ORIGINAL PAPER

Comparison of learning and memory of *Apis cerana* and *Apis mellifera*

Qiu-Hong Qin · Xu-Jiang He · Liu-Qing Tian · Shao-Wu Zhang · Zhi-Jiang Zeng

Abstract The honeybee is an excellent model organism for research on learning and memory among invertebrates. Learning and memory in honeybees has intrigued neuroscientists and entomologists in the last few decades, but attention has focused almost solely on the Western honeybee, *Apis mellifera*. In contrast, there have been few studies on learning and memory in the Eastern honeybee, *Apis cerana*. Here we report comparative behavioral data of color and grating learning and memory for *A. cerana* and *A. mellifera* in China, gathered using a Y-maze apparatus. We show for the first time that the learning and memory performance of *A. cerana* is significantly better on both color and grating patterns than that of *A. mellifera*. This study provides the first evidence of a learning and memory difference between *A. cerana* and *A. mellifera* under controlled conditions, and it is an important basis for the further study of the mechanism of learning and memory in honeybees.

Q.-H. Qin · X.-J. He · L.-Q. Tian · Z.-J. Zeng (✉)
Honeybee Research Institute, Jiangxi Agricultural University, Nanchang 330045, People's Republic of China
e-mail: bees1965@sina.com

S.-W. Zhang (✉)
ARC Center of Excellence in Vision Science, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia
e-mail: shaowu.zhang@anu.edu.au

Abbreviations

A. mellifera	*Apis mellifera*
A. cerana	*Apis cerana*
CCD	Colony collapse disorder

Introduction

The honeybee offers several advantages as a model organism for studying learning and memory, including a relatively simple brain structure, social organization allowing easy rearing, and a complex behavioral repertoire, which is readily manipulated. Although a honeybee has only a tiny brain that has only about 1 million nerve cells in it (only one hundred-thousandth of the human), it has an amazing ability to learn and remember tasks and objects (Menzel 1990, 2001; Giurfa et al. 1996, 2001; Zhang et al. 1996, 1999, 2000, 2004; Giurfa 2007). Learning and memory performances in honeybees were quantified in a color learning experiment by Menzel (1967, 1968) for the first time; this initiated an interest in honeybees learning and memory research. Since then, it has been found that honeybees can not only learn to distinguish different colors and orientations (Hateren et al. 1990; Zhang and Srinivasan 1994), but can also extract abstract concepts from visual patterns (Giurfa et al. 2001; Zhang et al. 2005; Pahl et al. 2007; Gross et al. 2009; Avargues-Weber et al. 2011; Avargues-Weber et al. 2012). However, most research attention in this field has centered on Western honeybees, and as a result, the learning and memory capabilities of most tropical bees have not been properly investigated. Some research has recently started on stingless bees in South America (McCabe et al. 2007; McCabe and Farina 2009, 2010), but Asian bees still need to be characterized.

As prototypical honeybees of the East and the West, *A. cerana* and *A. mellifera* are two important honeybee species that are widely bred and studied. Recent work on the two species has revealed that both geographical isolation and long-term evolutionary divergence are responsible for differences between the two species in key biological characteristics including shape, individual development and living habit, etc. (Chen 2001; Zeng 2009). However, to our knowledge, there have been hardly any direct comparative studies on their learning and memory capabilities up to now.

Foraging by honeybees is a social enterprise, one in which thousands of foragers in a colony cooperate to find and exploit rich patches of flowers. The natural food sources of honeybees, the flowers, differ dramatically in their availability and predictability in both biotopes of *A. cerana* and *A. mellifera*—the tropical Asian forest and that of the European one. While in Europe honeybees exploit large flower fields that are predictable and stay in the same location for several days, in the tropical forest there are no such massive concentrations of flowers, and these may appear and disappear in a rather unpredictable way. As a result of natural selection in their respective homeland, *A. cerana* is adept in collecting sporadic nectar flowers in the mountain and forest region, but *A. mellifera* exploits large flower fields well (Chen 2001; Zeng 2009). Furthermore, collecting propolis is peculiar to *A. mellifera* (Chen 2001). Honeybee foragers have to fly several kilometers—up to more than 10 km away—to collect pollen and nectar, and therefore it is necessary for them to learn and remember not only the color and shape of flowers, but also how to get to them (Chittka et al. 1993; Menzel et al. 1996; Vorobyev and Menzel 1999; Zhang et al. 1999; Collett et al. 2003; Pahl et al. 2011). Therefore, we expect that the ability to track food sources may differ between both species, and thus, their ability to learn about them should also be different. Besides, in tropical regions—except those with homogeneous vegetation that provides adequate nectar and pollen—*A. cerana* colonies are likely to abscond to another area in response to cessation of flowering, while *A. mellifera* would rather be starved in the hive than migrate (Gong and Zhang 2000). Honeybees must learn and adapt to new surroundings when migrating, which we hypothesize may also cause some difference in the learning and memory between the two species.

In an attempt to contribute some baseline information about the *A. cerana* learning and memory behavior, and comparative visual learning performance in *A. cerana* and *A. mellifera*, this study was conducted by using a Y-maze apparatus to test the visual learning and memory of these two honeybee species in China.

Materials and methods

General

The Eastern honeybee, *A. cerana*, and the Western honeybee, *A. mellifera*, were used throughout this study. The honeybee colonies were raised at the Honeybee Research Institute, Jiangxi Agricultural University, Nanchang, China (28.46u N, 115.49u E), according to standard beekeeping techniques. All experiments were carried out on fine days in the summer when the temperature was 27 ± 3 °C and with a relative humidity of 82 ± 5 %.

