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ORIGINAL ARTICLE

Bumblebees attend to both the properties of the string and the target in string-pulling tasks, but prioritize the features of the string

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Abstract Previous studies have demonstrated that associative learning and experience play important roles in the string-pulling of bumblebees (*Bombus terrestris*). However, the features of the target (artificial flower with sugar reward) and the string that bees learn in such tasks remain unknown. This study aimed to explore the specific aspects of the string-flower arrangement that bumblebees learn and how they prioritize these features. We show that bumblebees trained with string-pulling are sensitive to the flower stimuli; they exhibit a preference for pulling strings connected to flowers over strings that are not attached to a target. Additionally, they chose to pull strings attached to flowers of the same color and shape as experienced during training. The string feature also plays a crucial role for bumblebees when the flower features are identical. Furthermore, bees prioritized the features of the strings rather than the flowers when both cues were in conflict. Our results show that bumblebees solve string-pulling tasks by acquiring knowledge about the characteristics of both targets and strings, and contribute to a deeper understanding of the cognitive processes employed by bees when tackling non-natural skills.

Key words associative learning; non-natural skills; strategy; visual search

Introduction

Bumblebees employ a range of visually guided skills with complex cognition, including an exquisite navigational system (Lihoreau *et al.*, 2016; Woodgate *et al.*, 2016), the ability to assess gaps of geometry for flying through (Ravi *et al.*, 2020), and numerical abilities (MaBouDi *et al.*, 2020). To explore the flexibility of animal behavior, scholars in the field of comparative cognition emphasize the importance of intelligence-testing tasks that animals do not routinely encounter in their daily lives (Chittka, 2017; Chittka & Rossi, 2022). Interestingly, bumblebees have demonstrated a variety of remarkable non-natural skills, including relocating a ball to the defined location to obtain food (Loukola *et al.*, 2017), and pulling strings to access rewards (Alem *et al.*, 2016).

String-pulling is one of the most widely utilized problem-solving paradigms in animal cognition research, and has been used to investigate animal intelligence and

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behavioral flexibility (Jacobs & Osvath, 2015; Wang *et al.*, 2019). Animals are tested on pulling a non-valuable string to move an otherwise inaccessible reward within their reach (Taylor *et al.*, 2010; Hofmann *et al.*, 2016; Lamarre & Wilson, 2021). In the past decades, string-pulling behavior has been demonstrated in numerous species, mainly in mammals and birds (Range *et al.*, 2012; Riemer *et al.*, 2014; Jacobs & Osvath, 2015; Wakonig *et al.*, 2021). The mechanisms involved in string-pulling paradigms can range from simple trial-and-error exploration, and perceptual feedback to means-end understanding, causal cognition, and insight (Taylor *et al.*, 2012; Jacobs & Osvath, 2015; Wang *et al.*, 2019). The complexity of the string-pulling paradigm can be altered by varying the number and mutual positions of the strings and reward, allowing the investigation of different aspects of cognition (Jacobs & Osvath, 2015; Wang *et al.*, 2019).

To our knowledge, the bumblebee is the only invertebrate that has been trained to pull strings to access rewards. Multiple cognitive mechanisms can be simultaneously involved during the string-pulling process, such as proximity principle, perceptual feedback, means-end understanding, and generalization across conditions (Jacobs & Osvath, 2015; Wang *et al.*, 2019). However, the more proximate learning mechanisms (which features are learned, etc.) are rarely addressed. Previous work on string-pulling in bumblebees suggested that such performances are based on associative mechanisms and trial-and-error learning (Alem *et al.*, 2016). However, we still do not know what features of flowers or strings bumblebees have learned to solve string-pulling tasks. The present experimental design aimed to explore which features are actually learned by bumblebees in such tasks. To investigate this, 7 horizontal string-pulling experiments were conducted. We presented bumblebees with a range of string discrimination problems, in which we varied the presence of the artificial flower (discs with reward at the center, henceforward "flowers") and the feature of flowers, as well as string arrangement and color in the training and test, to determine: (i) whether bumblebees pay attention to the presence of flowers; (ii) whether bumblebees attend to the cues of artificial flowers or attached strings; (iii) whether visual features of artificial flowers or the attached strings are more important.

Materials and methods

Animals and arena

The data collection for the string-pulling experiments was conducted from October 2022 to August 2023. *Bombus terrestris* colonies, each containing a queen, were obtained from commercially available stocks provided by a distributor in China (Biobest Shouguang Biotechnology Co., Ltd). The bees were housed in a plastic box (40 cm × 28 cm × 11 cm [$L \times W \times H$]) connected to a flight arena (100 cm × 75 cm × 30 cm [$L \times W \times H$]) through a Plexiglas corridor (length = 25 cm, 3.5 cm × 3.5 cm in cross-section). The bees were provided with 20% w/w sucrose solution and ~5 g commercially obtained pollen (Changge Yafei Beekeeping Professional Cooperative, China) every day. The floor of the flight arena was painted green to enhance the contrast between the strings and flowers in the background. Plastic sliding doors were located along the corridor to control the bees' access to the arena (Fig. 1A). All the training and tests were conducted in the flight arena.

General methods

Before the training phase, all bees were pretrained to associate artificial flowers (3 cm diameter discs with an inverted Eppendorf cap at the center) with the sugar water (50% sucrose solution, w/w). In this stage, bees could freely return to the hive when satiated. Bees that seemed to forage frequently were marked with number tags (Bienen-Voigt & Warnholz GmbH & Co. KG, Germany) for individual identification. In detail, a forager was gently transferred to a small cage (diameter = 3.8 cm, length = 7.7 cm) with mesh, pressing the bee against the mesh, and then tagged to the dorsal thorax with a small amount of glue. All the training and testing sessions were conducted between 9 am and 7 pm at room temperature (23 ± 4 °C). Illumination was provided by fluorescent lighting (YZ36RR, 36W, T8/765, FSL, China) equipped with high-frequency ballasts (T8, YZ-36 W, 50 Hz, FSL, China). This lighting setup can simulate natural light, making its flicker frequency greater than flicker fusion frequency of bumblebee compound eyes (Skorupski & Chittka, 2010).

