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Visuo-motor biases in buff-tailed bumblebees (Bombus terrestris)

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Visuo-motor biases in buff-tailed bumblebees (*Bombus terrestris*)

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ABSTRACT

Bees provide a good model to investigate the evolution of lateralization. So far, most studies focused on olfactory learning and memories in tethered bees. This study investigated possible behavioural biases in free-flying buff-tailed bumblebees (*Bombus terrestris*) by analysing their turning decisions in a T-maze. Bees of various size were trained to associate a syrup reward with a blue target placed at the centre of the T-maze. The bees were then tested over 16 trials by presenting them with blue targets at the end of the maze's arms. The maze was rotated 180° after the first 8 trials to control for environmental factors. The number of turnings to the left and right arms were analysed. The bees sampled exhibited a population-level rightward turning bias. As bumblebees vary significantly in size with large bees being better learners than smaller ones, we measured the thorax width to identify a possible relationship between size and bias. No significant correlation was identified. This study shows that bees present lateralization in a visuo-motor task that mimics their foraging behaviour, indicating a possible specialisation of the right side of the nervous system in routine tasks.

Keywords: Behavioural bias, side-turning, T-maze, bee, lateralization

INTRODUCTION

With the growing evidence of brain and behavioural asymmetries in invertebrates, insects have become a very good model to investigate the evolution of lateralization (Niven and Frasnelli, 2018). Directional biases have been identified for prey capture, escape response, motor tasks, and interspecific interactions in several species of invertebrates (summarised in Frasnelli, 2013, see also Frasnelli et al., 2012; Frasnelli, 2017). For example, cuttlefish (*Sepia officinalis*) favour the left visual field to scan for potential predators and the right visual field for prey attack (Schnell et al., 2016). Ants (*Lasius niger*) show a rightward turning bias on their foraging trails (Heuts and Brunt, 2005), whereas rock ants (*Temnothorax albipennis*) display a left turning bias when searching for nest spaces in a branching maze (Hunt et al., 2014). Leaf-cutting ants (*Acromyrmex lundii*) have a similar leftward turning preference when tested in a Y-maze (Endlein and Sitti, 2018), and so do giant water bugs (*Belstoma flumineum*) tested in a T-maze (Kight et al., 2008). Overall, the studies conducted in invertebrate animals suggest that even organisms with smaller brains benefit from the different, and often complementary, functional specialisation of the left and right side of the nervous system. Moreover, they prove similarities with vertebrates in the way lateralization is manifested, helping to understand the evolutionary basis of this very interesting characteristic widespread in the animal kingdom.

Given their rich social life and astonishing cognitive abilities (Menzel and Giurfa, 2001), bees have become a valuable model to investigate lateralization and its evolutionary basis (Frasnelli et al., 2014). Honeybees (*Apis mellifera*) display an asymmetrical use of their antennae in the recall of olfactory memories (Rogers and Vallortigara, 2008; Frasnelli et al., 2010), in sucrose responsivity (Baracchi et al., 2018), and in social interactions (Rogers et al., 2013). Some bumblebee species (*Bombus lapidarius*, *Bombus lucorum* and *Bombus pascuorum*) show a preferred circling direction when visiting florets arranged around an upright inflorescence (Kells and Goulson, 2001). Although buff-tailed bumblebees (*Bombus terrestris*) do not exhibit such a preferred circling direction (Kells and Goulson, 2001), they show lateralization in the recall of olfactory memories as honeybees do (Anfora et al., 2011).

Despite the increasing evidence of brain and behavioural asymmetries in bees, most of the studies have so far focused on testing tethered bees in simple olfactory learning (e.g. Anfora et al., 2011) or in visual conditioning (Letzkus et al., 2008). Very little is, instead, known about visuo-motor biases in free-flying bees and how lateralization may affect the bees' performance in visually guided tasks. A couple of recent studies (Ong et al., 2017; O'Shea-Wheller, 2019) suggest that bees display behavioural asymmetries also in the visuo-motor domain. This is not surprising given that bees rely heavily on vision to forage and navigate, tasks where a lateralised brain may be beneficial. When honeybees are

tested in a tunnel with a left and a right aperture of equal width, 26.5% of the individuals prefer to go through the right aperture, 24.5% through the left aperture, whereas 49.0% of the bees have no preferential bias (Ong et al., 2017). However, honeybees present a population-level rightward turning bias when entering unknown open cavities, with reduced decision latency for bees exhibiting a strong rightward turning bias (O'Shea-Wheller, 2019).