Two identical Y-maze setups were placed in a large indoor laboratory with two windows on opposite sides. The experimental twin-choice Y-maze that was constructed from perspex was divided into three chambers (Fig. 1a), an apparatus well established in honeybee behavioral research (Srinivasan and Lehrer 1988; Zhang et al. 1992, 1995, 1996, 1999; Zhang and Srinivasan 1994). These two Y-mazes were located close to the windows, and one was used for *A. cerana* experiments, while the other was for *A. mellifera*. The beehives of these two species were kept outside and housed the honeybees used in the experiments. The distance between each hive and the corresponding Y-maze was about 20 m. For each experiment, up to 20 honeybees were individually marked and trained to visit a feeder with sugar solution in the Y-maze. During training, the feeder was initially placed at the entrance of the maze and then moved step-by-step to the decision chamber (advancing about 20 cm every 10 min). Honeybees entering the Y-maze were trained to choose one of two patterns, which indicated the position of the feeder reward. The feeder was placed on the floor just under the entrance and was thus invisible to the honeybee from the entrance. Training was carried out by reinforcement: if the honeybee made a positive decision by flying through the correct pattern (termed "positive"), it would find a feeder with sugar solution as a reward; if the honeybee chose the wrong pattern (termed "negative"), it would find an empty chamber without reward, and would then be released to try again. The trained honeybees flew regularly between the hive and the maze, averaging about three visits every 8 min. Experiments on the two honeybee species were carried out simultaneously.

Visual stimuli

We investigated the ability of *A. cerana* and *A. mellifera* to discriminate color and orientation in visual patterns. The visual stimuli were presented as 18-cm-diameter circles (color and grating patterns, Fig. 1b) at the entrance of the chambers and subtended a visual angle of 37°. All of the
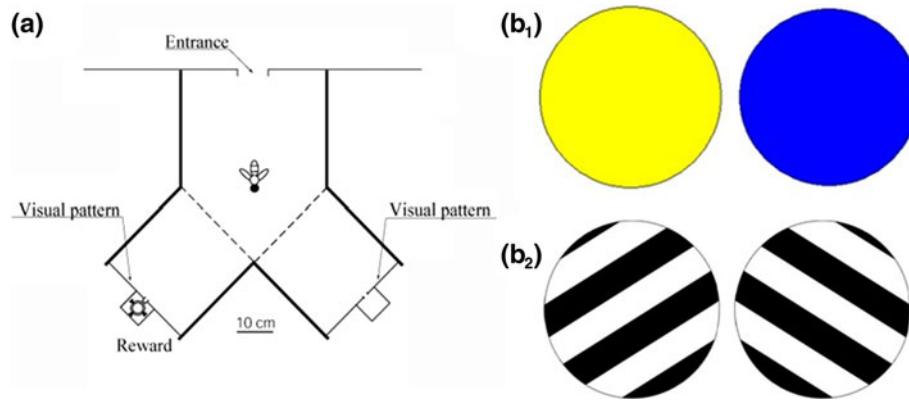
Fig. 1 The experimental setup and visual patterns. **a** The experimental twin-choice Y-maze was divided into three chambers in which honeybees were required to discriminate between rewarded and unrewarded patterns. **b₁** *Yellow* and *blue patterns* were used in the training and testing procedure for color. **b₂** Grating patterns in *black/white* (period 6 cm, 13°) oriented at 45° versus 135° were used in the grating learning and memory experiment. See "Materials and methods" for details

visual stimuli had a central orifice, 2 cm in diameter, which allowed access to a reward chamber containing the feeder or not. The color stimuli were yellow and blue (Fig. 1b₁). The yellow pattern had a plateau reflecting wavelengths from 580 nm, and the blue pattern had a peak value of 460 nm (Fig. 2); these were measured in Zhejiang University using a Shimadzu UV3150 UV–visible-infrared spectrophotometer. Grating patterns in black/white were created on a computer using a graphics program, and then they were printed on normal copy paper using an OKI C3200n color laser printer. Additionally, the gratings had a spatial period of 6 cm, and the period subtended a visual angle of 13°. What's more, grating patterns were oriented at 45° versus 135° (Fig. 1b₂) during training.

Training and testing procedure

We assessed learning and memory performance using a Y-maze association paradigm in which honeybees are required to discriminate between rewarded and unrewarded patterns. We guided honeybees to collect sugar solution in the Y-maze at the beginning of each experiment and then
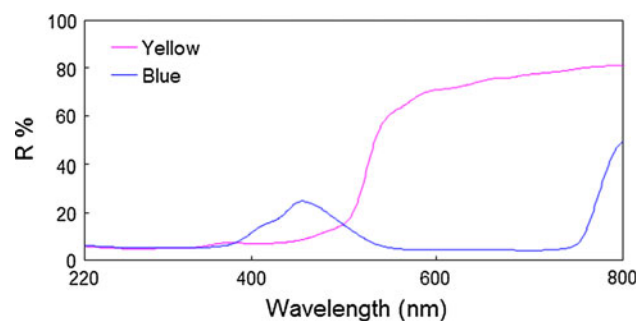

Fig. 2 Spectral reflectivity curves of the color patterns. **a** Spectral reflectivity curves of yellow pattern, with a plateau reflecting wavelength from 580 nm. **b** Spectral reflectivity curves of blue pattern, with a peak value of 460 nm

marked them with different colors. When up to 20 honeybees had been individually marked, and most of them were acquainted with the way to obtain the reward in the Y-maze, visual stimuli were presented and the training commenced.

The trainings were conducted for a period (termed "block") of about 16 min, during which the positions of the patterns (left or right) were swapped at around 8 min (after an average of 3 rewarded visits per honeybee), i.e., halfway through a block. Thus, each honeybee was rewarded six times in a block, on average: three times with the positive pattern on the left and three times with it on the right. Frequent swapping of the positions of the two comparison stimuli minimized the effects of possible biases caused by asymmetrical lighting or external landmarks. This ensured that the honeybees learned to obtain a reward by visual comparison, not by associating the feeder location with a particular chamber. Furthermore, a break of half an hour after each 2 h training was needed to prevent fatigue in the honeybees. During these breaks, another feeder, with a diluted sugar solution, was provided at the entrance of the maze.