Bees were trained with artificial flowers (diameter = 3 cm) connected to a string (length = 5.5 cm, diameter = 0.15 cm) under a transparent Plexiglas table (here forward tables; 15 cm × 10 cm × 0.4 cm [$L \times W \times H$]) in the flight arena. The table was 0.6 cm above the ground, preventing bees from reaching the artificial flowers by squeezing underneath the table. The training protocol followed the procedure used by Alem *et al.* (2016). Initially, selected bees were trained to obtain the reward when the flowers were gradually positioned further under the table. The first step involved placing half of the artificial flower disk under the table, allowing bees to access the sucrose solution without moving the flowers. Subse-

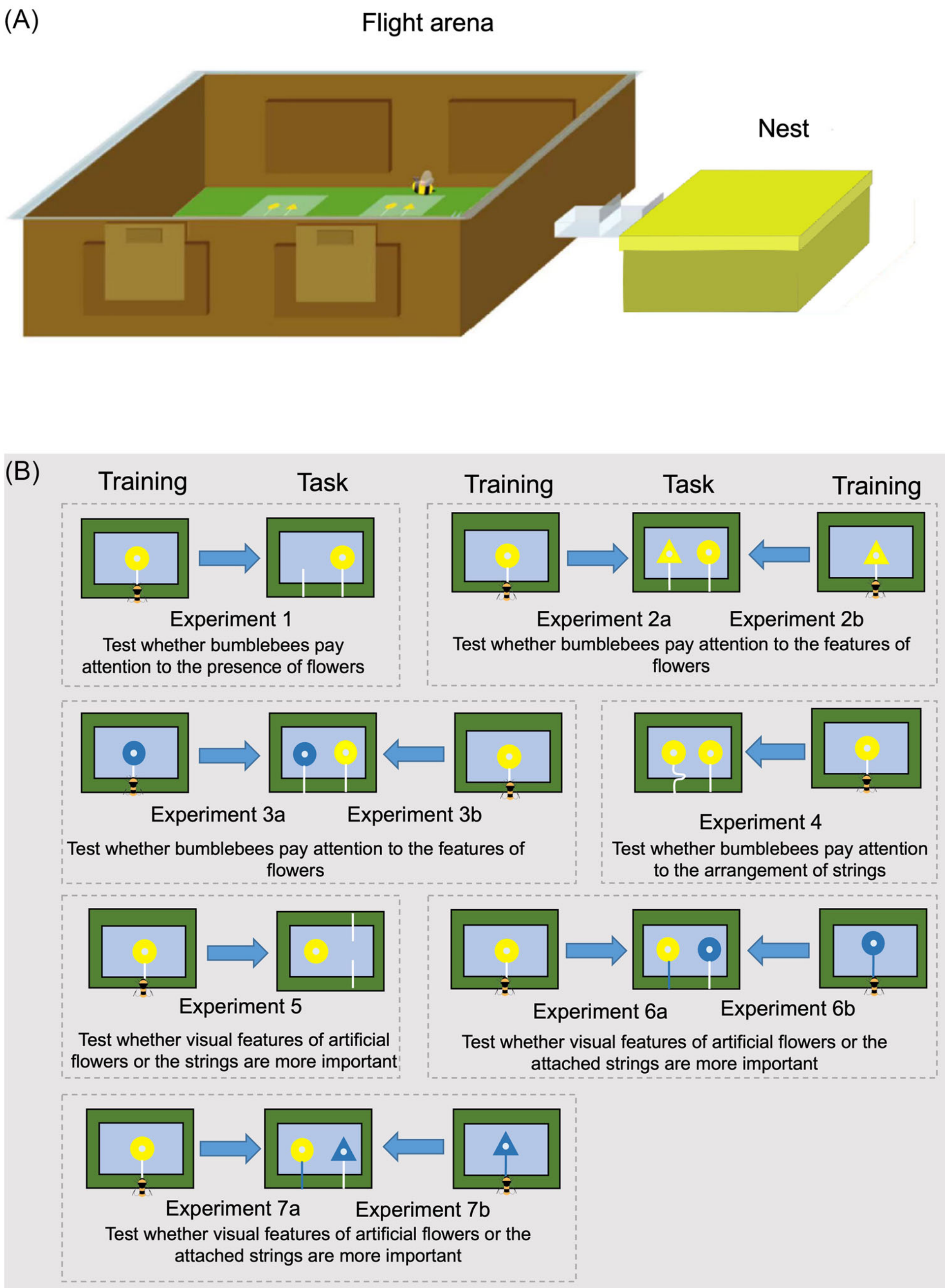

Fig. 1 Experimental apparatus (A) and summary of training and tasks in different experiments (B). The experimental setup consisted of a flight arena connected to a hive *via* a Perspex corridor. During the test, 2 options were presented to bees, and their locations varied randomly from left to right under each table. For further details and descriptions of each experiment, see the main text.

quently, the task required the bees to move the flowers (75% and 100% covered) to access the reward. In the final step, bees had to pull the strings which extended 2 cm outside the edge of the table. The training phase was completed when a bee successfully pulled out the strings 30 times after the first occurrence of string-pulling during the final step.

For each test, bumblebees were individually tested in the flight arena, and presented with 4 transparent tables (each table has 2 options). The strings were glued to the floor to prevent alterations in the direction and position of the flowers and strings caused by the air flow generated by flying bumblebees. The choice and the duration of bees attempting to pull each string were recorded. To prevent bees from developing a side bias, the position and direction of the strings or flowers varied randomly from left to right for each table. To rule out any influence of chemosensory cues, the strings and artificial flowers were used only once in each test. This measure also served to prevent learning behavior within one test. The tested bees were removed from the colonies and euthanized by freezing in a −20 °C freezer. All tests were video recorded with an iPhone 12 (Apple, Cupertino, CA, USA) placed above the arena, with each test having a maximum duration of 10 min.

