Retrieved from http://www.blender.org). The image depicting the training of paired- and control individuals was made with the use of Clip Studio Paint.

2.3.0 Pretraining

During pretraining, bees could freely enter the arena and feed from one of the fake flowers in the arena floor where they could obtain 30% w/v sucrose solution via a hole drilled in the center of the flower (a reservoir of 30% w/v sucrose solution was attached the underside of the arena floor).

2.4.0 Training

Bees that that collected sucrose solution from the flowers and returned home regularly were individually tagged with colored number tags. The most active and motivated foragers (5-10 individuals) of those tagged were chosen for step one of training. During training, the individuals were given 50% w/v sugar solution as a motivator. Training was separated into two steps, with an additional step 1.5 used in specific situations if needed.

In step one, the bees were trained individually to feed from a flower that was covered with a block in a stepwise manner, with the block moving to cover more of the flower upon the completion of the previous, easier step. (Fig 2a). As the block was moved to partially cover the feeding hole, the bumblebees would push the block to get into a better feeding position, teaching them that they could move the blocks by pushing. Ultimately, the bees would learn to push a block which covered almost the entire flower to gain access to the sucrose solution. This step with most of the flower covered was repeated 3-5 times, depending on individual performance, until the individual's proficiency was established. A small portion of the blue flower was left uncovered to help attract the bees to the blocks quickly and give their push a focal point. Two bees from this training phase who had foraged most successfully and regularly were chosen for step two of training.

In step two, training continued in the same manner, but two bricks (twice the size of the blocks) replacing the block (Fig. 2d). In addition, the paired bees were released simultaneously into the arena to start the trial. However, to gain access to the flower and sucrose solution, bees were required to push the brick simultaneously for at least one second.

Pushing was defined as an action where an individual touched the brick with its head continually for 1 second with its body perpendicular to the brick and with the perceivable intention of moving the brick. This action may have or may not have been assisted with the use of proboscis. Once both bees had simultaneously pushed for one second, the experimenter would move the brick from the flower. This was made possible by moving a magnet underneath the arena that would then move the bricks.

Training step 1.5 (Fig. 2c) was introduced to the training programme to smoothen the transition from step 1 to step 2 of paired training pairs that, for any reason, could not or would not tolerate the presence of their paired bee on the same brick. In this step, the pair was given only a single brick to encourage tolerance between paired bees.

Control bees were trained in the same way as the test bees, but bricks were used in both steps and the control individuals could push the bricks alone (with the assistance of the experimenter).

Step 2 of training was repeated 25 times for both the paired and control groups.

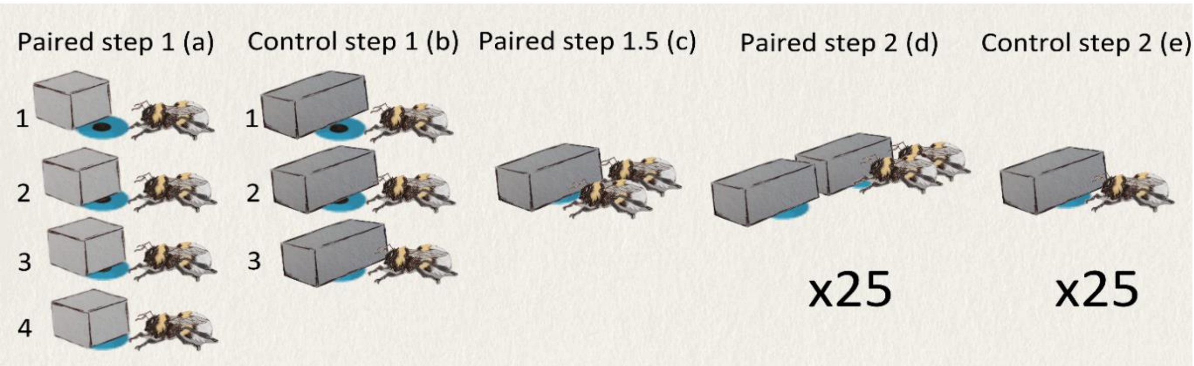

Figure 2: Training steps used for the paired- and control individuals. Paired step one: a block is moved incrementally to cover more and more of the flower, eventually covering the hole. Control step one: A brick is moved incrementally to cover more and more of the flower, eventually covering the hole. Paired step 1.5: A pair trained individually with paired step 1 is presented with a single brick covering a hole. Paired step two: A pair is presented with two bricks covering holes. Control step 2: A control individual is presented with a single brick covering a hole.