Our study comprised two series of experiments, and the training procedures are described separately as follows:

Series 1: color learning and memory

These experiments investigated whether *A. cerana* was able to distinguish between different types of colors as well as *A. mellifera* could (Giurfa 2004), and we wanted to explore some differences between these two honeybee species simultaneously. There were two experiments in this series. In experiment 1, we set the yellow pattern as "positive." Here, a pair of color patterns, namely yellow versus blue, was presented at the entrances of the reward chambers. Honeybees that had flown through the Y-maze and made a correct choice of the yellow pattern in the decision chamber obtained access

to a reward of sugar solution, while the ones that chose the blue pattern would reach an empty chamber without reward. For experiment 2, we set the blue pattern as "positive." In this series, each experiment was carried out for 1 day and included ten training blocks. For each experiment, five independent biological replicates were performed as described, i.e., the experiments were carried out repeatedly five times, and each replicate was carried out by training a fresh group of bees.

Series 2: grating learning and memory

Here, we tested the ability of honeybees to discriminate gratings of different orientations. In this experiment, a pair of black/white gratings oriented at 45° versus 135° to the horizontal was used at the entrances to the chambers, and we set the 45° grating pattern as "positive." Each experiment was carried out for about 2½ days and included 28 or 32 blocks. We also performed five independent biological replicates for each experiment using fresh groups of untrained honeybees. Considering the results of Chandra et al. (1998) in which the response of the bees was independent of the orientation of the trained orientation, we only used a 45° grating pattern as the positive pattern in the experiments.

Control experiments

Controls were carried out at the end of each experiment to check whether the trained honeybees had developed any side bias in their choice behavior, which could have been caused by asymmetrical lighting or external landmarks. The control experiment was also necessary for checking whether olfaction was involved in honeybees' choices (Zhang et al. 2005; Pahl et al. 2007). The honeybees' choice performance was measured while two identical visual patterns were presented at the entrances of chambers, but with the feeder removed. The stimulus used in series 1 was a pair of identical yellow patterns and then identical blue patterns; in series 2 it was a pair of identical white/black horizontal gratings and then identical vertical gratings. Each control experiment lasted for about 2–3 min, during which time the trained honeybees could make two independent choices, on average, and then the pair of testing patterns was changed for another pair. Honeybees' choices for the left or the right chamber were recorded separately. A preference for one side or the other would indicate a side bias.

Data collection and analysis

Each type of experiment was performed on five independent biological replicates to gather sufficient data. We used two methods to analyze the choices of honeybees, both of which are well described in honeybee behavioral research (Zhang et al. 2004, 2006; Baird et al. 2005; Pahl et al. 2007). In the first method, only the first choice of each honeybee on each visit was taken into account to eliminate the possibility that the second choice in the same training block might be influenced by the outcome of the first. This was particularly important if the first choice was incorrect, as now the second choice could be made by a simple process of elimination. In the second method, we analyzed all choices on each training block.

During training, every "positive" and "negative" choice of the honeybees was recorded in each block. We performed ANOVA to check the homogeneity of data across all blocks and for individual honeybees for a given type of training using the statistical software SPSS Statistics Base 17.0. Based on these tests, the performance of each honeybee was evaluated separately by pooling its correct choices and visits over all blocks and calculating the ratio of the number of correct choices to the number of visits. The average performance for a given type of training was obtained by averaging choice frequencies across the honeybee. The sample size (N) was the total number of bees in five replicates, and N was specified in the figures. For each type of experiment, the data of five replicates were analyzed to obtain mean values of choice frequency, standard deviations (SD) and standard errors of means (SEM). In the text and figures, performance is indicated by the mean choice frequency ($\pm$SEM). A series of learning curves for these experiments was drawn based on the mean values of choice frequency of five replicates in every training block.

To resolve the question of whether there was any significant difference in the performances of A. cerana and A. mellifera, the Kruskal-Wallis test was used in our analyses. In addition, we analyzed the data for significant differences in the performances of A. cerana between yellow and blue patterns, and a similar analysis was carried out for the performances of A. mellifera. Student's t tests were used to determine whether each choice frequency was significantly different from the random choice level of 50 %. The 2 × 2 McNemar tests were used for all of the control experiments (Zhang et al. 2005; Pahl et al. 2007).

Results

Both species of trained honeybees learned the visual discriminations

The honeybees learned the visual discriminations very well. In each training procedure, their preference gradually moved to the "positive" pattern after several visits, and eventually their choice frequency in favor of the "positive"
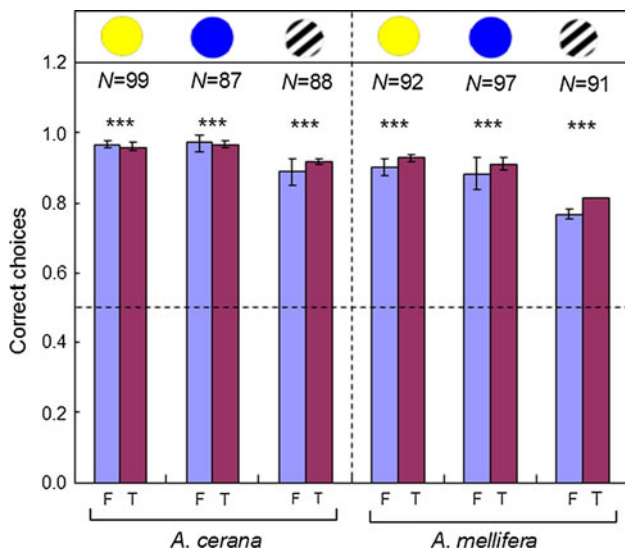
Fig. 3 The choice frequency in favor of the "positive" stimulus for color and grating training in the last three blocks of the training. We analyzed the learning performance of the last three blocks for each experiment and obtained their average values as shown in Fig. 3. Results show that the choice frequency of these two species for the "positive" pattern was above 90 % for color learning and above 80 % for the gratings for both the first (*F*) and total (*T*) choice ratios. All of the choice frequencies are significantly greater than the random choice level of 50 % ($P < 0.001$). Color and grating patterns at the top denote the "positive" stimulus in each experiment. *N* denotes the total number of bees that were tested in five replicates of each experiment. *Asterisks* denote statistically significant differences from the random choice level of 50 % (*broken horizontal line*). ***P < 0.001. Values are mean ± SEM of the data