We investigated whether bees show any directional bias when presented with a coloured target, previously associated with a food reward. To do so we tested buff-tailed bumblebees (*Bombus terrestris*) in a T-maze. This allowed us to use free-flying bees in a task that mimics their natural foraging behaviour, but likely also requires the recall of visual memories, given that bees were trained to associate the coloured target with a reward. Moreover, bumblebees permitted to provide further evidence of visuo-motor asymmetries in support to previous findings in honeybees. Lastly, unlike honeybees, bumblebees vary massively in size, with bees having a thorax width ranging from 2.5 mm to 6.9 mm (Goulson et al., 2002). Large bees seem to be the main foragers within the hive as they provide the colony with the heavier sucrose and pollen loads than do smaller ones (Goulson et al., 2002). Large bees are not only more efficient foragers, but they also seem to be better learners than smaller bees (Mares et al., 2005). Thus, we wonder whether large bees are more strongly lateralised than their smaller sisters.

MATERIALS AND METHODS

Animals

Two colonies of buff-tailed bumblebees (*Bombus terrestris*) were purchased from Koppert Biological Systems (UK). Each colony was connected with the experimental apparatus described below. We waited three days before starting the experiments to let the bees acclimitise and allow only foragers to emerge. Forty foragers were randomly selected across the two colonies, but only 20 completed all trials (7 from the first colony and 13 from the second colony). The colonies were kept in the boxes as provided by Koppert and stored in a laboratory in Minster House (University of Lincoln, UK). The bees were fed with 10ml of syrup every day in the evening and 2 tsp of pollen every other day. The syrup (provided by Koppert Biological System, UK in a bag connected to the colony) and the pollen (also provided by Koppert Biological System, UK) were stored in glass jars in a refrigerator at 4ºC.

Experimental Set-Up

The box containing the bumblebee colony was placed into a custom-made set-up (Figure 1). A Plexiglas chamber (15 x 10 x 15 cm; l x w x h) allowed the experimenter to visualise and select the bees when they spontaneously emerged from the colony. A Plexiglas corridor (24.5 x 4.5 x 4.5 cm) connected the chamber to the T-maze. The corridor had two vertical sliding doors to allow the selective release of marked bees. A T-maze (100 x 100 x 20 cm) was used, with each arm of the 'T' being 40 cm long (Figure 1). During the test phase, a vertical wooden panel with a squared hole in it (5 x 5 cm) was placed at the end of the main arm of the maze in order to focalise the bees' flight trajectories (Figure 1). In this way, we prevented any bias in the choice of the left or the right arm being induced by a potential bias in the flight trajectory before reaching the T-maze intersection. Red and white chequered paper lined the inside of the maze to provide the bees optic flow and allow them to stabilise their flight (Linander et al., 2017). Red was used because bees cannot see the colour of the paper due to their trichromatic vision but compared to black, this helped the experimenter to better visualise the bee against the background (Barth and Biederman-Thorson, 1985). Two black lines on the chequered paper marked the intersection between the central area and the left and right arms of the maze, allowing us to record the choice made by each bee (Figure 1).

To train the bees to associate a visual target with a food reward we used an Eppendorf tube's cap glued onto a blue acrylic sheet (60 x 60 mm), filled with syrup (the same used to feed the bees and provided by Koppert Biological System, UK together with the colony) – we will refer to this as "blue target" (Figure 1). The apparatus was cleaned after each trial with ethanol, 95% (v/v) (Fisher Chemical, UK) to avoid any effects of pheromones produced by the bees on later training and testing (Dornhaus et al., 2003).

Figure 1 - about here

Experimental Procedure

Naïve bees spontaneously emerging from the hive were individually released into the T-maze. After travelling forwards for 80 cm, the bees reached the T-junction of the maze, where they turned either left or right and travelled for an additional distance of 40 cm until they reached the terminus of the chosen arm. Here, each bee was collected in a tube through a door and the initial spontaneous choice of either the left or the right arm of the T-maze was noted. Once in the tube, each bee was tagged for identification

purposes with a unique coloured number glued onto the thorax. After being tagged, the bee was placed back in the colony.