2.5.0 Testing

2.5.1 Delay test

For the Delay test, trained bees from the Paired group were individually released into the arena with two bricks each covering a flower (Fig 3). When the focal bee landed within 1 cm of a brick, a 30 second timer was started, after which its pair was released into the arena. The duration from the bee's entry to the arena to the landing was recorded, as was the time from the landing until its first push attempt. Whether or not the focal individual attempted to push before its pair had arrived was recorded, as well as if the bee's first attempted push after the pair had arrived was in the vicinity (within 4cm) of the pair. The test was identical for the control individuals, but since they had no training pair, a randomly picked forager would be released into the arena 30 seconds after the focal control bee landed within 1cm of a brick. The control individuals could solve the test on their own and were allowed to drink after a successful push.

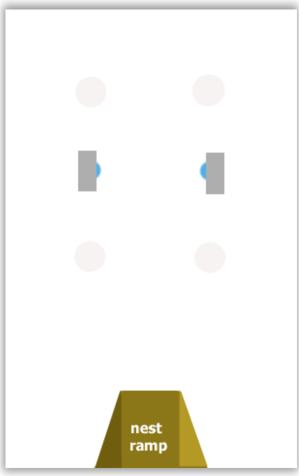

Figure 3: Arena state during Delay test

2.5.2 Black ball test

For the Black ball test, two bricks were placed as shown in figure 4 (or its mirror opposite) and a black ball was placed in front of the furthermost brick from the arena entrance. Bees were let into the arena individually and their behavior towards the ball and bricks was observed. Once Test-subject had spent 5 minutes on the task, the ball was removed and the partner released onto the arena, allowing the bees to solve the trial for a reward. Alternatively, the bee was allowed to leave the arena after that time if it was attempting to return to the nest on its own. Control bees could solve the test at their own and weren't forced to stay in the arena for the whole three-minute duration. Within the first five minutes, whether or not the focal bee examined the ball before pushing and which brick they pushed first was recorded.

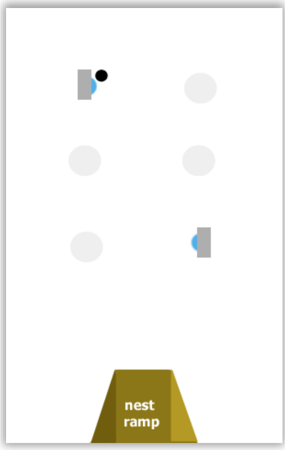

Figure 4: Arena state during Black ball test

2.5.3 Bricks or Blocks

For the Bricks or Blocks test, two bricks and two blocks were placed as in figure 5 (or its mirror opposite) and each subject was tested individually. Each bee was allowed entrance to the arena, and the target for its first attempted push was recorded.

To test the control-subjects, who had been previously trained and tested with only bricks, were trained to push blocks until they could accomplish the task with ease. They were then refreshed with a single brick pushing test before attempting the Bricks or Blocks test.

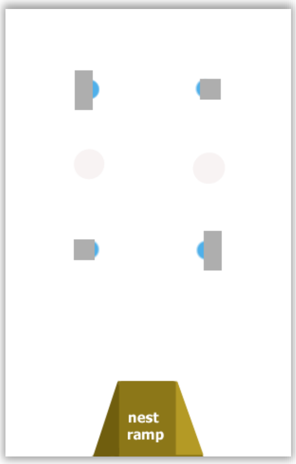

Figure 5: Arena state during Bricks or Blocks test

3.0.0 Results

3.1.0 Delay test

In the Delay test, it was expected that the paired individuals would not immediately attempt to push the bricks, understanding that they could not accomplish the task alone and instead wait for a potential pair. Although there was no significant difference between how long the focal subjects of the paired individuals and the control individuals took to land next to a brick after entering the arena (Fig 6. glmmTMB: Dispersion estimate for Gamma family (sigma^2): 0.734, z = 0.03 p = 0.98), with paired individuals waiting on average 16.5 seconds and control individuals waiting on average 11.5 seconds. Paired bees waited significantly longer to attempt a push after the approach (Fig. 7, glmmTMB: Dispersion estimate for Gamma family (sigma^2): 1.49, z = -3.68, p = 2.37e-4).

