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Consistent Interindividual Differences in Discrimination Performance by Bumblebees in Colour, Shape and Odour Learning Tasks (Hymenoptera: Apidae: Bombus terrestris)

Helene Muller & Lars Chittka

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Muller H & Chittka L (Biol Chem Sci, Queen Mary University of London, London, E1 4NS) **Consistent Interindividual Differences in Discrimination Performance by Bumblebees in Colour, Shape and Odour Learning Tasks (Hymenoptera: Apidae: Bombus terrestris).** – Entomol Gener **34** (1/2): 000–000; Stuttgart 2012-09 – – – [Article]

The ability to discriminate between flower-related stimuli is essential to bumblebees foraging in a natural environment, given the variety of flower features and profitability. Using differential conditioning tasks in a foraging context, it turns out that some individual bumblebees (*Bombus terrestris* Linnaeus 1758) were consistently better than others at discriminating rewarding from punishing stimuli across modalities (visual or olfactory) or dimension (colour or shape) of the cues used. The size of the bees did not have a significant effect on the individuals' discrimination abilities but there was a significant difference between the two colonies tested. Bees therefore appeared to differ in their ability to discriminate between stimuli both individually and at the colony level. The here presented results have implications for the study of insect cognition as well as for the study of bees' foraging behaviour and task specialisation in social insects.

Keywords: *Bombus terrestris* (Linnaeus 1758) – cognition – individuality – olfaction – vision

Muller H & Chittka L (Biol Chem Sci, Queen Mary University of London, London, E1 4NS) **Différences Interindividuelles en termes d'Apprentissage des Couleurs, Formes et Odeurs chez le Bourdon (Hymenoptera: Apidae: Bombus terrestris)**. – Entomol Gener 34 (1/2: 000–000; Stuttgart 2012-09 – – – [Article]

La capacité de distinguer entre différents stimuli floraux est essentielle à la survie des bourdons dans leur environnement naturel, où les caractéristiques et le degré de profitabilité des fleurs sont susceptibles de varier. Nous avons montré que, lorsque des bourdons (*Bombus terrestris* Linnaeus 1758) sont soumis à des tests de conditionnement différentiel, certain individus sont constamment meilleurs que d'autres, et ce, indépendamment de la modalité (visuelle ou olfactive) ou de la dimension (couleur ou forme) des stimuli. La taille des individus n'avait pas d'effet significatif sur ces résultats. Par contre, nous avons montré qu'il y avait une différence significative, en termes de capacité à discriminer, entre les individus provenant de deux colonies différentes. Notre étude suggère qu'il existe des différences de capacité de discrimination au niveau individuel et au niveau de la colonie chez les bourdons. Cette étude pourrait donc se révéler d'une grande importance dans le domaine de la cognition de l'insecte, de l'étude du comportement de fourragement chez les *Apidae* et dans l'étude de la division des tâches au sein des colonies d'insectes sociaux.

Mots clés: *Bombus terrestris* (Linnaeus 1758) – cognition – individualité – olfaction – vision

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1 Introduction

Most animals, from the roundworm *Caenorhabditis elegans* [Rankin, Beck & Chiba 1990] to humans, are capable of some form of learning and there is evidence that variations in learning abilities can impact fitness [Dukas & Bernays 2000, Ohashi, Leslie & Thomson 2008, Raine & Chittka 2008, Snell-Rood, Davidowitz & Papaj 2011, Kawecki 2010]. Individuals of a species vary substantially in their learning abilities [Chittka & Thomson 1997, Kolata, Light, Townsend et al 2005, Raine, Ings, Dornhaus et al 2006, Raine, Ings, Ramos-Rodriguez et al 2006, Dukas 2008, Muller & Chittka 2008, Kotrschal & Taborsky 2010]. However, for most species, relatively little is known about individual consistency in learning abilities across time or across dimensions and modalities. In honeybees, Page & Scheiner [2006] reported a significant correlation between a sensory response (sensitivity to sucrose concentration) and learning performance (learning speed and highest learning asymptote) during an olfactory task. In this species, it is also known [Page & Scheiner 2006] that sensory thresholds are correlated with each other across some modalities (gustatory, olfactory and visual), but not others [Roussel, Carcaud, Sandoz et al 2009].

