Male bumblebees, *Bombus terrestris*, perform equally well as workers in a serial colour-learning task

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The learning capacities of males and females may differ with sex-specific behavioural requirements. Bumblebees provide a useful model system to explore how different lifestyles are reflected in learning abilities, because their (female but sterile) workers and males engage in fundamentally different behaviour routines. Bumblebee males, like workers, embark on active flower foraging but in contrast to workers they have to trade off their feeding with mate search, potentially affecting their abilities to learn and utilize floral cues efficiently during foraging. We used a serial colour-learning task with freely flying males and workers to compare their ability to flexibly learn visual floral cues with reward in a foraging scenario that changed over time. Male bumblebees did not differ from workers in both their learning speed and their ability to overcome previously acquired associations, when these ceased to predict reward. In all foraging tasks we found a significant improvement in choice accuracy in both sexes over the course of the training. In both sexes, the characteristics of the foraging performance depended largely on the colour difference of the two presented feeder types. Large colour distances entailed fast and reliable learning of the rewarding feeders whereas choice accuracy on highly similar colours improved significantly more slowly. Conversely, switching from a learned feeder type to a novel one was fastest for similar feeder colours and slow for highly different ones. Overall, we show that behavioural sex dimorphism in bumblebees did not affect their learning abilities beyond the mating context. We discuss the possible drivers and limitations shaping the foraging abilities of males and workers and implications for pollination ecology. We also suggest stingless male bumblebees as an advantageous alternative model system for the study of pollinator cognition.

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Bumblebees, *Bombus terrestris audax*, originated from four commercial colonies provided by BIOBEST (Biobest Belgium N.V., Westerlo, Belgium). Each nestbox was connected to a bifurcated Perspex tunnel system leading to two identical flight arenas (100 × 70 cm and 30 cm high), in which males and workers were pretrained to freely forage for 20% sucrose solution (w/w) from 12 clear square Perspex chips (25 × 25 mm and 5 mm high) placed horizontally on top of an upright clear glass tube (height: 40 mm; Fig. 1a). Males and workers successfully feeding on these Perspex feeders were individually marked with number tags (Opatlithplättchen, Warnholz & Bienenvogt, Ellerau, Germany). Pollen was provided ad libitum directly into the colony.

**Methods**

**Bees and Experimental Set-up**

For the training we replaced the Perspex feeder chips in one of the two flight arenas, the training arena, with coloured square plastic chips (25 × 25 mm and 5 mm high), while the other arena remained set up with Perspex feeders as a foraging arena (Fig. 1a). With shutters in the tunnel system connecting the colony to the two arenas we could divert workers for the colour training individually into the training arena, while allowing normal colony foraging on the clear feeders in the other arena.

The training arena contained 12 feeders of two types. One feeder type (six feeders) was rewarding (CS⁺) and provided 2 µl of sucrose solution (20% w/w), whereas the unrewarding feeder type (CS⁻) contained a 2 µl droplet of water. The small reward volume was chosen to encourage multiple feeder choices during each foraging bout in both workers and males, while the low sucrose concentration ensured that satiated males would become hungry again after short periods of food deprivation.

We used four different feeder colours in this study. To human observers these were yellow, green, blue and purple. The reflectance for each of those colours was measured in 1 nm increments over a wavelength range from 300 to 700 nm using a spectrophotometer (Ocean Optics S2000, Dunedin, FL, U.S.S.) with a deuterium/halogen light source with feeder reflectance values varying from 0 (no reflectance) to 1 (100% reflectance). Combining these reflectance curves with the spectral sensitivity functions of the three photoreceptors of *B. terrestris* (maximal sensitivity  in UV: 348 nm; blue: 435 nm; green: 533 nm; Chittka, Spaethe, Schmidt, & Hickelberger, 2001; Skorupski, Döring, & Chittka, 2007) we determined the positions of the feeder colours within the bee colour hexagon (Chittka, 1992). The distances between the feeder colour loci range from a small colour distance of 0.061 hexagon units between yellow and green, a medium colour distance between purple and blue of 0.189 to large colour distances of 0.3 hexagon units for blue-yellow (0.333) and purple-green (0.391; Fig. 1b).
Feeder Colour Sequences

Mimicking a temporal change in the floral market similar to that experienced by both workers and males, we used two sequences of successively presented feeder colour pairs. Colour sequence A started with rewarding yellow feeders (yellow+/−) and nonrewarding blue feeders (blue+/−) and subsequently progressed to green+/−/green− and blue+/−/purple−. In sequence B we reversed the colour sets and rewarding colours starting with purple, rewarding (purple+/−), and blue, nonrewarding feeders (blue+/−) followed by green+/−/green− and blue+/−/yellow− (Figs. 1c and 2). In this way we balanced the use of feeder colours controlling for potential differences in colour preferences between males and workers.