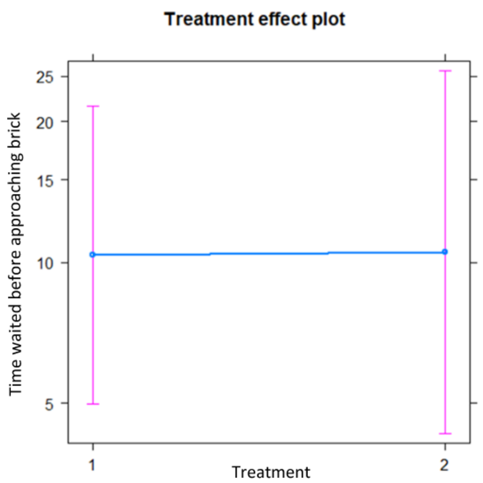

Figure 6: Time waited (sec) before approaching a brick (within 1 cm while on ground) by the two groups. Treatment 1 = Paired, treatment 2 = Control. Standard error marked with red columns.

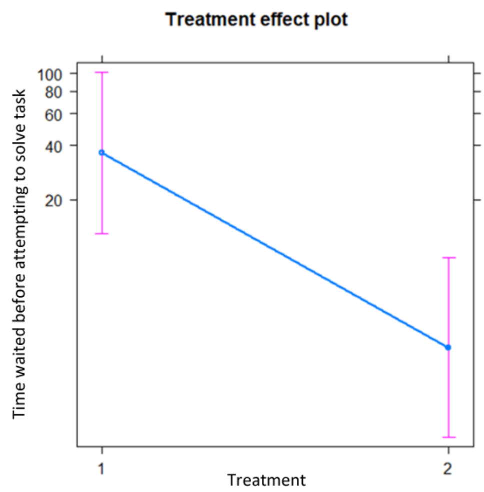

Figure 7: Time waited before an attempted push (sec) after approaching a brick (within 1 cm while on ground) by the two groups. Treatment 1 = Paired, treatment 2 = Control. Standard error marked with red columns.

Paired bees were also significantly (Fig 8; FETCD: p = 0.008366) more willing to wait for their pair to arrive to the arena, compared to controls. The testing order of individuals of pairs was not significant (glmmTMB, z = 1.624, p = 0.10443).

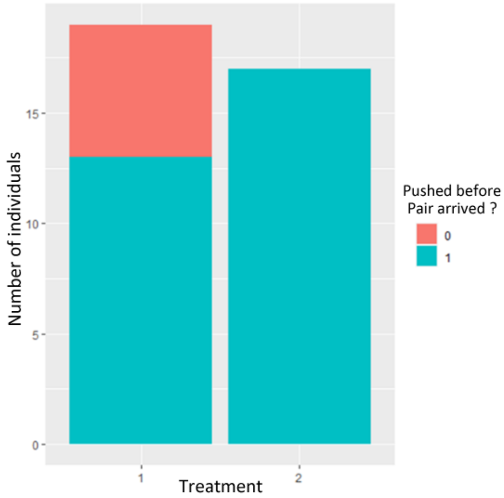

Figure 8: Number of individuals who pushed a brick before their pair had arrived during the delay test. Treatment 1 = Paired, treatment 2 = Control. Color 0 = Had not pushed a brick before pair had done so, Color 1 = pushed a brick before pair did.

Once the partner bee had arrived to the arena, the focal bee would be likely (Fig. 9; FETCD: $p = 0.001952$) to attempt to push a brick while the partner was in the near vicinity (within 4cm).

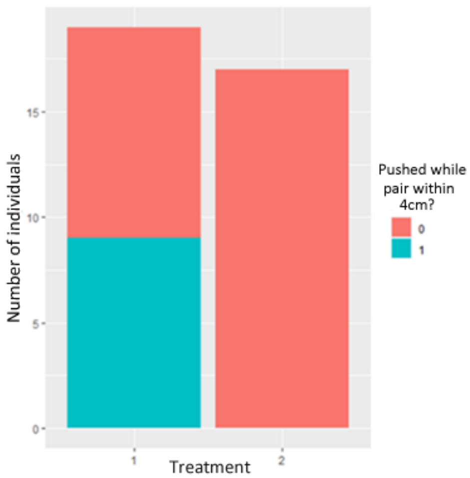

Figure 9: The number of individuals whose first push was made in the vicinity (within 4 cm) of the pair. Treatment 1 = Paired, treatment 2 = Control. Color 0 = attempted a push while away from the pair, Color 1 = attempted a push in the vicinity of the pair.