Individually tagged bees were then trained to associate the blue target with a syrup reward, which was placed at the T-junction of the maze as shown in Figure 1. Each bee was released into the maze and was given 5 minutes to locate the blue target and start feeding from it. During these 5 minutes, the first directional choice (left or right) taken at the intersection of the maze, and the time taken to do this, was noted. If the bee had not found the syrup within the 5 minutes (which happened for 17 out of 20 bees), it was collected into a tube and gently placed onto the blue target for a first training session. After finishing feeding, the bee had 5 minutes to go back to the colony by itself, after which it was caught and placed back within the colony. This process was repeated for each tagged bee until the bees could find the blue target by themselves. Three bees found it without any training, one bee after 1 training trial, another bee after 2 trials, most of the bees (n = 12) required 3 trials, and the remaining 3 bees required 4 training trials. Once completed the training, the bees were tested to see whether there was any bias in their left/right arm choice. In order to motivate the bee's behaviour, we placed three blue targets, as the one used during the training, but that did not provide any reward, on the wall of the T-maze in three points: at the T-junction, and at the end of the left and right arms of the maze (Figure 1). Upon reaching the T-junction, it was noted whether the bee turned left or right as well as the time taken to make the choice. Once the bee made its choice, it was collected and, after 2 minutes, was given a small amount of syrup to encourage its foraging behaviour in the following trials. The bee was then placed back in the colony. This process was repeated 8 times per individual. The second part of the experiment was run following the same protocol, but with the apparatus rotated 180º, to reduce any possible effects of environmental cues such as lighting or electromagnetic fields. A further 8 trials were run for each bee. All bees in the sample population were tested with the apparatus at 0º for the first 8 trials.

The thorax size of each of the individuals tested was taken by measuring the intertegula span (Spaethe and Chittka, 2001) using a calliper (Mitutoyo Absolute Digimatic NTD12-6"C), to determine any possible relationship between the direction chosen and the size of the bee.

Data Analyses

A laterality index (LI) was calculated using the formula (L-R)/(L+R), where L and R represent respectively the number of turns to the left and to the right (Bisazza et al., 2000). After checking that our LI data were normality distributed, we tested for the possible effect of the maze's position using a Friedman test. A one sample T-test was

then performed to determine whether our data showed overall a significant bias at the population level in the turning direction. Individual level lateralization was assessed performing binomial tests. We also looked at possible correlations between: i) LI and the bees' size; ii) the strength of the bias (i.e. the absolute value of LI) and the bees' size; and iii) LI and the time taken to make a choice in the T-maze (averaged across the 16 trials), using either Pearson or Spearman rank correlation tests (depending on the data's distribution). In addition, we conducted binomial tests to check for potential spontaneous biases in the first turning choice (i.e. when the bees were released for the first time before being tagged) and in the second turning choice (i.e. when the bees were released for the second time before being trained). When considering the first and the second turning choice, we tested whether the direction of turning (left or right) influenced the time taken to make the choice, using Kruskal-Wallis tests.

RESULTS

Out of the 40 bees selected, 20 completed all 16 testing trials, so the analyses were performed on this sample. The laterality index (LI) data from these 20 bees resulted normally distributed for the trials with the maze in its original, i.e. 0º, position (Shapiro-Wilk, p = 0.093), but not for the trials with the maze rotated 180º (Shapiro-Wilk, p = 0.011). The mean LI value was -0.238 (SE = 0.082) when the maze was at 0° and -0.288 (SE = 0.134) at 180°. The 180° rotation of the maze did not have an effect on the turning decision of the bees ($\chi^2(1)$ = 2.250, p = 0.134), allowing us to exclude any possible effect from variations in environmental cues such as light intensity that might have caused the bee to travel down the arm that was more illuminated. As there was no effect of the maze position on LI, the data were merged. The resulting LI values resulted normally distributed (Shapiro-Wilk, p = 0.066) and revealed a significant rightward bias within the tested bees (One-sample T-test, t (19) = -2.666, p = 0.015). Figure 2 shows the distribution of the laterality indices of the sampled bee population (N = 20): overall, bumblebees preferentially turned towards the right arm of the maze. To evaluate how many individuals showed a lateralization at the individual level, we performed additional analysis on single individual choices. The results showed that 4 individuals were significantly biased towards the right (2-tailed binomial test, p = 0.021; p = 0.004; p = 0.004; p = 0.001) and 1 individual was significantly lateralized towards the left (2-tailed binomial test, p = 0.004).