Training Procedure

Workers and males differ fundamentally in their foraging behaviour. Workers repeatedly move between the foraging grounds and the colony (Sladen, 1912), where they regurgitate their harvest before they resume foraging again. Although hunger may also be a contributing factor, their motivation to forage is largely determined by the nutritional status of the colony (Dornhaus & Chittka, 2001; Dornhaus & Chittka, 2005; Molet, Chittka, Stelzer, Streit, & Raine, 2008). For the colour training we only chose workers that commuted between the feeders and the colony at least three times during pretraining, indicating an active and motivated forager. Self-provisioning male bumblebees, on the other hand, only forage to satisfy their own energetic needs and commence foraging as soon as they are sufficiently hungry (Goulson, 2003). For the experiment we selected only those males that had been observed feeding on the Perspex feeders on at least three different occasions.

To provide comparable training conditions we not only tested workers in their usual foraging mode (shuttling between colony and artificial flower meadow) but we also trained isolated workers such as the males under a regime of controlled food deprivation of 2 h prior to testing and between foraging bouts to motivate them to visit the feeders. Once a bee entered the training arena it had to distinguish between six rewarding feeders indicated by a colour and six differently coloured nonrewarding ones. We recorded the number of landings on nonrewarding feeders before the first correct choice (‘latency to switch’). After a rewarding feeder had been visited for the first time, we recorded feeder choice (defined as landings on the feeder) and feeding events for the next 50 visits. Depleted rewarding feeders were refilled by hand with 2 μl of sucrose solution from an electronic dispenser pipette (HandyStep electronic, BrandTech Scientific, Inc., Essex, CT, U.S.A.) after the bee had left the feeder and had landed on another rewarding feeder. Recording continued until the bee was satiated and ceased foraging (foraging bout) or reached 50 visits and was caught.

Workers tested in their natural foraging mode (e.g. Raine & Chittka, 2008; Raine & Chittka, 2012; Raine, Ings, Ramos-Rodriguez, & Chittka, 2006; Wolf, Roper, & Chittka, 2015) were free to return to the colony after completing a foraging bout or were returned to the colony after 50 visits were completed. Their reentering the training arena from the colony was regulated by means of shutters in the connecting tunnels. In contrast, all males and the group of workers trained using between-bout food deprivation were transferred into a separate and empty flight arena where they were allowed to move about freely but had no access to food for 2 h before being transferred back to the training arena.
Between foraging bouts all feeders were cleaned and the locations of the rewarding and unrewarding feeder types were randomized. If the bee had not completed the training on a colour pair (i.e. visited less than 50 feeders) in the previous bout the bee was allowed to complete training on the previously encountered feeder colours. Alternatively, if the training on the previous colour pair had been completed, the feeders’ colours were exchanged for the next colour pair in the training sequence (Fig. 1c).

Sixty males were trained, of which 30 completed the full training sequence. In addition we trained 59 workers (48 with natural foraging and 11 with food deprivation). Forty of the natural foragers and 10 of the food-deprived individuals engaged with the full training sequence.

After completion of the last colour pair in the training sequence each bee was allowed to continue foraging to satiation (no limitation of visits) on these feeders while the recording of visits and feeding events continued. Using the largest volume of sucrose collected in a single bout by a bee we estimated the bees’ crop capacity even if this exceeded 100 μl (maximum collectable volume in 50 visits) as a potential factor influencing foraging behaviour.

**Data Analysis and Statistics**

Learning curves were generated from the mean choice accuracies (±SD) of the bees for every consecutive block of 10 visits starting from the first correct visit in each of the four colour sets per training sequence (Fig. 2a, b). Latencies to switch were not plotted in the learning curve.

Following the approach of previous studies on bumblebee learning (Ings, Raine, & Chittka, 2009; Raine & Chittka, 2008, 2012)
we estimated individual learning speed (LS) from the rate at which the number of incorrect visits decreased over the course of the training fitting first-order exponential decay functions of the form
\[ y = y_0 + A e^{-kt} \]
using Origin Pro 8 v.8.0.7724 (OriginLab Corporation, Northampton, MA, U.S.A.). In this function \( x \) represents the number of flower choices starting from the first correct choice, \( y \) is the number of errors, \( y_0 \) is the number of incorrect choices at the end of the training and was constrained to 0 (100% correct feeder choices). Learning speed is reflected by the decay constant \( t \) where high \( t \) values indicate slow learning and vice versa. The curve amplitude \( A \) corresponds to the maximal number of incorrect choices the bee made and was constrained to 10 (100% incorrect choices). An exponential decay function could be successfully fitted to the behavioural performance in 71.9% of the workers' and 76.4% of the males' learning curves, respectively. The proportion of cases allowing for a successful curve fitting versus cases where no curve could be fitted was considered as an additional measure for potential performance difference.

