© 2016. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2016) 219, 412-418 doi:10.1242/jeb.126920

RESEARCH ARTICLE

Accelerated behavioural development changes fine-scale search behaviour and spatial memory in honey bees (*Apis mellifera* L.)

Tomokazu Ushitani[1], Clint J. Perry[2,3], Ken Cheng[2] and Andrew B. Barron[2,*]

ABSTRACT

Normally, worker honey bees (*Apis mellifera*) begin foraging when more than 2 weeks old as adults, but if individual bees or the colony is stressed, bees often begin foraging precociously. Here, we examined whether bees that accelerated their behavioural development to begin foraging precociously differed from normal-aged foragers in cognitive performance. We used a social manipulation to generate precocious foragers from small experimental colonies and tested their performance in a free-flight visual reversal learning task, and a test of spatial memory. To assess spatial memory, bees were trained to learn the location of a small sucrose feeder within an array of three landmarks. In tests, the feeder and one landmark were removed and the search behaviour of the bees was recorded. Performance of precocious and normal-aged foragers did not differ in a visual reversal learning task, but the two groups showed a clear difference in spatial memory. Flight behaviour suggested normal-aged foragers were better able to infer the position of the removed landmark and feeder relative to the remaining landmarks than precocious foragers. Previous studies have documented the cognitive decline of old foragers, but this is the first suggestion of a cognitive deficit in young foragers. These data imply that worker honey bees continue their cognitive development during the adult stage. These findings may also help to explain why precocious foragers perform quite poorly as foragers and have a higher than normal loss rate.

KEY WORDS: Temporal polyethism, Spatial memory, Navigation, Social insect, Precocious forager, Reversal learning

INTRODUCTION

Honey bees (*Apis mellifera* L.), like many social insects, demonstrate temporal polyethism: changing their role in the colony as they age. The sequence of tasks is predictable, with young adult bees working initially on in-hive tasks such as brood care and nest construction and commencing foraging later in life (Seeley, 1995). This is considered an adaptive behavioural strategy as foraging is the most high-risk of bees' roles (Dukas, 2008; Rueppell et al., 2007; Sakagami and Fukuda, 1968; Visscher and Dukas, 1997) and delaying high-risk tasks to later in a worker's life increases the total contribution of that worker to colony growth and reproduction (Jeanne, 1986; Tofilski, 2002; Woyciechowski and Moron, 2009). Foraging is also arguably the most energetically and cognitively demanding role for a social insect worker. In both bees and ants, physiological and behavioural developmental processes precede the onset of foraging to prepare the worker for that role. In honey bees, there is extensive adaptation of worker physiology and metabolism, with changes in flight muscle (Correa-Fernandez and Cruz-Landim, 2010; Herold, 1965), a reduction in brood-feeding glands (Winston, 1987) and fat stores (Toth and Robinson, 2005), and an overall increase in metabolic rate (Harrison, 1986). There are also behavioural changes, with workers establishing a clear diurnal rhythm to activity (Bloch et al., 2001), and engaging in orientation flights (Becker, 1958; Capaldi et al., 2000) prior to foraging. During these observation flights, bees are presumed to learn about their landscape and the location of the hive.

Foraging is also associated with changes in brain structure. In honey bees, foraging is correlated with growth of the mushroom bodies (Farris et al., 2001; Ismail et al., 2006; Withers et al., 1993), which are paired structures in the insect central brain involved in sensory processing and integration and learning (Menzel, 2001; Menzel and Giurfa, 2001). The calyx of the mushroom body (the region of sensory input) is larger in forager bees than in same-aged nurse bees (Farris et al., 2001; Withers et al., 1993). This growth is due to an increase in the size and complexity of dendritic arbours within the calyx and synaptic density, rather than neurogenesis (Fahrbach et al., 1995; Farris et al., 2001; Groh et al., 2012), and the calyx continues to grow throughout the foragers' active life. Similar changes have been documented in ant brains, with the onset of foraging being associated with an increase in the size and complexity of mushroom bodies (Gronenberg et al., 1996; Stieb et al., 2012).

In honey bees, foraging typically begins when a bee is between 2 and 3 weeks old as an adult (Fukuda and Sakagami, 1968; Seeley, 1995; Winston, 1987). The pace of behavioural development is, however, highly flexible and if an individual bee or their colony is stressed, a very common reaction is for workers to begin foraging earlier. Starvation or malnutrition (Free, 1961; Janmaat and Winston, 2000; Schulz et al., 1998; Toth and Robinson, 2005), diseases or environmental toxins (Goblirsch et al., 2013; Higes et al., 2008; Søvik et al., 2015; Woyciechowski and Moron, 2009) and a loss of foragers (Huang and Robinson, 1996; Robinson et al., 1994) can all lead to an early onset of foraging in young bees.