stimulus was significantly greater than the random choice level of 50 % ($P < 0.001$, Fig. 3). Furthermore, trained honeybees obtained the reward rapidly, and the accuracy eventually reached a high level for both species.

Series 1: a comparison of color discrimination in two species

For a comparison of color learning between these two honeybee species, the visits for "positive" and "negative" pattern were recorded in each training procedure, including the first and total choices. The ratio of the number of choices of the "positive" pattern to the number of total choices was calculated for each training procedure, i.e., the correct choice ratio. These ratios were then pooled according to the experiment type for *A. cerana* and *A. mellifera* separately. Our data show that the choice frequency of these two species for the "positive" pattern was above 90 % at the end of the learning phase in both experiments 1 and 2. When we compared the average choice frequency in favor of the "positive" pattern between *A. cerana* and *A. mellifera*, the results showed that the learning and memory performance to color was

significantly different between these two species for both the first and total choice ratios ($P < 0.05$, Fig. 4). The performance of *A. cerana* was significantly better than that of *A. mellifera*. Furthermore, there was no significant difference in the performance of *A. cerana* between yellow and blue patterns ($P > 0.05$, Fig. 5a), and a similar conclusion was reached when we compared the performance of *A. mellifera* ($P > 0.05$, Fig. 5b).

Series 2: a comparison of grating-orientation discrimination in two species

The results of this experiment reveal that honeybees' preference for the "positive" pattern was significantly better than the random choice level of 0.50 after repeated visits ($P < 0.001$). At the end of the learning phase, the choice frequencies of these two species for the "positive" pattern were above 80 %. Kruskal-Wallis test results showed that the performance of *A. cerana* was significantly better than that of *A. mellifera* for both the first and total visits ($P < 0.001$, Fig. 6).

Control experiments

The control experiments were carried out at the end of each experiment. We used a 2×2 McNemar test to determine whether there was any side bias during training (Zhang et al. 2005; Pahl et al. 2007). In these control tests, the choice frequency for any one of the two (identical) comparison stimuli was not significantly different from the random choice level of 0.50 ($P > 0.05$) in all series 1 and 2 experiments. This result demonstrates that honeybees did not have any significant side preference, i.e., exhibiting no preference for the stimulus that was associated with the feeder. Thus, the honeybees' choices in our experiments were driven only by the visual cues provided by the patterns and not by any other cues.

Discussion

The findings of this study quite clearly demonstrate that *A. cerana* were able to distinguish between different types of colors and orientation of gratings patterns at least as well as *A. mellifera* (Zhang et al. 1992; Srinivasan et al. 1994; Giurfa et al. 2001). Furthermore, the results of the present study showed that there were significant differences between *A. cerana* and *A. mellifera* in learning and memory performance on color and orientation learning and that the performances of *A. cerana* were significantly better than that of *A. mellifera*.

As shown by Chen (2001), *A. cerana* has a better sense of smell than *A. mellifera*. In this study, we attempted to