Figure 2 – about here

We then determined whether there was any correlation between the bees' size (calculated as thorax width) and their laterality index. The thorax width measurements ranged from 4.09 mm to 6.96 mm, with a mean of 4.83 mm (SE = 0.129). Because of one outlier (the value of 6.96 mm), the data resulted not normally distributed (Shapiro-Wilk, p < 0.0001). There was no significant correlation between the bees' size and their LI (Spearman rank, rho = -0.113, p = 0.636; Figure 3a), nor between the bees' size and the absolute LI (│LI│), indicating the strength of the bias (Spearman rank, rho = 0.202, p = 0.393; Figure 3b). When the outlier was removed, the correlation between the bees' size and their LI was still not significant (Pearson rank, rho = -0.020, p = 0.936), and the same was true for the correlation between the bees' size and the absolute LI (│LI│) (Pearson rank, rho = 0.254, p = 0.294).

Figure 3 – about here

Overall, the LI had no effect on the time taken to make a choice in the T-maze, averaged across the 16 trials, (Pearson rank, rho = 0.032, p = 0.893). The same was found for the │LI│ (Pearson rank, rho = -0.125, p = 0.601), suggesting that nor the strength or the direction of the bias influenced the time the bees needed to make a choice.

When released for the first time into the maze, 14 bees chose the left arm and 6 bees chose the right arm, but this was not significant (2-tailed binomial test, p = 0.115). Interestingly, when released for the second time, 9 out of the 19 bees (one bee went straight to the blue target) changed their initially preferred arm. Indeed, 6 bees turned towards the left arm and 13 bees turned towards the right arm, but again this was not significant (p = 0.167). The direction of turning (left or right) did not influence the time taken to make the choice, nor in the first turning choice (Kruskal-Wallis test, p = 0.710) or in the second turning choice (p = 0.114).

DISCUSSION

The results of this study suggest that bumblebees (*Bombus terrestris)* present a directional bias to turn rightwards when tested in a T-maze. This is in line with previous findings in honeybees. Indeed, honeybees show the same directional bias when they are

tested to explore unknown open cavities (O'Shea-Wheller, 2019). Moreover, when honeybees are presented with a left and right aperture to fly through, 45% of the individuals tested show biased side choice for one aperture, either the one on their left or the one on their right (Ong et al., 2017). Although the results of this study seem quite different from ours, when we look carefully at them, we can notice that for the exit aperture, the distribution on the bias within the sample analysed is slightly biased towards the right with 40.19% of the bees showing a bias towards the right aperture and only 25.49% of them showing a bias towards the left aperture (see Figure 5b; Ong et al., 2017).

Very interestingly, honeybees are more likely to turn right when entering an open cavity, but when presented with a branching aperture, they do not exhibit any bias (O'Shea-Wheller, 2019). This suggests that directional approaches of honeybees are context-dependent and supports the idea that lateralization manifests itself in behaviour depending on the context and task animals are tested in (Frasnelli and Vallortigara, 2018). This likely depends on the complexity of the task: a brain may be lateralized only for those functions that require a certain cognitive complexity. Studies into other invertebrate species have demonstrated similar results too. Cuttlefish (*Sepia officinalis*) show a leftward bias when released into a T-maze but only when shelters are provided at the end of the two lateral arms of the apparatus (Jozet-Alves et al., 2012). When tested for spontaneous behaviour while exploring a T-maze, octopuses do not display any significant bias (Frasnelli et al., 2019). This is likely due to the lack of a specific motivation, such as looking for protection or foraging, in the task.

Preliminary data we collected showed that when bumblebees are tested for spontaneous choices in the same T-maze we used in this study, they do not display any bias in their turning direction (Dimitriou, 2018). Although bumblebees naturally leave the colony to forage, it is difficult to control their motivation once they are released into a completely new environment such as a T-maze, which partially limits the space they can navigate through. Thus, we decided to test them in a task that could mimic their foraging behaviour in order to narrow down the motivation behind their behavioural choices. By training them to associate a coloured target placed in the T-maze with a syrup reward we reinforced their motivation to forage when out of the colony and, importantly, their familiarity with the T-maze environment. We then assumed that when leaving the colony, forager bees were exploring the T-maze to find the previously rewarded blue target. The bias towards the right arm of the T-maze indicates that when foraging they prefer to approach the rewarding target on their right than on their left. This could be possibly associated with a specialisation of the right side of the nervous system for tasks related to foraging/prey catching.