This response is considered adaptive because it would increase (or rapidly restore) the foraging force in response to acute stressors, and increase the capacity for resource collection, but two recent studies have shown that precocious foragers perform extremely poorly (Chang et al., 2015; Perry et al., 2015). Perry et al. (2015) found that precocious foragers live less long, complete fewer foraging trips, take longer on foraging trips and are more likely to die in their first flights from the hive than bees that began foraging at >14 days old as adults. Accelerating foraging onset with the juvenile hormone methoprene had similar results on foraging performance (Chang et al., 2015). The consequence of this is significant as a

[1]Department of Cognitive and Information Sciences, Faculty of Letters, Chiba University, 1-33 Yayoi-cho, Inage, Chiba 263-8522, Japan. [2]Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia. [3]School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK.

*Author for correspondence (Andrew.Barron@mq.edu.au)

Received 17 June 2015; Accepted 12 November 2015

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 412-418 doi:10.1242/jeb.126920

chronic colony stressor (such as pesticide contamination or malnutrition) could result in a decrease in the average age of the forager force. The reduced efficiency of a younger foraging force could render the colony population unsustainable (Perry et al., 2015).

It would appear that there is a cost to the worker bee of accelerated behavioural development to become a forager (Chang et al., 2015; Perry et al., 2015), but it is not clear why precocious foragers perform so poorly. One interpretation is that they are not physiologically and metabolically equipped for foraging (Vance et al., 2009), but it is also possible that precocious foragers are not cognitively equipped for foraging. To explore this latter hypothesis, we tested the performance of precocious and normal-aged foragers from the same colony in tests of colour learning and visual spatial learning.

MATERIALS AND METHODS

All experiments were performed with bees maintained at Macquarie University (Sydney, Australia) between October 2012 and May 2013 (the southern hemisphere summer and autumn). Bees originated from the commercial standard stock available to Australia (mostly a *Ligustica* background) containing a naturally mated queen.

Experiment 1: comparison of performance in a visual reversal learning task

We established a small single-age cohort colony (SCC) consisting of >4000 bees by collecting 800–1200, 1 day old bees every day for 4 days. All of the bees were marked with small spots of enamel paint on the dorsal thorax. A different colour was used each day so that the age of all bees was known. We set the SCC in a 150 m^2 flight cage in which bees were provided with a dish of pollen and an *ad libitum* 1 mol l^{-1} sucrose feeder as forage. Some of the bees from the colony started foraging precociously. Here, we classed precocious foragers as bees that were observed foraging when <16 days old (Perry et al., 2015). We tested 23 precocious foragers from the first SCC. When the first SCC was 16 days old, we established a second SCC using the same method and from this tested a further 17 precocious foragers. During this time, bees from the first colony had aged, and we were able to test 26 bees that had begun foraging at ≥16 days old.

Bees were selected for testing once they were observed foraging at the *ad libitum* 1 mol l^{-1} sucrose feeders. From there, individual bees were trained to enter cylindrical open-topped decision chambers (Fig. 1A,B) placed 8.6 m from the colony. These were illuminated by sunlight and covered with insect screen. The entrance was a 4 cm hole and the inner dimensions were 20×25 cm. The inside of each cylinder provided a white, UV-reflecting background for stimuli. Stimuli (‘flowers’) were 5 cm coloured (CMYK yellow, blue and green) discs with radiating chromatic ‘black’ lines. All stimuli were printed with a high-resolution laser printer on paper. Stimuli, feeding containers and sugar solutions were cleaned with ethanol and water between each learning trial and test. Bees were tested within 3 days of commencing foraging.

A differential conditioning protocol was used in training. Three artificial flowers were placed equidistant from each other at the bottom of the testing cylinder. At the centre of the rewarded colour flower, we placed 50 µl of a 2 mol l^{-1} sucrose solution. The other two flowers contained 50 µl water. Bees were first trained on one colour as rewarding for 20 trials, at which point the rewarded colour was switched so that the trained colour was now unrewarding and one of the alternative flowers was rewarding for 20 more trials. Only one bee was allowed in the decision chamber at any one time. Positions of flowers were changed pseudo-randomly for each trial.