We used a general linear model (GLM) fitted to a normal distribution with training procedure ('TP': with and without food deprivation), training sequence ('seq': A, B), colour distance of each feeder colour pair ('CD') and sex ('sex': male, worker) as fixed models to compare the choice accuracies in increments of 10 visits over the course of the training and learning speeds and latencies to switch (LAT) of the bees on each training regime (feeder colour pairs). Furthermore, using the global data set, we analysed the relationship of learning speed and latency to switch for both males and workers using a linear regression model. All statistical tests were done using Statistica 7.0 (Statsoft, Tulsa, OK, U.S.A.).

Ethical Note

Prior to the experiments all bees were in their natal colony environment and were able to freely forage in a flight arena. Colonies were kept in naturally dark conditions and the only experimenter actions at the colony were the provisioning with pollen every 2nd day. Males voluntarily left the colony and spent most of their time foraging, flying around or resting on the arena walls. Casual observations indicated that the time spent between actual bouts of flower visits was about 2 h; hence we used this interval of 2 h for our controlled food deprivation periods to ensure that individuals were not starved for periods that exceeded their voluntary breaks between bouts of foraging activity.

Workers, likewise, were not forced to leave the nest and were only trained and tested if they voluntarily engaged in foraging behaviour. In workers, a 2 h interval between foraging bouts has also been successfully used in other experimental work (Ravi, Crall, Fisher, & Combes, 2013). Food deprivation typically resulted in the bees ceasing to fly around and resting on the arena walls. They spontaneously commenced foraging when given the opportunity after 2 h. All bee handling procedures and transfers were carefully done using plastic pots rather than forceps to reduce stress of handling.

RESULTS

Effects of Training Protocol

Both males and workers could be trained on the coloured feeders. Comparing the choice accuracies of workers in the context of the training procedure ('TP'), we found no significant difference between the 11 workers subjected to the interbout food deprivation of 2 h and the 40 workers allowed to return to the colony between bouts (choice accuracy during the initial and final 10 visits per colour pair: GLM: blue-yellow/yellow-blue: initial: \( \tau_{55} = 0.39 \), final: \( \tau_{55} = 0.31 \), \( P = 0.69 \); yellow-green/green-yellow: initial: \( \tau_{55} = 0.06 \), \( P = 0.93 \); initial: \( \tau_{55} = 0.74 \), \( P = 0.46 \); green-purple/purple-green: initial: \( \tau_{55} = 0.25 \), \( P = 0.80 \); final: \( \tau_{54} = 0.33 \), \( P = 0.74 \); purple-blue/blue-purple: initial: \( \tau_{57} = 0.87 \), \( P = 0.38 \); final: \( \tau_{56} = 0.65 \), \( P = 0.52 \); overall: \( \tau_{1113} = 1.04 \), \( P = 0.30 \) (Fig. 2). We thus used the more natural uninterrupted training procedure to collect the majority of the worker data.

Foraging Characteristics of Males and Workers

Overall, males visited more feeders per foraging bout than workers, requiring an average of 1.3 ± 0.46 bouts to complete training on a colour set (50 visits) whereas workers needed 1.6 ± 0.54 bouts (\( t \) test: \( t_{207} = 5.84 \), \( P < 0.001 \)). While 84% of the males completed more than 40 visits in the first bout per colour set (70.3% finished the task in a single bout), only 41.3% of the workers accomplished more than 40 visits in the first bout (39.4% single bout tasks). Within the first bout on each colour pair, males accomplished on average more visits than workers (41.2 ± 10.9 visits) finishing the bout after 46.9 ± 7.9 visits (\( t \) test: \( t_{180} = 3.144 \), \( P < 0.01 \)). These differences can be explained by the significantly larger crop capacities we found in males than workers: the mean maximal crop fill in males (\( N = 20 \)) was 118.5 ± 43.8 µl whereas workers (\( N = 20 \)) only collected 80.9 ± 20.5 µl in a single bout (\( t \) test: \( t_{18} = 3.35 \), \( P < 0.01 \)).