However, many of these differences were observed between specialised groups of honeybees (e.g pollen versus nectar foragers or especially established breeding lines) [Chandra, Hosler & Smith 2000, Page & Scheiner 2006], and learning was quantified as a product of group behaviour; individual learning performance was not typically quantified [Muller, Grossmann & Chittka 2010]. For bumblebees, one of the model organisms in the study of the evolutionary ecology of learning, inter-individual differences in learning performance have been measured in a variety of tasks [Chittka & Thomson 1997, Chittka, Dyer, Bock et al 2003], but it is not known if the learning performances are correlated with each other across modalities. Raine & Chittka [2008] found that learning speed in a colour discrimination task correlated with fitness as assessed by foraging performance in the wild. To explore the generality of this correlation, it is essential to demonstrate that better learners are not limited to superior performance in a single task, but that performance is individually consistent across tasks. The ability to learn to discriminate between two stimuli within a particular dimension/modality might reflect a more general ability to learn, independently of the task, dimension or modality considered [Boogert, Anderson, Peters et al 2011]. Alternatively, it is conceivable that there are tradeoffs between performance levels across tasks, so that superior performance in one task comes at the expense of poor performance in another [Papaj & Snell-Rood 2007].

The here presented study therefore aims to explore whether individual discriminatory abilities are consistent across and within sensory modalities. To achieve this, simple differential conditioning was used based on a foraging paradigm in which the type of cues could be varied which the bees had to learn. Each individual bee was tested on her ability to differentiate between pairs of stimuli belonging to a given modality (visual or olfactory) or dimension of the visual modality (shape or colour).

2 Material and methods

Two colonies of *Bombus terrestris* containing approximately 40 workers each were obtained from Syngenta Bioline (Weert, Netherlands). Queens of this species mate only once in their life; therefore all the foragers within a colony are full sisters [Schmid-Hempel & Schmid-Hempel 2000]. Upon delivery, colonies were transferred into bipartite wooden nest boxes (28 × 16 × 11 cm). All tested bees were individually tagged with coloured dots or Opalith number tags (Christian Graze KG, Germany).

The nest box in which the colony was housed was connected through a plastic tube to a foraging flight arena (120 × 100 × 35 cm) covered with a UV-transparent Plexiglas lid (**Fig. 1a**).

Bees could be allowed one at a time into this arena using a system of shutters built in the connecting tube. The room in which the colonies were kept had an average ambient temperature of 21 ºC. Controlled illumination for laboratory experiments was provided by high-frequency fluorescent lighting (TMS 24F lamps with 4.3 kHz ballasts; Philips, The Netherlands) fitted with Activa daylight tubes (Osram, Germany) to simulate natural daylight, and equipped with special ballasts for high frequency lighting above the bee flicker fusion frequency [Dyer & Chittka 2004].

The maximum thorax width was measured three times per individual bee and took the average as an estimate of the bee's size (thorax width is the most common measure of body size for bumblebees) [Spaethe & Weidenmuller 2002, Spaethe & Chittka 2003]. This information is essential since sensory performance in some tasks can be correlated with body size; for example the visual-spatial resolution of the eye is superior in larger workers [Spaethe & Chittka 2003]. Foragers were initially allowed to collect 50 % (w/w) sucrose solution from translucent gravity feeders [Von Frisch 1967, p 19, Fig 18] which provided unlimited supplies for two days.