Four paired individuals waited both for their pair to arrive and for them to attempt a push before joining in their pair's attempt themselves, showcasing ideal behavior during the delay test.

3.2.0 Black ball test

In the Black ball test, the paired bees were expected to show interest in the provided bead, but not push either brick. As can be seen in fig. 10, while a slightly greater number of paired bees chose to examine the black ball first, the difference is not significant (glmmTMB: z =0.686, p = 0.82, Fig.10). Paired individuals also pushed the brick associated with the black ball first more readily, and the differences between paired- and control population were significant (glmmTMB: z = -2.82, p = 0.005, fig.11). Three of the 19 paired individuals did not attempt a push during the observation period.

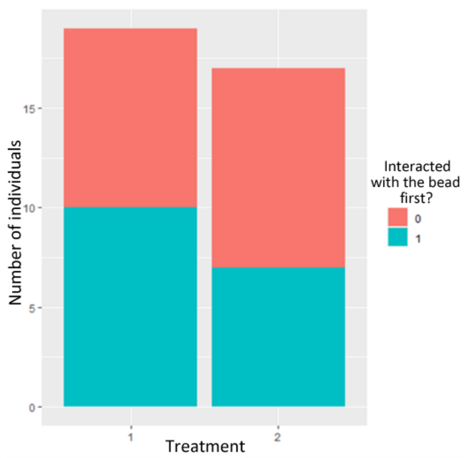

Figure 10: Number of individuals who interacted with the black ball before interacting with either of the bricks. Treatment 1 = Paired, treatment 2 = Control. Colour 0 = 1st interaction after arriving to the arena was with one of the two bricks , Colour 1 = 1st interaction after arriving to the arena was with the Black ball

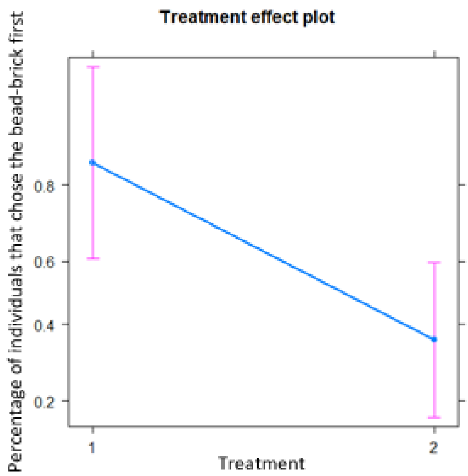

Figure 11: Percentage of paired-and control individuals whose first choice of bricks to push was the brick next to the black ball. 3 of the 19 paired individuals did not attempt a push during the test. Treatment 1 = Paired, treatment 2 = Control. Standard error marked with red columns.

3.3.0 Bricks and Blocks test

In the Bricks and Blocks test, the paired bees were expected to understand their limitations and choose one of the blocks. Control individuals were expected to show no differentiation between bricks or blocks, as they were able to move either. The paired bees were indeed more likely to choose blocks, whereas control individuals showed a preference for bricks (Fig. 12), but the variation between Paired- and control individuals were not significant (glmmTMB: $z = 1.671$ $p = 0.095$).

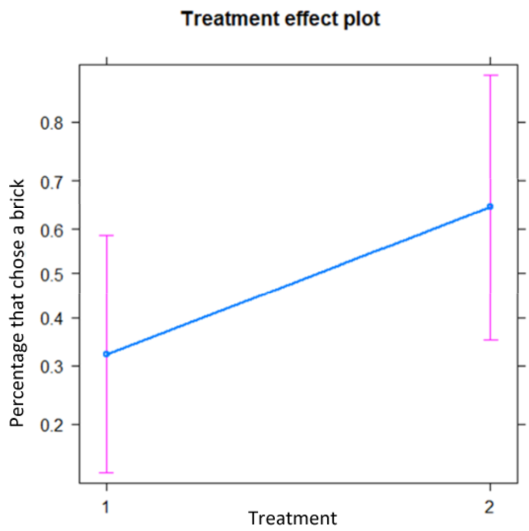

Figure 12: Percentage of paired- and control individuals whose first choice was to attempt to push a brick during the Bricks and Blocks test. Treatment 1 = Paired, treatment 2 = Control. Standard error marked with red columns.