Learning Performance of Males and Workers

Looking at feeder choice, we found that, independent of training protocol, both males and workers clearly improved their choice accuracy over the course of the training for each colour pair used (Fig. 2).

Throughout the training there was no significant difference in the choice accuracy of males and workers (effect of sex on choice accuracy on the initial and final 10 visits of the sequentially presented colour pairs in the sequence: first colour pair: initial: \( t_{112} = 0.51 \), \( P = 0.61 \); final: \( t_{110} = 0.04 \), \( P = 0.97 \); second: initial: \( t_{97} = 0.65 \), \( P = 0.52 \); final: \( t_{95} = 0.95 \), \( P = 0.35 \); third: initial: \( t_{80} = -1.59 \), \( P = 0.12 \); final: \( t_{85} = -0.84 \), \( P = 0.41 \); fourth: initial: \( t_{81} = -0.47 \), \( P = 0.64 \); final: \( t_{79} = 0.11 \), \( P = 0.91 \) (Fig. 2). Already at the end of the first bout on each colour pair both sexes reached similarly high mean choice accuracies (% correct of the last 10 visits) with 87.7 ± 12.9% (males) and 86.5 ± 13.9% (workers) correct choices (\( t_{109} = 0.48 \), \( P < 0.63 \)). There was no significant difference between the sexes in the learning speed (GLM: Wald test = 0.08, \( df = 1 \), \( P = 0.77 \)) or in the latency to switch (GLM: Wald test = 0.05, \( df = 1 \), \( P = 0.82 \)) for any of the colour pairs (Fig. 3a, b).

In addition to our analyses based on bees for which the learning speed could be quantified using exponential decay curve fitting with Microloric Origin (OriginLab Corporation), we also found no significant difference between the sexes in the prevalence of learning curves, to which no decay function could be successfully fitted, which was the case for 42 of 178 (males) and 47 of 167 (workers) learning curves (\( \chi^2_{1} = 0.93 \), \( P = 0.33 \)).

Effects of Colour Distance on Foraging Performance

In both sexes the overall foraging performance highly significantly depended on the colour distances (CD) of the feeder colours presented (GLM: LS: Wald test = 76.53, \( df = 3 \), \( P < 0.001 \); LAT: Wald test = 54.61, \( df = 3 \), \( P < 0.001 \)). Similar feeder colours (smaller colour distances in the hexagonal bee colour space) entailed faster switching than highly different colours in both males and workers, with colour distance being highly significantly correlated with the latency to switch to a novel colour (LAT-CD: males: \( R^2 = 0.25 \), \( P = 0.02 \); females: \( R^2 = 0.24 \), \( P = 0.03 \)).
Our results show that the ability to respond adequately to changes in foraging conditions was highly similar in males and workers, with the bees' sex not having significant effects on the recorded behavioural responses. In contrast, colour distance and experience proved to have highly significant but sex-independent effects on the foraging behaviour.

This study provides the first assessment of the learning abilities of freely foraging bumblebee males, to explore how sex-specific behavioural differences may affect the cognitive abilities in a nonnaming context. Comparing the learning performance of bumblebee males on temporally changing floral colour cues with that of the much better studied workers (Clarke, Whitney, Sutton, & Robert, 2013; Dyer, Whitney, Arnold, Glover, & Chittka, 2006; Nicholls & de Ibarra, 2014; Raine & Chittka, 2007, 2008; Raine, Ings, Dornhaus, Saleh, & Chittka, 2006; Raine, Ings, Ramos-
We found no difference between the two sexes. This is supported by recent findings on PER conditioning to colour stimuli of harnessed bumblebee males and workers (Lichtenstein et al., 2015). The similarity in learning ability between the sexes is surprising considering the workers’ near exclusive focus on foraging in contrast to the males’ need to integrate foraging abilities into a much broader behavioural repertoire. Trading off foraging behaviour with mate searching, self-provisioning males clearly benefit from being able to efficiently exploit available floral resources. Males face considerable energy costs of patrolling behaviour which is a prerequisite for mating involving sustained flights over large distances (Alcock et al., 1978; Frank, 1941; Haas, 1949; Svensson, 1979). Bertsch (1984) showed that males in a flight room fly up to 17 km a day, incurring substantial energetic costs. In our study, the ability to learn and memorize flower rewards facilitated the acquisition of high-quality food while also freeing up time for mate search. Bumblebee males have to frequently interrupt their patrol routes for foraging trips (Frank, 1941). During these breaks males will have no access to potentially available queens and may miss mating opportunities. This is aggravated by the fact that queens of most European bumblebee species mate only once (Schmid-Hempel & Schmid-Hempel, 2000). With several males often sharing patrol routes (Auyang et al., 2001; Stiles, 1976), competition for mating opportunities might well be severe. Assuming that queen encounters are rare events, efficient male foraging will be tightly linked to male mating success.