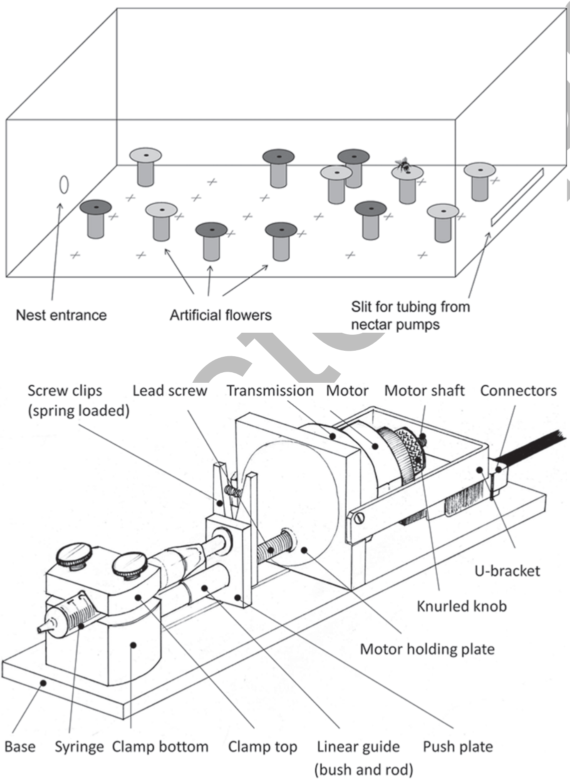

Fig 1: Experimental setup: flight arena (a), and nectar-pump (b). **(a)** The bees were released one at a time in the arena (dimensions: 120 × 100 × 35 cm) containing the artificial flowers distributed in a random pattern. The sides of the arena were made of wood whereas the top lid was UV-transmitting Plexiglas. The position of the flowers was changed after every foraging bout. Crosses on the floor mark the 6 x 5 position grid onto which artificial flowers were randomly allocated. **(b)** Each 'flower' was connected to a nectar-pump [Leadbeater & Chittka 2008]. A pump consisted of a glass syringe the plunger of which was connected to a lead screw. This screw was rotated by a mains-operated motor (500 rpm with gearbox set for 1 rev/12 minutes; RS, London, UK), causing the plunger to continuously squeeze minute amounts of 40 % (w/w) sucrose solution out of the syringe into the tubing and out into the cup of the flower. Drawing of the pump mechanism by Sara Blackburn.

Thereafter, sucrose solution was provided through 'nectar pumps' connected via flexible plastic tubing to artificial flowers. Each 'flower' consisted of a cut-out shape of laminated coloured paper ('corolla') mounted on a grey plastic cylinder (height: 6 cm, diameter: 3.1 cm). The 'corolla' part of the flower was pierced in its centre to allow space for a small plastic cup containing the sucrose. The cup was embedded into the plastic cylinder so that the amount of nectar available in it could not be seen by the bee. The cup was connected to a pump (**Fig 1b**) delivering 40 % sucrose (w/w) through the tubing at a rate of 0.363 ml/h. The 'corolla' part of each flower was cleaned with 70 % ethanol between foraging bouts so as to ensure there were no scent marks left from previous visits by the bee [Saleh, Scott & Bryning et al 2007].

The position of the flowers on the arena was shuffled between foraging bout using computer-generated random spatial patterns on a 6 × 5 square grid (with 14 cm between positions). This shuffling of the position of the flowers was necessary to prevent the bees from associating location with reward [Chittka, Kunze, Shipman et al 1995]. Bees were pre-trained collectively to obtain sucrose from the artificial flowers without any paper 'corolla'. The grey cylinders supporting the cup and tubing were achromatic and pre-training to achromatic stimuli has been shown not to affect colour preference during subsequent exposures to coloured stimuli [Giurfa, Núñez, Chittka et al 1995]. A focal bee was then allowed alone into the arena with 'corolla'-less flowers for one foraging bout prior to the experiment itself.