Experiment 1: Investigating whether string-pulling of bumblebees is influenced by the presence of the artificial flower

Bumblebees ($n = 20$) were trained with yellow artificial flowers (diameter = 3 cm) attached to white strings (diameter = 0.15 cm; length = 5.5 cm) under transparent tables. Subsequently, during the test phase, 4 tables were placed in the flight arena, and 2 strings were glued on the floor 3 cm apart under each table, both strings extended 2 cm from the edge of the table. The free end of 1 string connected to a yellow flower as before, and another string only was not connected to a flower. Both options were simultaneously presented to the bees (Fig. 1B). If the string-pulling of bumblebees is influenced by the presence of the artificial flower, their preference for a particular option should depend on whether such a target is attached to the end of the string.

Experiments 2−3: Investigating whether bumblebees recognize the features of flowers

We manipulated 2 features (shape and color) of the flowers in the training and test to see how they influenced the performance of bumblebees in string-pulling tasks (Fig. 1B). To investigate the impact of flower shape on string-pulling behavior, bumblebees ($n = 20$) were trained with yellow artificial flowers in Experiment 2a. In the test, 2 strings were glued under each table, with 1 string connected to a round flower, and another string connected to a triangular flower (Fig. 1B). In contrast, bumblebees ($n = 15$) were trained with white strings connected to triangular yellow flowers in Experiment 2b. The trained bees were tested in the same tasks as Experiment 2a (Fig. 1B). If the bees prefer to pull the strings connected to a flower with the same shape as in training, this would indicate that bees note the shape of flowers.

To investigate whether the bumblebees ($n = 10$) memorize the flower color during the string-pulling process, they were trained with white strings connected to blue flowers in Experiment 3a. In the test, 2 strings were glued under each table, with 1 string connected to a yellow flower, and another connected to a blue flower (Fig. 1B). In contrast, bumblebees ($n = 20$) were trained with white strings connected to yellow flowers in Experiment 3b. The trained bees were tested in the same tasks as Experiment 3a (Fig. 1B). If the bees show a preference for the string-connected flowers with the same color as in the training, this would indicate that they recognized the cues of flowers in this condition.

Experiment 4: Investigating whether bumblebees recognize the arrangement of strings

To study whether bumblebees ($n = 17$) memorize the features of strings during training, they were trained with yellow flowers connected to white straight strings. In the test, 2 yellow flowers were placed 3 cm apart under each table, with 1 flower connected to a coiled string (length = 8 cm); this pattern was visually different from straight strings during training. Another flower was attached to a short straight string the same as in the training (Fig. 1B). If the bees preferred to pull the straight strings, this would indicate that bees recognize the arrangement of strings in this task.

Experiments 5−7: Investigating whether strings' or flowers' features are more important for bumblebees

In previous experiments, bumblebees could use either the visual cues of flowers or strings in their string-pulling behaviors (see Results). We designed 5 more experiments to test bumblebees' preferences where the cues of strings and flowers conflicted. Bumblebees ($n = 16$) were trained with white strings connected to yellow artificial flowers in Experiment 5. But in the test, 1 artificial flower was placed under each table (no string connected), and 2 strings (length = 5.5 cm, perpendicular to the wide edge of the table) were glued on the floor 3 cm apart from the flower. Both non-connected strings extended 2 cm outside the edge of the table (Fig. 1B). We investigated whether bees chose to pull on the non-connected strings, or attempted to reach the artificial flower by directing their activities to the location closest to the artificial flowers.

Bumblebees ($n = 18$) were trained with white strings connected to yellow flowers in Experiment 6a. During the test, 2 artificial flowers were placed 3 cm apart under each table, with 1 yellow flower (flower was consistent with training) connected to a blue string, and another a white string connected to a blue flower (Fig. 1B). If the bees prefer to pull the blue strings connected to the yellow flower, this suggests that the bees are primarily focused on the cues of the flowers. However, if bees show a preference for pulling white strings, this indicates that the color of strings is more critical for the bees. Similarly, bumblebees ($n = 10$) were trained with blue strings connected to blue flowers in Experiment 6b. The trained bees were tested in the same tasks as Experiment 6a (Fig. 1B). If the bees preferred to pull the white strings, this indicated the bees note the color of the flowers, while if bees showed a preference for pulling blue strings, this suggests that the color of the strings is more critical for the bees.

To further study the effect of flower and string cues on string-pulling, we conducted the next 2 experiments in which we changed both the color and shape of the flowers. In Experiment 7a, bumblebees ($n = 16$) were trained with white strings connected to yellow round flowers. In the test, 2 flowers were placed 3 cm apart under each table, with one a blue string connected to a yellow round flower, and another was a white string connected to a blue triangular flower (Fig. 1B). If bees showed a preference for pulling the white strings, it demonstrated that bees pay more attention to the cues of the strings. Similarly, bumblebees ($n = 10$) were trained with blue strings connected to blue triangular flowers in Experiment 7b. The trained bees were tested in the same tasks as Experiment 7a (Fig. 1B).

Statistical analysis

All statistical analyses were conducted with R version 4.3.2. The proportion of choices and the duration of each choice were used to determine the preference in different experiments. The proportion of choices in different experiments was analyzed using a generalized linear model (GLM) with binomial distribution and logit function. Each choice was a response variable and no independent variables were used in the model. The duration of each choice was tested using a generalized linear mixed model (GLMM) (package "glmmTMB") with Gamma distribution and log function. Duration of each choice was the response variable, choice was the fixed effect, and bees' IDs were considered a random effect, accounting for intercept effect. To test the effect of different training in the same tasks, we combined Experiments 2a and 2b, as well as Experiments 3a and 3b, and used GLM with binomial distribution and logit function. The choice was the response variable and training treatment was set as fixed effect. A *post hoc* test was used to analyze the performance of bees in different training conditions.