4.0.0 Discussion

4.1.0 Do bumblebees understand their partners role?

As to whether a bumblebee understands their partners role in the task or not, the results of this experiment remain, in part, inconclusive. While the testing set-up shows that the bumblebees respond to the absence of their partner in a significant manner, whether they recognize the need for a bee partner remains undetermined.

The results of the Delay test leave little room to doubt as to the bumblebees' ability to comprehend their inability to accomplish the task without a partner. Paired individuals spent a significantly longer time waiting before attempting a push than control individuals (Fig. 7), which was to be expected given that controls had neither a reason to wait nor a concept of needing a partner, and difference between averages times that the paired- and control group waited after approaching a brick without attempting to solve the task was approximately 57 seconds. This is notable since, for example, dolphins who were tasked to push two buttons simultaneously struggled when the introduced delay was increased from previously tested two, to five seconds (Jaakkola et al., 2018b). Up until this point, the delay between the focal- and the partner dolphins had been increased only by a second at a time and had had increasingly successful trials. The test conducted with the dolphins did require a higher level of precision, as pushing a button simultaneously is a momentary action in contrast to the constant action that pushing the bricks used in this experiment presented, but the dolphins had had previous experience with the delayed release of their partner, unlike our bees, aside from delays that were born of the few second variations in the bees' arrival times to the arena during training step 2. Given that an increase of three seconds made a negative impact on the successfulness of the dolphin pairs, our 30 second delay and the on average minute wait before attempting to push after having approached a brick showcases a great amount of hesitation to attempt the task alone.

The B-ball results could be interpreted in one of two ways. Most paired individuals attempted to push the brick the black ball was, while control individuals had no preference, choosing either brick, bead or no, seemingly at random (Fig. 11). This could mean that paired subjects, either out of optimistic experimentation or simply because they felt all the pre-requisites had been fulfilled, chose the brick next to the black ball as it most closely resembled the state required for a reward. This showcases an understanding of the task parameters, but not necessarily the role of the bumblebee partner.

4.2.0 Are bumblebees aware of their own capabilities?

The results of the Bricks and Blocks test were not significant, as most individuals that chose blocks were from the same hive (see mistakes made for more info). Due to this, no conclusions can be made on the bees' ability to discern their own capability to move the different objects.

4.3.0 Bumblebee interactions during testing

The bumblebees often interacted with each other in ways that may suggest that an attempt at communication was being made, albeit through limited means. They would walk and fly around each other seeking physical contact, bumping into each other during flight or circling and pushing one another in the ground. The intensity of this behavior seemed to vary from bee to bee and was possibly tied to the level of individual comprehension. This behavior resembled the behavior described in communications used by bumblebees sharing the discovery of a new food source to their nest (Dornhaus & Chittka, 1999).

During the experiment, a test subject showcased the described behavior to a degree that indicated higher understanding of the task, and the role of its pair especially. Subject W22 was observed pushing its pair to the brick on multiple occasions during training and testing, and even retrieving its pair from a separate space on one occasion during subject G70's b-ball test. G70 had been allowed to exit the set-up, during which W22 slipped into the set-up from the enclosed waiting space. Noticing it was alone, W22 returned to the waiting space, pushed G70 back into the set-up and onto a brick to gain a reward. This implies not only that bumblebees can understand the task, but might be capable of recruiting other individuals, similar to chimpanzees (Hirata & Fuwa, 2007; Melis et al., 2006), or at least their designated pairs to complete the task. This behavior was limited to W22, which implies that this level of comprehension could be rare in bumblebees.

4.4.0 Challenges of paired training

As noted in training portion of Materials and methods, there were testing pairs that clearly avoided occupying the same brick during early step 2 training. This might be because the individuals had not yet experienced step 2 and had no reason to assume the need for the partners presence and instead behaved as they would in nature by avoiding an established food source already being foraged by seeking out another feeding location (Kawaguchi et al., 2007). Cooperation was usually quickly established between pairs when step 1.5 was used, and the parameters became clearer to the bees.