Fig. 4 Comparison of learning curves on color patterns between *A. cerana* and *A. mellifera*. Data show the results of blocks of ten consecutive training visits for each experiment. **a**, **b** The learning performance on *yellow* and *blue patterns* was significantly different between *A. cerana* and *A. mellifera* for both the first and the total choice ratios ($P < 0.05$), and the performance of *A. cerana* was significantly better than that of *A. mellifera*. **a₁**, **b₁** The first choice learning curves for color learning. **a₂**, **b₂** The total choice learning curves for color learning. *N* denotes the total number of bees that were tested in five replicates of each experiment. *Broken horizontal lines* denote the random choice level of 50 %. Values are mean ± SEM of the data
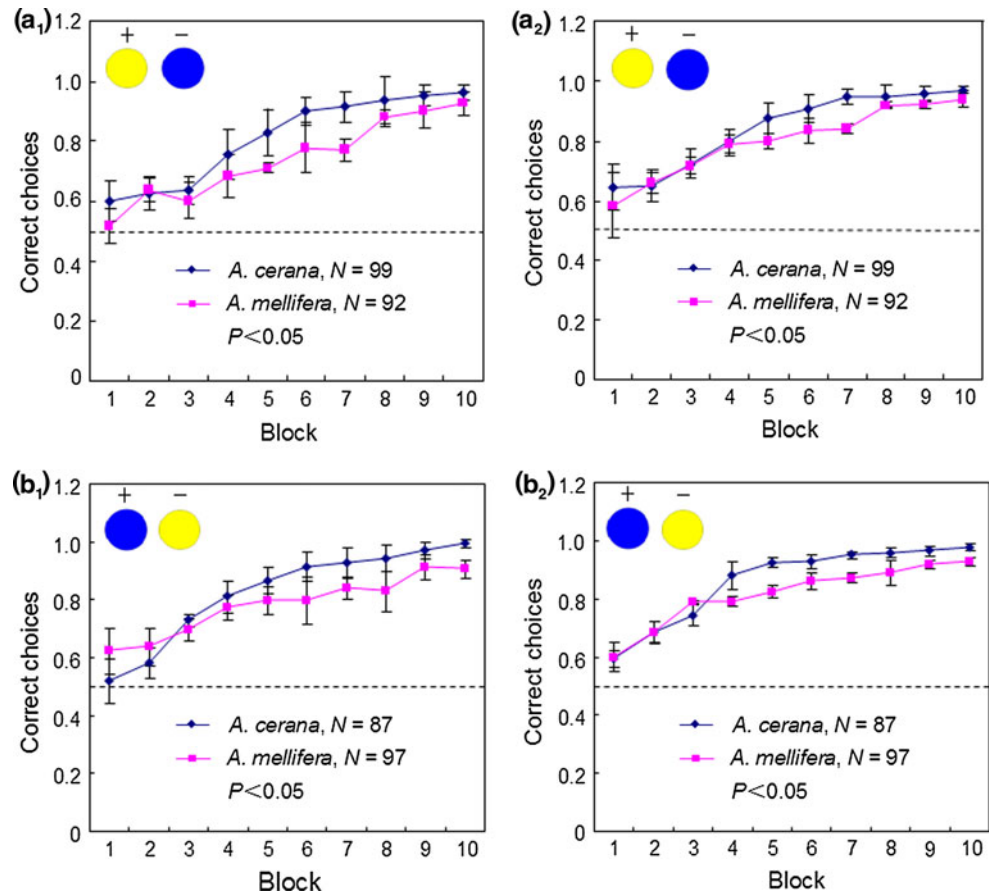
compare visual learning and memory between the two species. As a result, it is especially important to adopt some measures to avoid interference from olfactory cues. First, we used pure laboratory-grade sucrose rather than table sugar to minimize the smell of the sugar solution. Second, the feeder, the chamber and entrance were cleaned frequently with water or alcohol during the experiments. In any case, the results of the control experiments indicated that the honeybees had no preference for the chamber that contained the feeder when both chambers bore the same visual stimuli. Thus, it can be concluded that the honeybees' choices in our experiments were driven only by the visual cues provided by the patterns and not by any other cues.

At present, there are two main different analytical methods for data on the study of honeybee learning and memory. Giurfa et al. (1997) and De Ibarra et al. (2000) hold that no reward should be offered during the test and only the first choice calculated, as they insist that only in this way can we detect the real learning level of honeybees. Zhang et al. (2005) and Chen et al. (2003), however, consider that honeybees should obtain a reward if they make a correct choice to ensure that the honeybees' memory for the "positive" pattern is not influenced by unrewarded visits and that all of the honeybees' choices on

each training block are calculated. Only the first choice of each honeybee on each visit was taken into account in the first method to eliminate the possibility that the second choice in the same training block might be influenced by the outcome of the first. This is particularly important if the first choice is incorrect as now the second choice can be made by a simple process of elimination. However, this reduces the number of data points, and so the second method was utilized, i.e., analyzing the total choices. The use of both methods allowed us to analyze the preferences of *A. cerana* and *A. mellifera* more comprehensively.

A recent study has revealed that in mixed colonies of *A. cerana* and *A. mellifera*, the two species can understand each other's "dance language" (Su et al. 2008). In comparison to *A. mellifera*, however, *A. cerana* can decode the "dance language" more accurately and quickly. This demonstrates that *A. cerana* may have a stronger learning and memory capability than *A. mellifera*. Our result showing that the performances of *A. cerana* are significantly better than those of *A. mellifera* is in agreement with them.

As suggested by Zeng et al. (2010), depending on visual and olfactory sensation, *A. cerana* can distinguish and remove dummy larvae made of paraffin mixed with brood pheromone in the worker cells, while *A. mellifera* will seal

Fig. 5 Comparison of learning curves for *yellow* as positive pattern and *blue* as positive pattern in *A. cerana* and *A. mellifera*. **a**, **b** There was no significant difference in the performance of *A. cerana* between *yellow* as positive pattern and *blue* as positive pattern ($P > 0.05$), and a similar result was obtained for *A. mellifera* ($P > 0.05$). **a₁**, **b₁** The first choice learning curves for color learning. **a₂**, **b₂** The total choice learning curves for color learning. *N* denotes the total number of bees that were tested in five replicates of each experiment. *Broken horizontal lines* denote the random choice level of 50 %. Values are mean ± SEM of the data
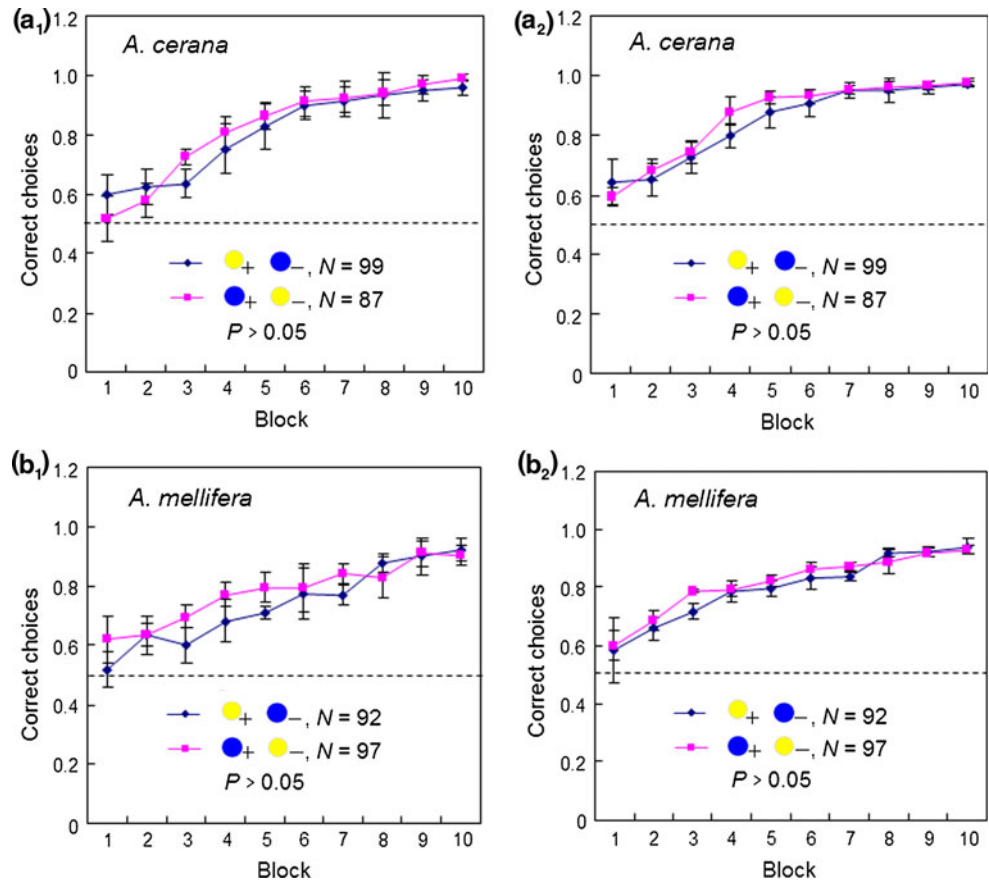
the cells of dummy larvae. This indicates that *A. cerana*'s ability to distinguish dummy larvae is better than that of *A. mellifera* because of their stronger visual and olfactory sensations. The reasons for *A. cerana*'s superior performance on color and grating learning are as yet unknown, but may well be related to a higher visual and olfactory sensitivity in *A. cerana*.