Of course behavioural differences do not inevitably translate into predictable variation in cognitive abilities, which may be the result of the interplay between multiple facilitating and limiting genetic, ontogenetic and environmental factors (Rowe & Healy, 2014). One possibility is that the efficiency of colour learning relies on identical genetic pathways in both sexes. This genetic correlation may be the ‘default scenario’, and any departure from this scenario (i.e. a sexual dimorphism) requires that the respective traits are controlled by different (or differentially expressed) genes in males and females. This may explain not only the males’ good learning abilities but also why workers, behaviourally highly specialized for near exclusive foraging, did not evolve superior learning abilities after losing the behavioural requirements of reproduction. As in foraging workers, male foraging performance was largely determined by the colour characteristics of the two feeder types presented. In line with previous studies on worker bees (Dyer & Chittka, 2004a, 2004b), highly similar flower colours (<0.1 hexagon units) resulted in slow learning and lower performance maxima in both workers and males as a result of poor colour discriminability (colour contrasts of only 0.04 hexagon units can only be discriminated by worker bumblebees in differential but not in absolute conditioning; Dyer & Chittka, 2004c) and bees generalizing over the two choices (Chittka, Gumbert, & Kunze, 1997). Under these conditions both males and workers very readily explored the novel feeder colour and learned this new rewarding colour only slowly. Additionally, as indicated by performance differences between the two training sequences, innate colour preferences seemed to play a role in choosing from highly similar feeders as compared to feeder types with large colour differences.

In contrast, males and workers were highly reluctant to abandon a previously learned colour in favour of a highly different novel colour (>0.18 hexagon units). The latency to switch was inversely correlated with the subsequently expressed learning speed. This is similar to the findings of Ings et al. (2009), showing that in workers of two B. terrestris subspecies, longer latencies to abandon innately preferred blue feeders also entailed faster learning rates of the rewarding nonblue feeders (Ings et al., 2009). In the current study, the high flow constancy for highly different feeder types induced bees to make significantly more unrewarded visits (to the previously learnt colour). This increasingly disassociated the previously formed reward expectation and the colour cue even before the novel rewarding feeder was discovered, ultimately facilitating the fast learning of the novel rewarding feeder type. In line with this, both sexes elicited fast and precise learning of the rewarding colour. Similar behavioural responses to medium to large colour distances have been reported for workers of B. terrestris (Dyer & Chittka, 2004a, 2004b) and several other bee species (Chittka et al., 2001) translating into high levels of flower constancy (Chittka, Thomson, & Waser, 1999; Waser, 1986), which facilitates conspecific pollen transfer. The similarity of the foraging behaviour of bumblebee males and workers supports the recent recognition of bumblebee males as valuable pollinators (Ogilvie & Thomson, 2015b; Östevik et al., 2010; Wolf & Moritz, 2014). Thus, our findings also have implications for the study of pollination ecology of both wild and commercial plants, which has thus far rarely considered the functional contribution male bees may make to the behavioural diversity of pollinator assemblages and, in consequence, to the quality of the pollination service (Garibaldi et al., 2013; Hoehn, Tscharntke, Tylianakis, & Stefan-Dewenter, 2008; Klein, Stefan-Dewenter, & Tscharntke, 2003).

Additionally, our findings highlight the possibility of using male bumblebees as an experimental model for learning and memory. In comparison to bee workers, a classical model organism for the study of learning and cognition, males have numerous practical advantages. For one, their foraging motivation does not rely on colony level factors and can be easily and reliably manipulated in the laboratory. The only amendment to traditional procedures with freely flying bees might be the use of very small reward quantities for males, as we have done here. More importantly, however, males do not have a stinger and the usual occupational hazards of bee stings and the common development of allergies to bee venom that often occur in experimenters can thus be avoided, which provides for ease in handling the animals, while possibly not forgoing any of the cognitive capacities that are typically studied in worker bees.

In summary, we have shown that worker and male bumblebees, although fundamentally different in lifestyle and behavioural needs, are very similar in their learning performances. This is possibly a result of similar underlying mechanisms facilitating and potentially limiting these cognitive abilities and the common need to exploit available resources efficiently irrespective of the natural behavioural repertoire.

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