Sensory modalities that bees would likely use when traversing the T-maze have been found to be lateralized to the right; the right antenna is superior for odour learning and short-term memory recall both in honeybees (Letzkus et al., 2006; Rogers and Vallortigara, 2008; Anfora et al., 2010; Frasnelli et al., 2010a) and bumblebees (Anfora et al., 2011), likely due to olfactory sensilla being more abundant on this side (Frasnelli et al., 2010b; Anfora et al., 2011). Honeybees turn towards isoamyl acetate (a component of the alarm pheromone), lemon and orange odours when these odours are presented on the right and away from them on the left, but they turn towards the rose odour on both sides (Rogers and Vallotigara, 2019). Furthermore, honeybees are more sensitive to sugar when they perceive it through the right antenna than the left antenna (Baracchi et al., 2018) and the right eye is primarily used for associative visual learning (Letzkus et al., 2008). Bees therefore appear better sensorially equipped for foraging on their right side, which could result in a motor bias in the same direction (Ong et al., 2017; O'Shea-Wheller, 2019).

The expression of lateralization seems to be also stimulus dependent. Honeybees respond better through the right antenna when presented with odours previously associated with a food reward, but this is not the case for all odours (Frasnelli et al., 2010a; Rigosi et al., 2011). Moreover, honeybees turn towards odours presented on both sides or only on the right side, depending on the olfactory stimulus (Rogers and Vallotigara, 2019). This furthers the concept that laterality is highly dependent on context, including the protocol and stimuli used in experiments (Frasnelli and Vallortigara, 2018). Bees have an innate preference for the colour blue (Chittka and Wells, 2004) and this is enhanced when the stimulus is increased in size (Naug and Arathi, 2007). As lateralization in honeybees' olfactory memory recall is odour-dependent, similarly lateralization of visual memory could also be dependent on the stimuli. Further studies need to investigate whether this is the case, by testing bees with different colours. Other invertebrate species have shown biases when tested in T- or Y-mazes. Some of them seem to be lateralized at the individual-level, i.e. each individual within the population tested has a significant preference for either the left or the right arm; this is the case of fruit flies (*Drosophila melanogaster*) (Buchanan et al., 2015) and seven-spotted ladybirds (*Coccinella septempunctata*) (Girling et al., 2007). Some other species present the same direction of bias in the majority of the individuals within the sample, i.e. at the population-level (Frasnelli, 2017). A leftward bias in turning was found in giant water bugs (*Belstoma flumineum*) (Kight et al., 2008), in the above-mentioned cuttlefish (Jozet-Alves et al., 2012), and in red bugs (*Dysdercus andreae*) (Rivero-Aragon et al., 2018). Interestingly, in this last study, when the bugs were blown with air before being placed in a T-maze, the bias was no longer found, suggesting that stress can affect the expression of

asymmetrical turning behaviour (Rivero-Aragon et al., 2018). A relationship between lateralization and stress has been shown in some vertebrate species. For example, Port Jackson sharks (*Heterodontus portusjacksoni*) exhibit lateralized turning in a T-maze, with more strongly lateralized individuals being more reactive to stress (Byrnes et al., 2016). Thus, not only the context and the stimuli animals are tested with, but also by their status can determine whether behavioural asymmetries are manifested or not and also at which level (individual- or population-) (Frasnelli and Vallortigara, 2018).

Population level lateralization has been thought to be linked to specific selective pressures pushing individuals to coordinate their behaviour (Ghirlanda et al., 2009; Frasnelli and Vallortigara, 2018). However, when coordination is not required, even social species can be lateralized at the individual level. At the same time, solitary species engaging in social interactions may align their bias in the same direction as shown in red mason bees (*Osmia cornuta)* (Rogers et al., 2016). In our task, the behaviour did not include any interaction among individuals; thus, our findings may be unexpected. However, as discussed above, the population-level rightward bias we observed may be induced by the specialisation of the right side of the brain in foraging behaviour.

When tested in consecutive trials, animals could just adopt the same strategy that proved to be successful. Bees use previous information from their initial choice to make subsequent tunnel choices (Macuda et al., 2001). In this study, all the testing trials were unrewarded to control for that. Our data show that none of the 20 bees we tested choose the same T-maze arm in all trials. Even more interestingly, when released for the first time into the maze, 14 bees chose the left arm and 6 bees chose the right arm, but when released for the second time, 9 out of the 19 bees changed their initially preferred arm. This confirms that the bias we found is not induced by an initial successful choice.