In autumn 2006, the Western honeybee *A. mellifera* disappeared across the United States, with half of the states affected and beekeepers losing 30–90 % of their colonies (van Engelsdorp et al. 2007). In the absence of a known cause, this syndrome was named colony collapse disorder (CCD) because the main trait was a rapid loss of adult worker bees. Since then, the problem has intensified in recent months, and CCD has been reported in Germany, Switzerland, Spain, Portugal, Italy, Greece and the UK (Lean and Shawcross 2007). Numerous causes of CCD have been proposed, however often with little or no supporting data (Oldroyd 2007). When CCD occurs, honeybees simply vanish relatively suddenly, with little or no dead adults in or near the colonies (Evans et al. 2009). In this context, it is interesting that there have been few reports to CCD in the Eastern honeybee, *A. cerana*. Further work is required to determine whether the differences in learning and memory capacity between *A. cerana* and *A.*

mellifera have a role to play in the development of CCD is the latter species but not the former.

In honeybees, the geographical races are the product of natural selection in their respective homeland. They represent very different genotypes adapted to dissimilar environments (Ruttner 1975). Inside the wide genotype races of honeybees, there are also ecotypes characterized by their adaptation to the environment in which they live (Louveaux 1969). In our experiments, the results showed that the learning and memory performance of *A. cerana* was significantly better than that of *A. mellifera*'s. We speculate that this can be largely explained by the different habitats of these two honeybee species, where there are different climates, weather conditions, food sources and the onsets of flower blooming. The Eastern honeybee, *A. cerana*, which is apparently better adapted to hot climates and able to avoid the oriental hornet, a serious predator, and is also good at collecting sporadic nectar flowers, is distributed from Afghanistan to Japan and Southeast Asia to the Wallace line; the Western honeybee, *A. mellifera*, on the other hand, is about one-third larger in size and is better adapted to extended periods of cold weather (Butler 1975; Dietz et al. 1986). According to reports, most of the European mainland is plains, with an area of more than 60 %, and its mountainous area is less than 2 % (Fan and
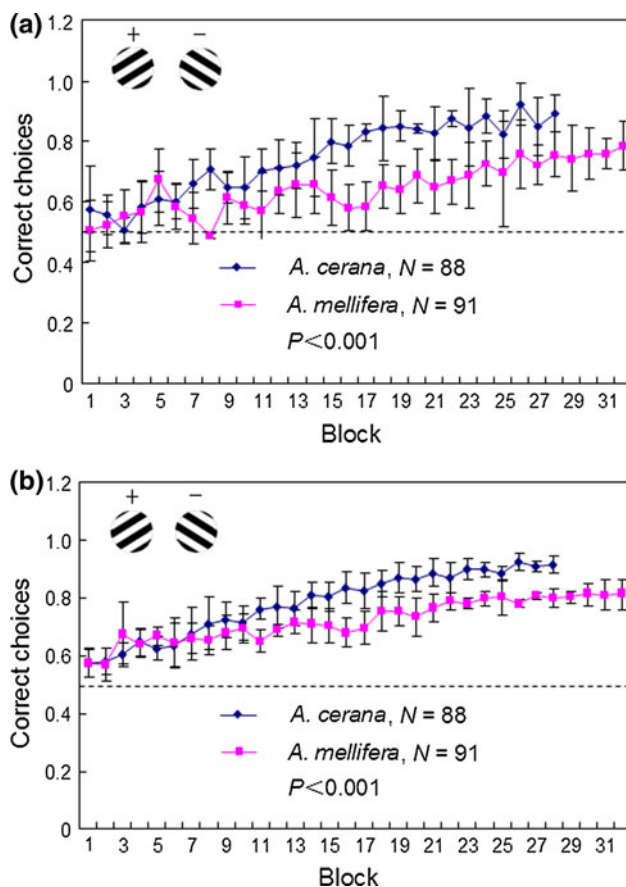
Fig. 6 Results of experimental series 2. **a**, **b** *Learning curves* obtained from the first and total choices of *A. cerana* and *A. mellifera* on *black/white gratings* oriented at 45° versus 135° to the horizontal are significantly different (*P* < 0.001). The performance of *A. cerana* was significantly better than that of *A. mellifera* for both the first and total visits. *N* denotes the total number of bees that were tested in five replicates of this series. *Broken horizontal lines* denote the random choice level of 50 %. Values are mean ± SEM of the data