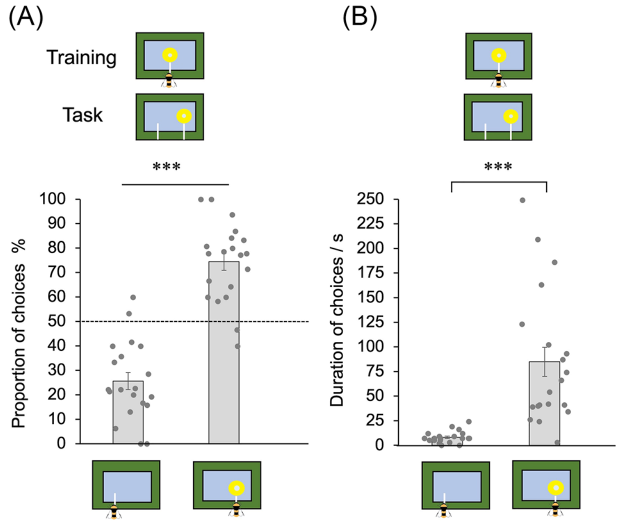

Fig. 2 The proportion of choices (A) and corresponding duration (B) of bumblebees ($n = 20$) on each choice in Experiment 1. The percentage of bumblebees pulling each string was compared with chance level (50%; horizontal dashed line). The proportion of choices was analyzed GLM with binomial distribution and logit function. The duration was analyzed using a GLMM with Gamma distribution and log function. Data are presented as mean ± standard error (*** indicates $P < 0.001$) and the circles indicate bees' individual data points. GLM, generalized linear model; GLMM, generalized linear mixed model.

Results

Bumblebees learned to search for artificial flowers in string-pulling tasks

Bumblebees ($n = 20$) were pretrained to associate artificial flowers (diameter = 3 cm) with the sugar water, and then trained with strings connected to flowers through 4 steps. Subsequently, each bee's preference between strings connected to flowers and non-connected strings was assessed. Bumblebees exhibited a stronger preference for strings connected to flowers than non-connected strings (GLM: 95% CI = 1.13 [0.88–1.40], $Z = 8.49$, $P < 0.001$; Fig. 2A; Video S1). Furthermore, bumblebees spent much more time attempting to pull the strings connected to flowers (84.80 ± 14.85 s) compared to the non-connected strings (8.15 ± 1.32 s) (GLMM: 95% CI =

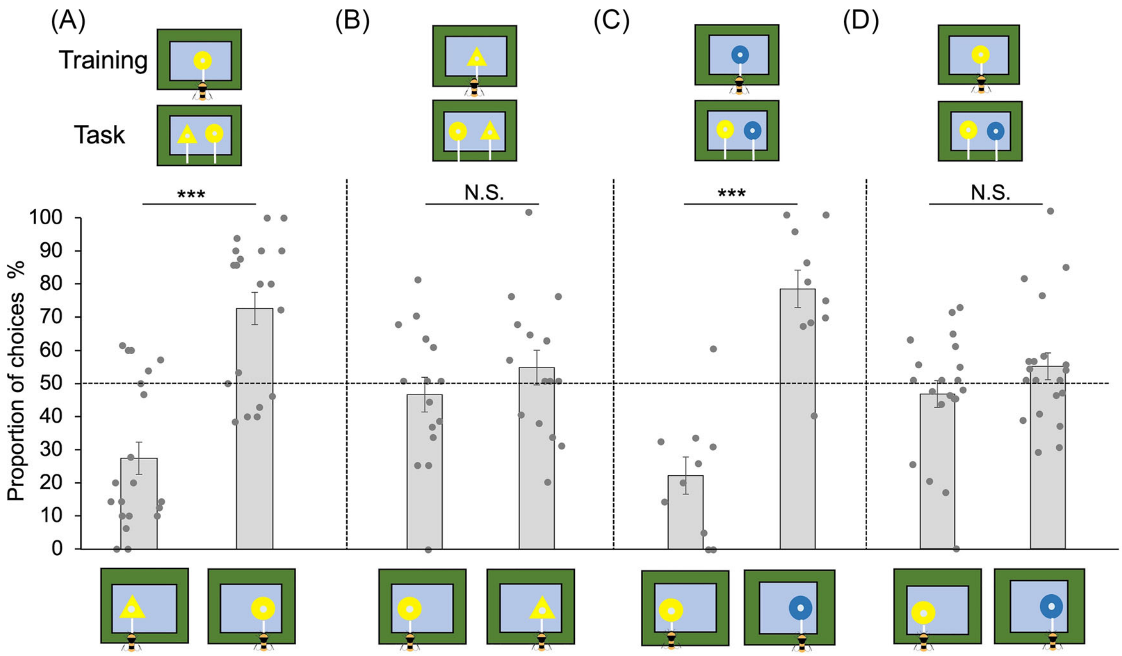

Fig. 3 The proportion of choice of bumblebees ($n = 20, 15, 10, 20$) in Experiments 2a (A), 2b (B), 3a (C), and 3b (D). The percentage of pulling each string compared with chance level (50%; horizontal dashed line). The proportion of choices was analyzed using GLM with binomial distribution and logit function. Data are presented as mean ± standard error (*** indicates $P < 0.001$, and N.S. indicates not significant at $P > 0.05$) and the circles indicate bees' individual data points. GLM, generalized linear model.

2.24 [1.79–2.69], $Z = 9.75$, $P < 0.001$; Fig. 2B). Taken together, these findings hint that bumblebees might have learned to look for the familiar artificial flower in this task.