A lateralized brain allows a more efficient use of neural circuitry, multiple stimuli to be processed discretely but simultaneously, more rapid responses because of lack of confliction between the two sides of the brain in responding to stimuli, and consequently increases cognitive efficiency (Rogers and Vallortigara, 2005). Support for this can be found in evidence linking the presence and strength of lateralization to cognitive performance (Frasnelli and Vallortigara, 2018). For example, locusts show an individual-level limb preference for crossing a gap (Bell and Niven, 2014) with the more strongly lateralized individuals making fewer mistakes that the less lateralized ones (Bell and Niven, 2016). Modelling suggests that seven-spot ladybirds (*Coccinella septempunctata*) that have a turning bias are more efficient foragers than those without a bias (Girling et al., 2007). At the same time, the lack of bias in red bugs when they are stressed could be an adaptive response to ensure the behaviour is not predictable when escaping predators (Rivero-Aragon et al., 2018).

Among vertebrates, strongly lateralized topminnows (*Girardinus falcatus*) show superior spatial learning abilities compared to non-lateralized individuals (Sovrano et al., 2005). In rainbowfish (*Melanotaenia duboulayi*) the direction of laterality affects learning; left-lateralized fish learned to associate a light with food faster than right-lateralized fish (Bibost and Brown, 2014). In zebrafish (*Danio rerio*), 54% of the individual tested exhibited a turning bias in a Y-maze, with lateralized fish showing increased associative learning during fear conditioning (Fontana et al., 2019).

The rightward bias showed by the bumblebees we tested had no effect on the time taken to make the choice in the T-maze: nor the strength or the direction of the bias influenced the time taken to make a choice. In honeybees, instead, the decision latency on right turns was significantly less than that for straight line or left turn decision (O'Shea-Wheller, 2019). Although the bias we found in bumblebees does not have an effect on their latency to make a choice, it can still provide them with an advantage when they engaged in a difficult and complex task such as foraging as it could allow them to simultaneously process different stimuli. Cuttlefish, for example, show a preference towards the left visual field to scan for potential predators during an anti-predatory task, and towards the right visual field for prey attack (Schneel et al., 2016). This right-side bias for predatory behaviour is in line with the bias we observed in the current study for foraging behaviour in bumblebees. Future studies may investigate whether bees, as cuttlefish, present a complementary left bias for anti-predator behaviour. A similar division of functions has been observed in several vertebrate species (Rogers et al., 2013). Toads, for example, clearly show a complementary right and left visual hemifield use for predatory and agonistic behaviour (Vallortigara et al., 1998). As the vertebrate brain is crossed-wired (i.e. the left hemisphere control the contralateral right side of the body and vice versa), this suggests that in vertebrates the left and the right brain hemispheres are specialised for routine behaviours (such as foraging, predatory behaviour) and for response to unexpected stimuli (such as predators, agonistic behaviour) respectively (Rogers et al., 2013). In invertebrates, instead, the neural projections between the nervous system and the body are ipsilateral (i.e. on the same side). Thus, the preferential use of the right visual hemifield for foraging/predatory behaviour would suggest a specialisation of the right side of the brain for such tasks.

As bumblebees vary significantly in size (Goulson et al., 2002) with large bees being better learners than their smaller sisters (Mares et al., 2005), we wanted to investigate whether the visuo-motor bias manifested by the bumblebee in this study was related to the size of the individuals tested. We found no significant correlation between the bees' size and their laterality index (LI) nor between the bees' size and the absolute LI (│LI│). We recognise, however, that we have tested a relatively small number of bees. Large

bumblebee workers have a significantly higher number of PDF neurons (the pigment dispersing factor is a gene that encodes the protein PDF, a neuropeptide which is used by neurons in communication) and a greater area of neuron soma (Weiss et al., 2009). Thus, the body size of a bumblebee is related to foraging behaviour (Goulson et al., 2002), but also to neural characteristics (Weiss et al., 2009). Further studies may investigate in a bigger sample size of bees showing a wider size range whether different body sizes also reveal different degrees of brain and behavioral lateralization.