Zhou 2005). However, China, the main habitat of *A. cerana*, is primarily a mountainous region (more than 75 % of the country) (Li et al. 2008; Cheng et al. 2009). Because of the complex terrain and variable climate, the mountainous area is a very harsh environment for *A. cerana*. Furthermore, mountainous areas have more rain and fog, more dispersed nectariferous plants and more natural enemies compared with plain areas. Consequently, the survival pressure on *A. cerana* may be heavier than that on *A. mellifera*, so we infer that *A. cerana* needs to have a greater learning and memory capability because of the complex environment. Moreover, this environment may also result in more opportunities for *A. cerana* to evolve in ways to adapt to the complex living environment. In conclusion, the pressures of survival may play an important role in the phenomenon that *A. cerana*'s performance in learning and memory is superior to that of *A. mellifera*.

Having been introduced for more than 100 years, *A. mellifera* are well adapted to the climate and nectar sources in most areas of China. However, without doubt, they are not quite as well adapted as *A. cerana*, whose homeland is here. Therefore, these experiments were performed in weather and temperature conditions, etc., that are probably much closer to normal biotope conditions for *A. cerana* than for *A. mellifera*. Besides, the summer in Nanchang, China, is excessively hot (average 27 °C) compared to European temperatures. This may have a negative influence on *A. mellifera*'s fitness and its capacity to negotiate maze discriminations to some extent. Therefore, our results were relative: perhaps if we carried out the same experiments in a European country in a summer where it might be 18 °C, the results would be different. Therefore, further work should be done in a different country with various climates to acquire more comprehensive data.

Another intriguing finding of our study was the improved performance of our trained bees after a short rest in comparison to the performance on the last training blocks before resting. As mentioned in "Training and testing procedure," we had a short break of half an hour after each 2-h training block, during which another feeder with a diluted sugar solution was provided at the entrance of the maze. We noticed that after prolonged training on the same set of visual patterns, the honeybees became inattentive, because they often passed into the right or left chamber with only a cursory scan of the patterns. After a break, however, they would spend more time scanning the patterns before passing into the chamber. Like the finding of Chittka et al. (2003), the more time an individual bumblebee invests in making a decision, the more accurate are its responses. It is possible that our bees, too, also achieved improved memory scores as a result. Gross et al. (2009) reported a similar finding. Moreover, the extended breaks during training, combined with the weaker sugar solution offered at the maze entrance during these breaks, had the effect of temporarily reducing the traffic of our 20 trained bees between the maze and the hive.

This is the first study to compare the learning and memory of *A. cerana* and *A. mellifera*. Our findings suggest that *A. cerana* and *A. mellifera* possess different abilities for color and grating learning. However, the study was just at the behavioral level. The exact reason why *A. cerana*'s performance was better than *A. mellifera*'s continues to be a mystery. A sound understanding of the differences in the learning abilities of the two species requires investigations of the underlying molecular mechanisms.

References

Avargues-Weber A, Dyer AG, Giurfa M (2011) Conceptualization of above and below relationships by an insect. Proc R Soc Lond Ser B 278:898–905

Avargues-Weber A, Dyer AG, Combe M, Giurfa M (2012) Simultaneous mastering of two abstract concepts by the miniature brain of bees. Proc Natl Acad Sci 109:7481–7486

Baird E, Srinivasan M, Zhang S, Cowling A (2005) Visual control of flight speed in honeybees. J Exp Biol 208:3895

Butler CG (1975) The honey bee colony-life history. In: The Hive and the Honey Bee. Dadant and Sons, Hamilton, pp 39–74

Chandra BCS, Geetha L, Abraham VA, Karanth P, Thomas K, Srinivasan MV, Gadagkar R (1998) Uniform discrimination of pattern orientation by honeybees. Anim Behav 56:1391–1398

Chen S (2001) The apicultural science in China. China Agriculture Press, Beijing

Chen L, Zhang S, Srinivasan M (2003) Global perception in small brains: topological pattern recognition in honey bees. Proc Natl Acad Sci 100:6884

Cheng W, Zhou C, Chai H, Zhao S, Li B (2009) Quantitative extraction and analysis of basic morphological types of land geomorphology in China. J Geo-Inf Sci 6:725–736

Chittka L, Vorobyev M, Shmida A, Menzel R (1993) Bee color vision—the optimal system for the discrimination of flower colours with three spectral photoreceptor types? In: Wiese K (ed) Sensory systems of Arthropods. Springer-Verlag, New York, pp 211–218

Chittka L, Dyer AG, Bock F, Dornhaus A (2003) Psychophysics: bees trade off foraging speed for accuracy. Nature 424:388

Collett TS, Graham P, Durier V (2003) Route learning by insects. Curr Opin Neurobiol 13:718–725

De Ibarra NH, Vorobyev M, Brandt R, Giurfa M (2000) Detection of bright and dim colours by honeybees. J Exp Biol 203:3289–3298

Dietz A, Krell R, Pettis J (1986) The potential limit of survival for Africanized bees in the United States. In: Proceeding of the Africanized Honey Bee Symposium, American Farm Bureau Research Foundation, Atlanta, GA, Feb 11–12, pp 87–100

Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y, Underwood R (2009) Colony collapse disorder: a descriptive study. PLoS ONE 4:e6481

Fan Y, Zhou M (2005) World Atlas. China Map Press, Beijing

Giurfa M (2004) Conditioning procedure and color discrimination in the honeybee Apis mellifera. Naturwissenschaften 91:228–231