Each focal bee was subjected to three tasks, after which she was removed from the colony. Two tasks were visual (learning to differentiate between colour and shape respectively) and one task was olfactory. During the 'shape' task, individual bees were presented with six cross-shaped flowers (4 × 4 cm; 4 arms, each 2 cm wide) and six octagon-shaped flowers (4 cm across, sides of 1.7 cm). Both shapes of 'corolla' were cut from green-coloured paper (khaki, Maya coloured card; Clairefontaine, Ottmarsheim, France) and were covered with transparent plastic film (Frisk Coverseal Film Rolls; Artcoe, Manchester, UK) for easy cleaning. Each of the octagon-shaped flowers was linked to a sucrose pump which delivered 40 % sucrose (reward) whereas the cross-shaped flowers contained saturated quinine hemisulfate salt (Sigma, UK) solution (punishment). In the 'colour' task, bees were presented with 12 flowers bearing 'corolla' (paper discs: diameter: 5 cm) of two different colours: six were ivory-white and six were barley-white (Daler Canford Card; Daler-Rowney, Bracknel, England), covered with transparent film. In the olfactory discrimination task, the 'corolla' of the flowers consisted of discs (diameter: 5 cm) of green coloured paper, covered in transparent film except for a small central area (disc diameter: 1.8 cm), where the paper was left uncovered so as to enable absorption of the scented solution. Five microlitres of scented solution (333 µL/L solution of peppermint or anise star essential oil; Essential Oils Direct, Oldham, UK) was deposited on the paper surface of each flower before each foraging bout. There were six anise-star-scented flowers, connected to the nectar-pumps and six peppermint-scented flowers, containing quinine solution. The flowers containing quinine were not linked to pumps. Unconnected tubing was used to mimic the appearance of the rewarding flowers. We varied the order in which the bees were subjected to the tasks to control for order effects (colony A contributed 21 bees in total and colony B contributed 17 bees in total, order1: shape-colour-scent $N_{colonyA} = 11$ and $N_{colonyB} = 10$; order2: colour-scent-shape $N_{colonyA} = 5$ and $N_{colonyB} = 3$; order3: scent-shape-colour $N_{colonyA} = 5$ and $N_{colonyB} = 4$).

Each task presented the bee with two different artificial flower types. For each individual bee, we recorded the first 50 choices, a 'choice' being defined as a landing on a flower. Landing on the rewarded stimulus (sucrose solution) was considered as a correct choice whereas landing on the stimulus associated with punishment (quinine solution) was considered as incorrect. Individual bees' performance was measured in the saturation phase of their learning curve, when performance increased no further (choices 31–50).

Kendall's *W* (also known as Kendall's coefficient of concordance) was used to assess the consistency of the bee's performance across all tasks. Kendall's *W* quantifies the agreement between ranks of two or more variable and has been used to assess the consistency of behavioural traits [Briffa, Rundle & Fryer 2008]. Kendall's *W* ranges from 0 (no agreement) to 1 (complete agreement).

In order to test for the effect of various factors (the nature of the task, the order of the task and the colony from which the bees originated), a Repeated Measures General Linear Model (SPSS 16.0) was built.

All tests were two-tailed and the alpha level was set to 0.05. Individual bees were used as within-subject factor with three levels, corresponding to the three tasks (shape, colour and scent discrimination). Two between-subjects factors were included: colony and the order in which the tasks were performed in a full-factorial model. Correlations between size and discrimination performances were investigated using Spearman's rank correlation coefficient.

3 Results

Individual scores for discrimination performance across tasks were significantly concordant (Kendall's $W_{38} = 0.530$, $P < 0.0001$, see **Fig 2**), meaning that the rank of a given bee for one task was consistent with her rank for the two other tasks. There was a significant within-subjects effect of Bee ($F_{2,64} = 32.89$, $P < 0.001$), demonstrating that bees differed significantly from one another. There was a significant between-subjects effect of colony ($F_{1,32} = 9.00$, $P = 0.005$), suggesting that the two colonies differed in their mean for discrimination performance. There was no significant between-subject effect of task order ($F_{1,32} = 1.88$, $P = 0.16$) and no significant interaction between colony and task order ($F_{2,32} = 0.92$, $P = 0.406$). There were no significant correlations between size and the discrimination performance for any task (Spearman correlation coefficients, all $P > 0.16$).

4 Discussion

The results show that there is consistent variation in discrimination performance in a colour, shape and scent learning tasks between individual foragers. In other words, bees which made the highest number of correct choices during their first task were also the ones which made the most correct choices during the second and third task. It is tempting to speculate that these findings support the view that learning is domain-general [Chiappe & MacDonald 2005] and adding support to the theory that evolution acts on 'generalized learning processes' (i.e learning a across a wide range of task differing in nature) rather than independent 'cognitive modules' [Kawecki 2010]. However, the observed discrimination abilities could be the result differences in sensory performance rather than learning. Scheiner & Erber [2009] showed in honeybee that sensory thresholds correlate across modalities [but see Roussel et al 2009]. The bees which had a low response threshold to a stimulus in the gustatory modality also had a low threshold response to stimuli in the olfactory and visual modalities [Scheiner & Erber 2009]. In the here presented study, not only could bees vary in their response threshold to the visual and olfactory stimuli, but also, they could vary in their response threshold to both gustatory stimuli (sucrose and quinine). It is therefore possible that some of the individual variation in discriminating abilities observed here could be attributable to differences in response threshold for conditioned as well as unconditioned stimuli.