Bumblebees recognize the features of flowers in string-pulling tasks

To test the cues by which bees recognized the flower, we carried out 4 additional experiments (Experiments 2–3). In Experiment 2a, we investigated the impact of flower shape on the string-pulling behavior. Bumblebees ($n = 20$) were trained with white strings connected to round flowers, and subsequently tested with round and triangular flowers connected to white strings. The percentage of bees pulling the strings connected to round flowers (73% ± 5%) was significantly higher than chance (GLM: 95% CI = 0.99 [0.68–1.30], $Z = 6.29$, $P < 0.001$; Fig. 3A; Video S2), and performed with longer duration on the strings connected to round flowers (61.20 ± 14.23 s) compared to strings connected to triangular flowers (11.95 ± 4.04 s) (GLMM: 95% CI = 1.53 [0.81–2.25], $Z = 4.17$, $P < 0.001$; Fig. 4A). However, when bees ($n = 15$) were trained with triangular flowers in Experiment 2b, we found no significant difference in the percentage of pulling both strings compared with chance (GLM: 95% CI = 0.04 [−0.28 to 0.37], $Z = 0.25$, $P = 0.80$; Fig. 3B). The same result was found in the duration between the bees pulling the strings connected to round and triangular flowers (GLMM: 95% CI = 0.07 [−0.59 to 0.74], $Z = 0.22$, $P = 0.83$; Fig. 4B). We speculate that the difference between the results of Experiment 2a and Experiment 2b (*post hoc* test: 95% CI = 1.03 [0.58–1.48], $Z = 4.50$, $P < 0.001$) might be attributed to an innate preferences for circular flowers over triangular flowers.

We explored whether bumblebees take note of the flower color during the string-pulling in Experiment 3. Bees ($n = 10$) were trained with white strings connected to blue flowers, and during the test the flowers' colors were yellow or blue, with both connected to white strings. The percentage of the bees pulling strings connected to the blue flowers (78% ± 6%) was significantly higher than chance (GLM: 95% CI = 1.23 [0.87–1.61], $Z = 6.48$, $P < 0.001$; Fig. 3C; Video S3), and the duration data also suggest that the bees preferred pulling strings connected to the blue flowers (GLMM: 95% CI = 1.23 [0.38–2.08], $Z = 2.84$, $P < 0.01$; Fig. 4C). In Experiment 3b, bees ($n = 20$) were trained with connected blue flowers. No significant differences were observed in

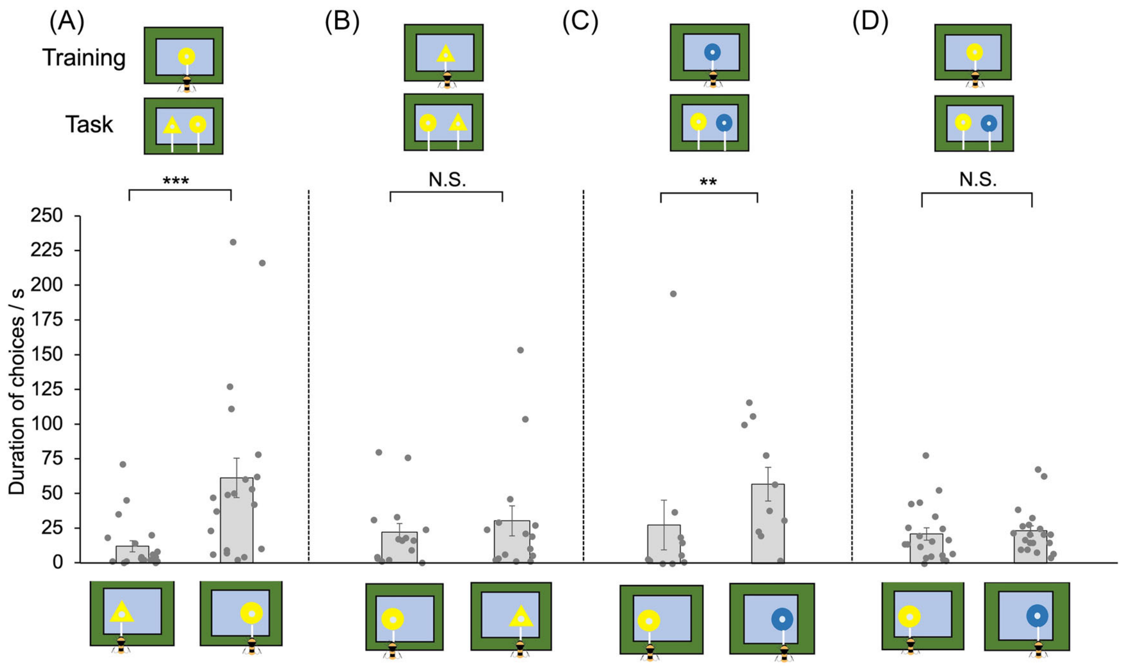

Fig. 4 Duration of the bumblebees ($n = 20, 15, 10, 20$) on each choice in Experiments 2a (A), 2b (B), 3a (C), and 3b (D). The duration was analyzed using a GLMM with Gamma distribution and log function. Data are presented as mean ± standard error (*** indicates $P < 0.001$, ** indicates $P < 0.01$, and N.S. indicates not significant at $P > 0.05$) and the circles indicate bees' individual data points. GLMM, generalized linear mixed model.

percentage (GLM: 95% CI = −0.05 [−0.31 to 0.21], $Z = -0.40$, $P = 0.69$; Fig. 3D) and duration (GLMM: 95% CI = −0.05 [−0.54 to 0.43], $Z = -0.21$, $P = 0.83$; Fig. 4D) of bumblebees pulling each string. The difference between the results of Experiment 3a and Experiment 3b (*post hoc* test: 95% CI = 1.18 [0.73–1.64], $Z = 5.09$, $P < 0.001$) could be explained by the bees' innate biases toward blue flowers rather than yellow flowers (Raine *et al.*, 2006).