Giurfa M (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. J Comp Physiol A 193:801–824

Giurfa M, Eichmann B, Menzel R (1996) Symmetry perception in an insect. Nature 382:458–461

Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A 180:235–243

Giurfa M, Zhang S, Jenett A, Menzel R, Srinivasan MV (2001) The concepts of 'sameness' and 'difference' in an insect. Nature 410:930–932

Gong Y, Zhang Q (2000) Classification and evolution of honeybee. Fujian science and technology press, Fuzhou

Gross HJ, Pahl M, Si A, Zhu H, Tautz J, Zhang S (2009) Number-based visual generalisation in the honeybee. PLoS ONE 4:e4263

Hateren JH, Srinivasan M, Wait P (1990) Pattern recognition in bees: orientation discrimination. J Comp Physiol A 167:649–654

Lean G, Shawcross H (2007) Are mobile phones wiping out our bees? News, The Independent [online]. http://newsindependentcouk/environment/wildlife/article2449968ece

Li B, Pan B, Han J (2008) Basic terrestrial geomorphological types in China and their circumscriptions. Quat Sci 28:535–543

Louveaux J (1969) Ecotype in honey bees. Pro. Int Beekeep Congr Munich 22:499–501

McCabe S, Farina W (2009) Odor information transfer in the stingless bee Melipona quadrifasciata: effect of in-hive experiences on classical conditioning of proboscis extension. J Comp Physiol A 195:113–122

McCabe S, Farina W (2010) Olfactory learning in the stingless bee Tetragonisca angustula (Hymenoptera, Apidae, Meliponini). J Comp Physiol A 196:481–490

McCabe S, Hartfelder K, Santana W, Farina W (2007) Odor discrimination in classical conditioning of proboscis extension in two stingless bee species in comparison to Africanized honeybees. J Comp Physiol A 193:1089–1099

Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (Apis mellifica). J Comp Physiol A 56:22–62

Menzel R (1968) Das Gedaechtnis der Honigbiene fuer Spektralfarben I.Kurzzeitiges und langzeitiges Behalten. J Comp Physiol A 60:82–102

Menzel R (1990) Learning, memory, and "cognition" in honey bees. In: Kesner RP, Olton DS (eds) Neurobiology of comparative cognition. Lawrence Erlbaum Associated, Hillsdale, pp 237–292

Menzel R (2001) Searching for the memory trace in a mini-brain, the honeybee. Learn Mem 8:53–62

Menzel R, Geiger K, Chittka L, Joerges J, Kunze J, Uuml U (1996) The knowledge base of bee navigation. J Exp Biol 199:141–146

Oldroyd BP (2007) What's killing American honey bees? PLoS Biol 5:e168

Pahl M, Zhu H, Pix W, Tautz J, Zhang S (2007) Circadian timed episodic-like memory—a bee knows what to do when, and also where. J Exp Biol 210:3559

Pahl M, Zhu H, Tautz J, Zhang S (2011) Large scale homing in honeybees. PLoS ONE 6:e19669

Ruttner F (1975) Races of bees. In: The Hive and the Honey Bee. Dadant and Sons, Hamilton, pp 19–38

Srinivasan M, Lehrer M (1988) Spatial acuity of honeybee vision and its spectral properties. J Comp Physiol A 162:159–172

Srinivasan M, Zhang S, Witney K (1994) Visual discrimination of pattern orientation by honeybees: performance and implications for cortical' processing. Proc R Soc Lond Ser B 343:199–210

Su S, Cai F, Si A, Zhang S, Tautz J, Chen S (2008) East Learns from West: asiatic honeybees can understand dance language of European honeybees. PLoS ONE 3:e2365

van Engelsdorp D, Underwood R, Caron D, Hayes J Jr (2007) An estimate of managed colony losses in the winter of 2006–2007: a report commissioned by the apiary inspectors of America. Am Bee J 147:599–603

Vorobyev M, Menzel R (1999) Flower advertisement for invertebrates: bees. In: Acher SN et al (eds) The Ecology of Vision. Kluwer Academic Publishers, Dordrecht, pp 537–553

Zeng Z (2009) Apiculture. China Agriculture Press, Beijing

Zeng Y, Zeng Z, Yan W, Wu X (2010) Effects of three aliphatic esters of brood pheromone on worker feeding and capping behavior and queen development of A. cerana cerana and A. mellifera ligustica. Acta Entomol Sin 53:154–159

Zhang S, Srinivasan M (1994) Prior experience enhances pattern discrimination in insect vision. Nature 368:330–332

Zhang S, Srinivasan M, Horridge G (1992) Pattern recognition in honeybees: local and global analysis. Proc R Soc Lond Ser B 248:55

Zhang S, Srinivasan M, Collett T (1995) Convergent processing in honeybee vision: multiple channels for the recognition of shape. Proc Natl Acad Sci 92:3029

Zhang S, Bartsch K, Srinivasan M (1996) Maze learning by honeybees. Neurobiol Learn Mem 66:267–282

Zhang S, Lehrer M, Srinivasan M (1999) Honeybee memory: navigation by associative grouping and recall of visual stimuli. Neurobiol Learn Mem 72:180–201

Zhang S, Mizutani A, Srinivasan M (2000) Maze navigation by honeybees: learning path regularity. Learn Memory 7:364–374

Zhang S, Srinivasan M, Zhu H, Wong J (2004) Grouping of visual objects by honeybees. J Exp Biol 207:3289

Zhang S, Bock F, Si A, Tautz J, Srinivasan M (2005) Visual working memory in decision making by honey bees. Proc Natl Acad Sci 102:5250

Zhang S, Schwarz S, Pahl M, Zhu H, Tautz J (2006) Honeybee memory: a honeybee knows what to do and when. J Exp Biol 209:4420