Whether the observed individual variation in discrimination abilities can be explained solely or partially by learning and/or sensory thresholds, the ability to discriminate stimuli in the environment is very likely to affect an individual's foraging performance [Raine & Chittka 2008] and therefore colony fitness. Indeed, learning plays a major role in the development of individual behaviour and in the way an individual responds to its environment [Ravary et al 2007, Chittka & Muller 2009] and learning cannot occur without some form of discrimination between the stimuli available in the environment.

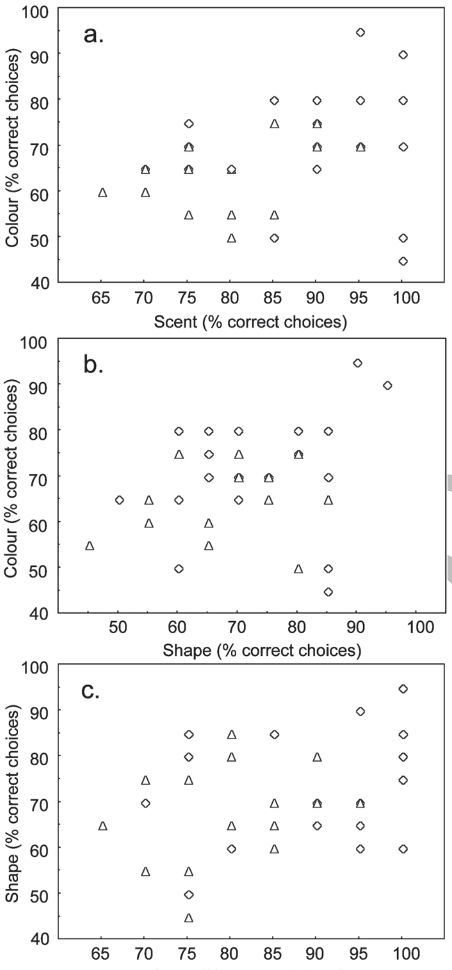

Fig 2: Pairwise correlations for discrimination performance in scent, colour and shape learning (percentage of correct choices between the 31st to the 50th choice). Spearman's correlation coefficient calculated for each pair of variables: a. Colour vs scent: RS = 0.33, P = 0.04; b. shape vs colour: RS = 0.31, P = 0.057; c. shape vs scent: RS = 0.43, P < 0.01. Circles represent bees from colony A and triangles represent bees from colony B.

In social insects, learning and the ability to discriminate between stimuli is essential to the performance of certain tasks, such as foraging. A forager has to learn and remember the location and features (stimuli) associated with the food sources [Gumbert, Kunze & Chittka 1999, Chittka & Raine 2006]. Other tasks appear to be much less demanding in terms of learning and discriminatory ability and may be largely governed by innate behaviour (e.g nursing the brood). Given this difference between tasks, it is natural to postulate that a bee's ability to perform a given task would be related to her ability to learn the task. Indeed, in honeybees, foragers and nurse bees are known to differ in learning abilities [Ben-Shahar, Thompson, Hartz et al 2000] and in ants, Ravary, Lecoutey, Kaminski et al [2007] showed that individual experience can generate task specialisation.

It is premature to conclude that the likelihood to perform a cognitively demanding task is linked to an individual's cognitive abilities, but the here presented study suggests that this is fruitful area for further research. The presented findings show that individual bumblebees consistently differ in their ability to learn to discriminate stimuli from the visual and olfactory modality. These results open the door to further research on the potential link between sensory/cognitive abilities and consistent individual behaviour, and more specifically, task specialisation in social insects.

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Authors' address – adresse des auteurs: Dr Helene Muller and Prof Lars Chittka, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, UK.
l.chittka@qmul.ac.uk