Bumblebees recognize the arrangement of strings in string-pulling task

In Experiment 4, we investigated the impact of the string arrangement on the performance of the string-pulling task. Bumblebees ($n = 17$) were trained with straight strings connected to flowers, and subsequently tested with yellow flowers connected to straight and coiled strings. The percentage of bees pulling the straight strings (70% ± 5%) was significantly higher than chance (GLM: 95% CI = 0.93 [0.61–1.28], $Z = 5.48$, $P < 0.001$; Fig. 5A; Video S4), and the duration also indicate that the bees exhibited a preference for pulling the straight strings (27.82 ± 5.50 s) than the coiled strings (8.76 ± 2.02 s) (GLMM: 95% CI = 0.93 [0.43–1.43], $Z = 3.67$, $P < 0.001$; Fig. 5B).

Properties of strings are more important than flowers' cues

Previous results indicate that bumblebees focus on the cues of both strings and flowers in the tasks. To test which is prioritized when the 2 cues are in competition, we designed 5 new experiments (5−7) in which we changed the string or flower cues between the training and the test. In Experiment 5, bees were trained with strings connected to flowers, and tested with the string or flowers only. We found that bees ($n = 16$) selected the strings (86% ± 3%) significantly more often than chance (GLM: 95% CI = −2.01 [−2.53 to −1.54], $Z = -8.00$, $P < 0.001$; Fig. 6A; Video S5), but the duration on each string was not significant (GLMM: 95% CI = −0.63 [−1.43 to 0.16], $Z = -1.56$, $P = 0.12$; Fig. 6B). The result implies that bumblebees did not focus on the flower targets but dedicated most of their efforts to the familiar strings in this task.

We studied the string-pulling preferences when one of the cues, either string or flower, matched the training, while the other differed from the training. In Experiment

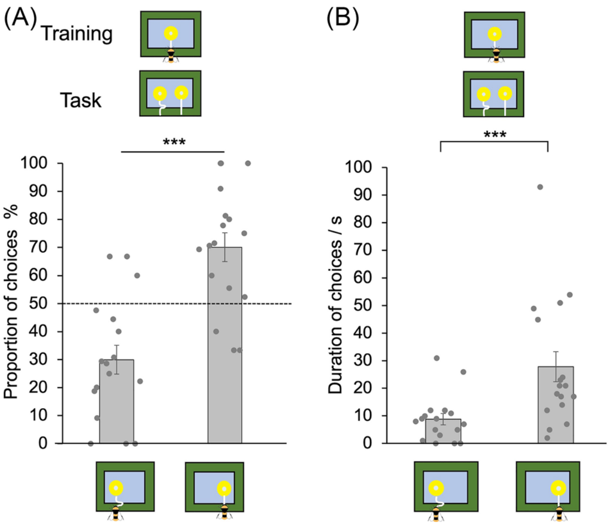

Fig. 5 The proportion of choice (A) and duration (B) of bumblebees ($n = 17$) on each choice in Experiment 4. The horizontal dashed line indicates chance level (50%; horizontal dashed line). The proportion of choices was analyzed with GLM with binomial distribution and logit function. The duration was analyzed using a GLMM with Gamma distribution and log function. Data are presented as mean ± standard error (*** indicates $P < 0.001$) and the circles indicate bees' individual data points. GLM, generalized linear model; GLMM, generalized linear mixed model.

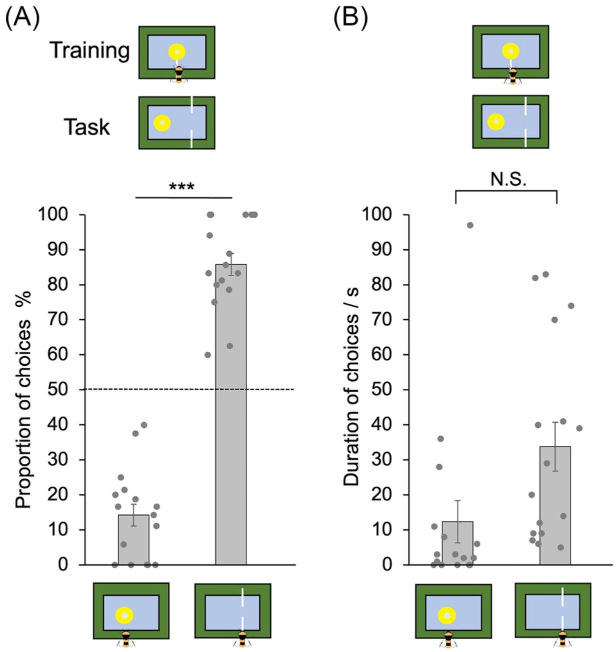

Fig. 6 The proportion of choice (A) and duration (B) of bumblebees ($n = 16$) on each choice in Experiment 5. The horizontal dashed line indicates chance level (50%; horizontal dashed line). The proportion of choices was analyzed with GLM with binomial distribution and logit function. The duration was analyzed using a GLMM with Gamma distribution and log function. Data are presented as mean ± standard error (*** indicates $P < 0.001$, and N.S. indicates $P > 0.05$) and the circles indicate bees' individual data points. GLM, generalized linear model; GLMM, generalized linear mixed model.

6a, bumblebees ($n = 18$) were trained with white strings connected to yellow flowers, and subsequently tested with blue strings connected to yellow flowers and white strings connected to blue flowers. Interestingly, bees chose to pull the white strings connected to the blue flowers (84% ± 4%) more often than chance (GLM: 95% CI = 1.56 [1.17–2.00], $Z = 7.39$, $P < 0.001$; Fig. 7A; Video S6), and the duration of the bees pulling the white strings was significantly longer than blue strings (42.33 ± 10.17 s) (GLMM: 95% CI = 0.89 [0.13–1.65], $Z = 2.29$, $P < 0.05$; Fig. 8A). Similarly, when bumblebees ($n = 10$) were trained with blue strings connected to blue flowers in Experiment 6b, the percentage of bees pulling the blue strings connected to yellow flowers (90% ± 3%) was higher than chance (GLM: 95% CI = 2.16 [1.58–2.84], $Z = 6.77$, $P < 0.001$; Fig. 7B; Video S7), and the duration (53.60 ± 10.15 s) was much longer than with the bees pulling the white strings connected to blue flowers (6.70 ± 2.66 s) (GLMM: 95% CI = 1.99 [1.53–2.45], $Z = 8.42$, $P < 0.001$; Fig. 8B).