Just being trained in pairs could also bring up training challenges. The efficiency of a learning situation is tied to the clarity of requirements for the task's completion and the speed at which the completion produces a reward. Hence, the more unpredictable variables are present in the learning environment, the weaker the subjects understanding of the task becomes. By introducing a pair and expecting cooperation, it might much more difficult for an individual to decide which variables are necessary for success. While the 25 trials used in step 2 minimize the variation within test- and control subject groups, it is possible that paired training resulted in greater variance of test results for the test subjects when compared to the variance between control individuals.

5.0.0 Future study

Given the vagueness of black ball test results, a more robust test using additional objects such as dead bees could be conducted to better gauge the focal bees interest towards another bumblebee specifically. This would give better insight into the bumblebees' comprehension of their partners in a cooperative task. This could also extend to the focal bee choosing between a naïve individual and its established pair.

The Bricks and blocks test was a side thought throughout the process, which shows in the results. A repeat with a more fleshed out procedure would be in order.

The details of the out-of-nest communication observed between paired bumblebees during training and experiments offer a possible venue for further research, and this subject is, in fact, under study right now.

Given that our bumblebees showcased apparent communication behaviors related food source communication outside of the location it has been previously linked with, it could be interesting to find out if this could also be induced in a more natural environment.

It might be interesting to determine how many bumblebee individuals would be able to participate in a task, and I would like to see how different participant numbers would affect the learning process. This might prove difficult however, due to reasons discussed in the "Challenges of paired training" section.

6.0.0 Additions

6.1.0 Benefits of using bumblebees as a model species

Bumblebees are a convenient model species. They have a more manageable hive-size (several dozen) when compared to, for example, honeybees (hundreds to thousands), which makes them more suitable for use within an enclosed laboratory environment. Their housing is easy to build and maintain, takes up little space and even a mass escape of bumblebees is unlikely to cause critical risk to the caretaker, since there would be far fewer individuals attempting to inflict painful, possibly life-threatening allergic reaction inducing stings.

Bumblebees are cute. While from a scientific or functionality viewpoint this aspect is largely immaterial, since whether the test subject is cute or not does not affect any of its other capabilities or the results of any tests, but the effect on public perception is undeniable. The current world state, where arthropods are experiencing great losses in both their biodiversity and abundance (Dirzo et al., 2014), has made it more important than ever to readjust the public opinion on insects and elevate them form indistinct pests into a more relatable, respected position. This is pivotal to sway not only the public opinion, but also the minds of company owners and law makers. To this end, it is beneficial to present an approachable "ambassador" to represent arthropods.

6.2.0 Mistakes made, lessons learned

Many things changed about the set-up throughout the experiment, which has given me some worries regarding the validity of the results. For example, my own capabilities as a trainer increased throughout the process, and those who assisted me during training naturally had their own touch and judgement of parameters despite active guidance. Naturally, these can also be argued to have also just been the result of nest- or individual level differences in bee behavior and could be dismissed as such, but this uncertainty does not sit well with me. In the future I would like to, if able, spend some time from a week up to a month to test out the viability of the testing set-up before beginning proper testing. Admittedly it could have been purely inexperience, but I often felt a testing period would have greatly helped by solidifying the set-up and procedure. This might have, overall, hastened the pace of the experiment and given some peace of mind.

Due to an oversight, the first seven of the test-subjects only had one brick in the Bricks and Block test, which might have resulted in them all choosing blocks in the test. A refresh on blocks could have been introduced to Paired individuals before testing. This was suggested, but only some time into testing when some pairs had already been tested. I chose not to implement it out of the fear that the inconsistency might further muddle the results of the test. I am unsure if I regret my choice.

For the control group, the bricks would have been minimally easier to move, as they were operated by magnet and required no true push from the bee. This could explain the slight preference towards bricks during the Bricks and Blocks test. To bypass this, I did my best to make them as proficient with block pushing as possible to make the task as effortless as possible.

In any possible future venture, I would be sure to mark individuals in a pair with different colored tags, to make following them in the recorded footage easier. In too many occasions, the data analysis was slowed down when tracking a bee in grainy footage became nearly impossible. Variance in tag colors would remove this problem.

6.3.0 Thanks

All my gratitude goes to my supervisors, who were kind and patient with me throughout this whole process, as well as to Juho Harmoinen and Sampo Tanskainen who assisted in the training and testing during the summer months. This was a wonderful experience, learning about the basic principles of experimental approach to comparative psychology, how to work with these insects, to coax and bribe them for experimental gain, and to appreciate long evenings in a greenhouse on a rainy day.

7.0.0 References

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