In summary, the results of this study show that buff-tailed bumblebees demonstrate a population-level rightward turning bias when completing a simple visuo-motor directional task. This rightward turning preference may indicate a specialisation of the right side of the nervous system to perform foraging-related behaviours. This specialisation within the population may allow for more collective decision-making by the colony, with large bees being more strongly lateralized and efficient in foraging tasks. Future studies may investigate the benefit of visuo-motor biases in foraging tasks as well as the relationship between bees' size and the degree of lateralization to provide additional insights and improve our understanding of the evolution of lateralization.

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DECLARATION OF INTEREST STATEMENT

The authors declare no conflict of interest.

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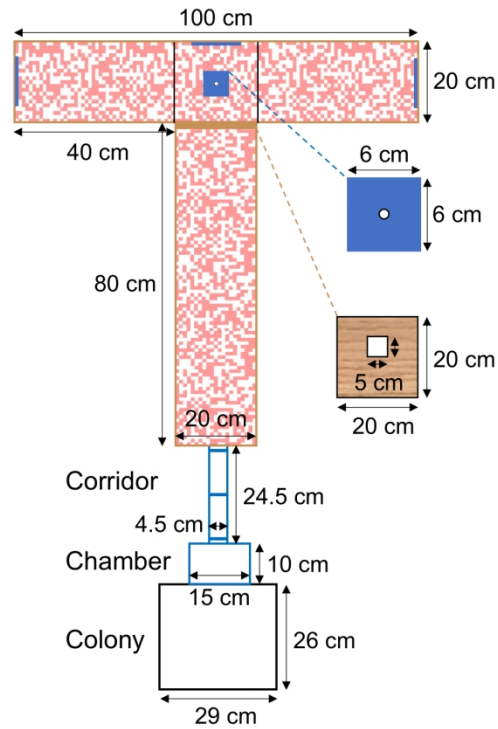43
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FIGURE CAPTIONS

Figure 1 – Schematic representation of the T-maze apparatus used in the experiment. The colony is connected to the T-maze through a Plexiglas chamber and tunnel. Vertical sliding doors, highlighted in red, allow the experimenter to select specific individuals. The blue target consists of a squared piece of plastic paper with an Eppendorf tube's lid glued on it, containing syrup.

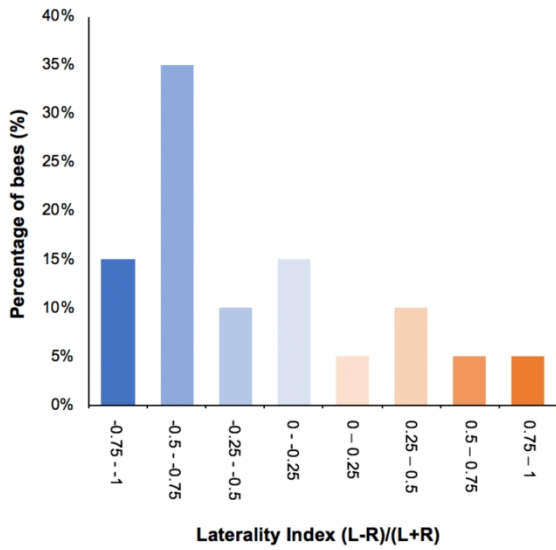**Figure 2** – Histogram of the laterality indices of the sampled bumblebee population (N = 20).

Figure 3 – Relationship between the bees' size (measured as thorax width) and a. the laterality index (LI); and b. the absolute laterality index ($|LI|$) (N = 20).
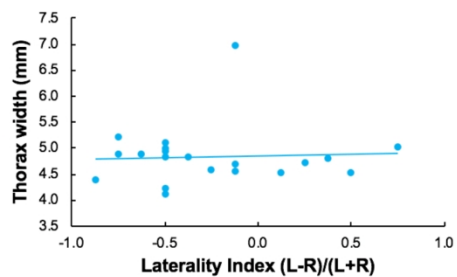
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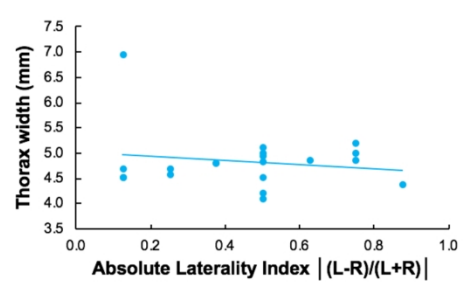51
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