In Experiment 7a, bumblebees ($n = 16$) were trained with white strings connected to yellow round flowers, and tested with blue strings connected to yellow round flowers and white strings connected to blue triangle flowers. We found that the bees preferred to pull the white string connected to blue triangular flowers (76% ± 5%) (GLM: 95% CI = 0.94 [0.60–1.30], $Z = 5.31$, $P < 0.001$; Fig. 7C; Video S8), but there was no significant difference in the duration spent on each string (GLMM: 95% CI = 0.46 [−0.26 to 1.18], $Z = 1.26$, $P = 0.21$; Fig. 8C). Similarly, when bees ($n = 10$) were trained with blue strings connected to blue triangular flowers in Experiment 7b, bees preferred pulling the blue strings connected to yellow round flowers (80% ± 7%) more than above chance (GLM: 95% CI = 1.41 [0.94–1.93], $Z = 5.66$, $P < 0.001$; Fig. 7D; Video S9), but the duration on each string was not significant (GLMM: 95% CI = 0.48 [−0.40 to 1.36], $Z = 1.07$, $P = 0.29$; Fig. 8D). In general, bees exhibited a consistent preference for pulling strings with the same color as in training, even though the flower cues differed. This suggests that bees prioritize the features of strings over those of flowers when the 2 cues are in competition.

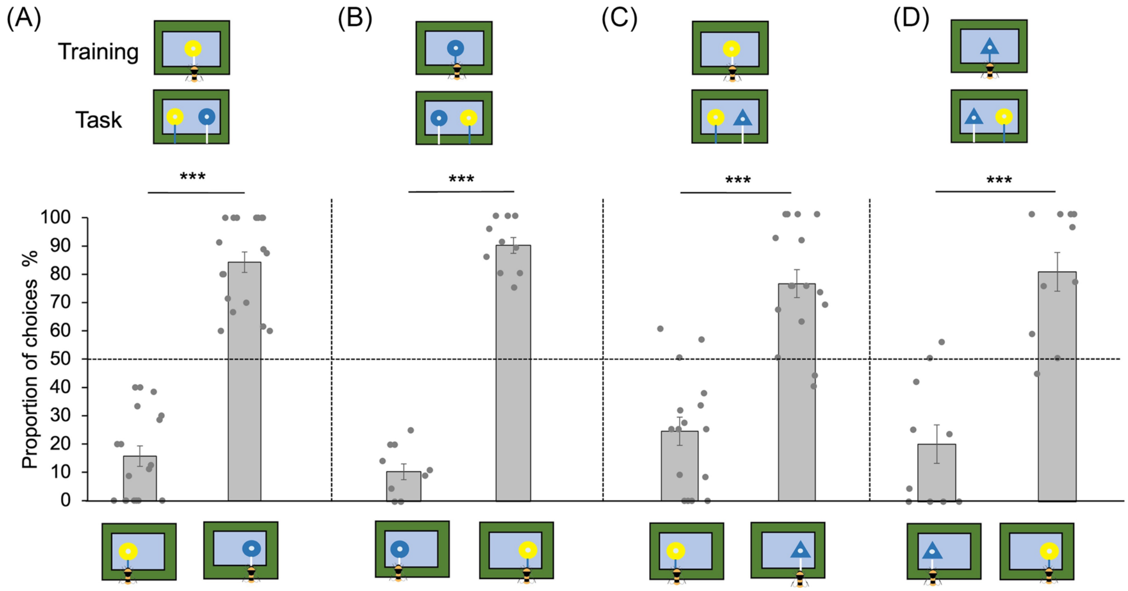

Fig. 7 The proportion of choice of bumblebees ($n = 18, 10, 16, 10$) in Experiments 6a (A), 6b (B), 7a (C), and 7b (D). The percentage of bumblebees pulling each string was compared with the chance level (50%; horizontal dashed line). The proportion of choices was analyzed using GLM with binomial distribution and logit function. Data are presented as mean ± standard error (*** indicates $P < 0.001$) and the circles indicate bees' individual data points. GLM, generalized linear model.

Discussion

We found that: (i) bumblebees' preference for pulling the strings is influenced by the presence of flowers; (ii) string-pulling in bumblebees relies on visual strategies, specifically involving the learning of multiple elements of both flowers and strings; and (iii) bumblebees prioritize the visual features of attached strings over those of flowers.

In our research, when given a choice between strings with artificial flowers and non-connected strings, bumblebees exhibited a preference for strings connected to flowers. This observation suggests that bumblebees could visually determine whether the desired item could be seen at the end of the string. A strong tendency to aim directly for the object at the end of the string has been shown in many other species in string-pulling tasks, such as dogs (*Canis lupus familiaris*; Osthaus *et al.*, 2005), keas (*Nestor notabilis*; Werdenich & Huber, 2006), azure-winged magpie (*Cyanopica cyanus*; Wang *et al.*, 2021), and peach-fronted conures (*Eupsittula aurea*; Ortiz *et al.*, 2019). It is worth noting that this does not mean that the animals understood the connection between string and rewards, but only demonstrates associative learning. Further experiments would be necessary to determine whether bumblebees truly understand the string as a means to obtain a reward.

Our research reveals that bumblebees focused both on flower and string features during string-pulling. There is substantial evidence that animals acquire knowledge about object affordances, learn perceptual features, and develop motor representations through previous memories (Matsuzawa *et al.*, 2005; Takeshita *et al.*, 2005). For example, an ant species (*Lasius niger*) combines object affordance with latent learning to make efficient foraging decisions (Poissonnier *et al.*, 2023). In bees, previous experience with flowers and strings might influence how patterns are learned and recognized, and individual preference for flowers by associating cues in floral displays with rewards (Nityananda & Pattrick, 2013; Hempel de Ibarra *et al.*, 2022). In a study of bumblebees passing through gaps, trained bees reoriented their in-flight posture while passing through, and successfully flew through narrow gaps. This suggests that bumblebees may have gauged the size of the gap relative to their own individual body dimensions (Brebner & Chittka, 2020; Ravi *et al.*, 2020).

We found that experienced bumblebees try to pull non-connected strings in Experiment 7. Experienced animals may pull a non-connected string as the result of operant conditioning as illustrated by the goldfinches' compulsive pulling behavior (Seibt & Wickler, 2006). Some animals may stop pulling as soon as they learn that there is no food reward; however, bumblebees persistently pulled

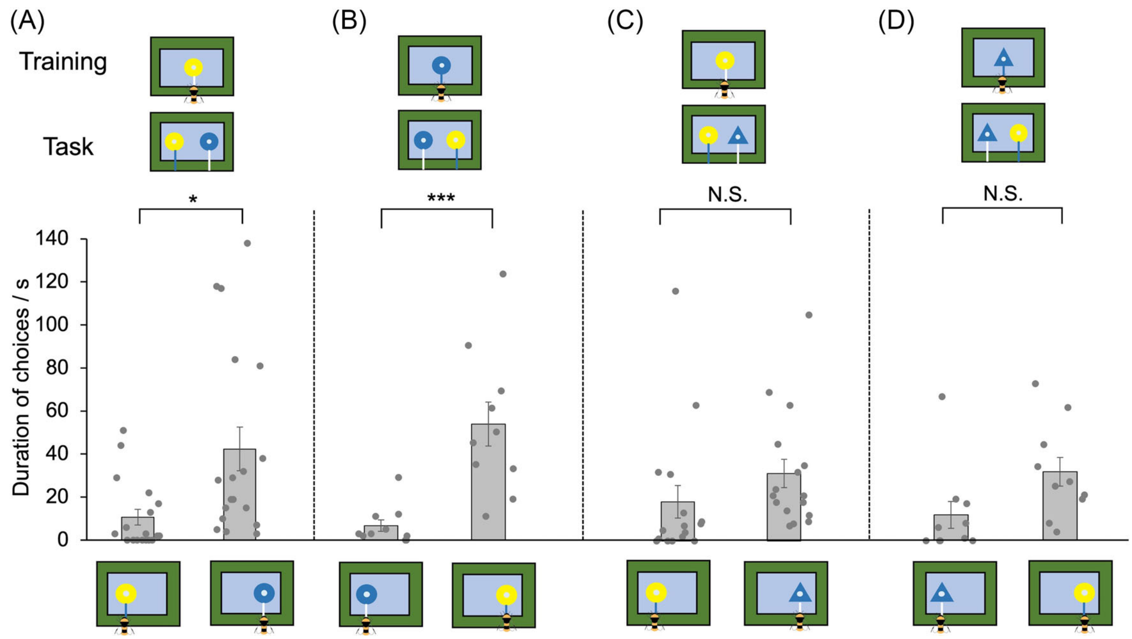

Fig. 8 Duration of the bumblebees ($n = 18, 10, 16, 10$) pulling each string in Experiments 6a (A), 6b (B), 7a (C), and 7b (D). The duration was analyzed using a GLMM with Gamma distribution and log function. Data are presented as mean ± standard error (*** indicates $P < 0.001$, * indicates $P < 0.05$, and N.S. indicates not significant at $P > 0.05$) and the circles indicate bees' individual data points. GLMM, generalized linear mixed model.

non-connected strings in this experiment (average duration 33.75 s). Bumblebees are stimulated to repeat the specific sequences of actions (moving the string with their legs or mandibles) on strings that induce the reward stimulus (i.e., the flower positioned under the table) to move a little closer. The movement of the rewarding object toward the animal produces positive feedback for continuing that action (Alem *et al.*, 2016). Such feedback may drive string-pulling performances if the sight of the target moves and then stays closer to an individual after a series of actions.

We also looked at how attention is deployed when more than one target type is present or how attention is divided across multiple cues. String-pulling of bumblebees resembles reward-based visual search more than goal-directed search. Bumblebees prioritize the strings' visual features when this conflicts with the features of the flowers previously experienced. Individuals appear to ignore cues of familiar flowers and attend to the features of the string they have learned to pull. One possible reason for prioritizing the string features could be that the string was extending 2 cm outside the edge of the table; bumblebees were first introduced to strings but not the flowers during the test phase. In conclusion, our results suggest that the ability of the bees to choose to pull the strings is based, at least in part, on associating learning with string and target during the string-pulling process. This perceptually based account agrees with previous studies suggesting that bumblebees rely on readily observable perceptual features when learning motor patterns such as string pulling (Chittka, 2017; Chittka & Rossi, 2022).

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Disclosure

The authors declare no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1 String-pulling test of bumblebees in Experiment 1.
Video S2 String-pulling test of bumblebees in Experiment 2a.
Video S3 String-pulling test of bumblebees in Experiment 3a.
Video S4 String-pulling test of bumblebees in Experiment 4.
Video S5 String-pulling test of bumblebees in Experiment 5.
Video S6 String-pulling test of bumblebees in Experiment 6a.
Video S7 String-pulling test of bumblebees in Experiment 6b.
Video S8 String-pulling test of bumblebees in Experiment 7a.
Video S9 String-pulling test of bumblebees in Experiment 7b.