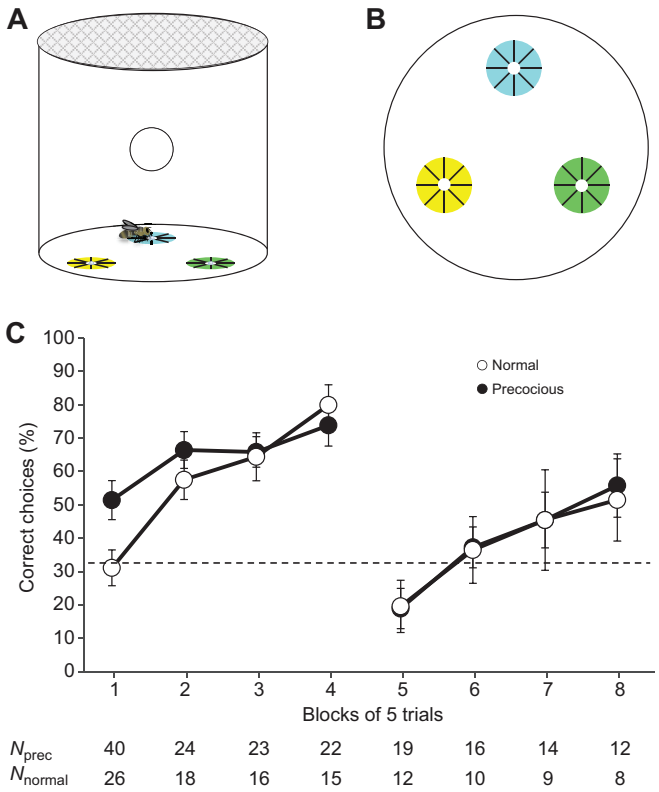

Fig. 1. Simple discrimination task set up and learning curves. (A) Training apparatus. (B) Aerial view of stimuli (‘flowers’). (C) Learning curves for initial discrimination and reversal learning for normal-aged bees and precocious foragers. Error bars represent s.e.m. The dashed line indicates the level of performance expected if bees were selecting flowers at random.

Experiment 2: comparison of performance in a spatial learning task

We established successively two SCC colonies in the same way as in experiment 1. We tested six (10–12 days old) precocious foragers from the first SCC and another six (8–11 days old) precocious foragers from the second SCC. In this experiment, we used a third colony of a similar size to the SCCs, but containing a normal demography of workers. We tested 12 foragers from this colony, whose age could not be identified, but they were presumed to start foraging at a normal age, which would be expected to be >16 days old as adults. Bees from the SCCs were selected for testing within 3 days of commencing foraging. The amount of foraging experience of bees from the normal demography colony could not be precisely determined, and may have been longer than 3 days.

The testing platform (Fig. 2A) was a 600×600 mm grey plastic square placed upon blocks 200 mm from the ground. The platform was divided into 36 equal square sections with light pencil lines. Experiments were done in the morning and afternoon (avoiding the time around noon) and care was taken to avoid any shadows on the platform during training and testing. Landmarks were spray painted (pink, green, blue) 5×7 cm cups. Bees were trained to a 5 mm diameter transparent cup containing 2 mol l^{-1} sucrose solution located next to a coloured landmark (proximal landmark) (Fig. 2A,B). Two differently coloured landmarks (distal landmarks) were always positioned so that all three landmarks formed a right-angle triangle with the sides adjacent to the right

Journal of Experimental Biology (2016) 219, 412-418 doi:10.1242/jeb.126920

angle parallel with the edges of the platform (Fig. 2B). Keeping this formation, between each trial, the feeder and landmarks were moved systematically through all possible positions on the platform without repeat. This allowed the bee to experience the feeder in 16 different positions on the platform and therefore also with respect to the background and skyline surrounding the platform, but ensuring that with each experience the feeder was in the same position with respect to the three coloured landmarks. Bees were trained and tested over 20 trials, 16 training trials and 4 test trials.

Every 5th trial, bees underwent a critical test and a control test (Fig. 2C,D), each for 30 s and alternating in order over the four test trials. During the critical test, the feeder and proximal landmark were removed (Fig. 2C). During the control test, only the feeder was removed (Fig. 2D). Each training and test trial was video recorded for analysis at 30 frames s^{-1}. The camera was suspended 77 cm directly above the platform outdoors with natural sunlight for illumination. The position of the bee in the landmark arena was continuously recorded during training trials and tests and analysed to create a density distribution of position calculated as the proportion of time each bee spent at each point in the arena (Fig. 3A–H).

RESULTS

Experiment 1: Comparison of performance in a visual reversal learning task

Normal-aged (greater than 16 days old) and precocious foragers (less than 16 days old) were trained to associate one of three differently coloured discs (flowers) with a sucrose reward (Fig. 1A,B), the other flowers offering water. Fig. 1C shows the percentage of correct choices by precocious and normal-aged foragers in each of the four, 5-trial blocks in the first learning phase (1st to 20th trial), and the second phase (21st to 40th trial) after the reversal of contingency, and the number of bees that completed each block; 30% (12 out of 40) of precocious foragers and 31% (8 out of 26) of normal-aged foragers that began the learning phase completed all 40 trials. There was no statistical difference between the completion rates (Fisher's exact test, P=1.000) and the drop-out rates of bees were similar for the two experimental groups (Fig. 1C). We noticed that most bees that dropped out of the test returned to foraging at the sucrose feeders provided to the colony in the flight cage.

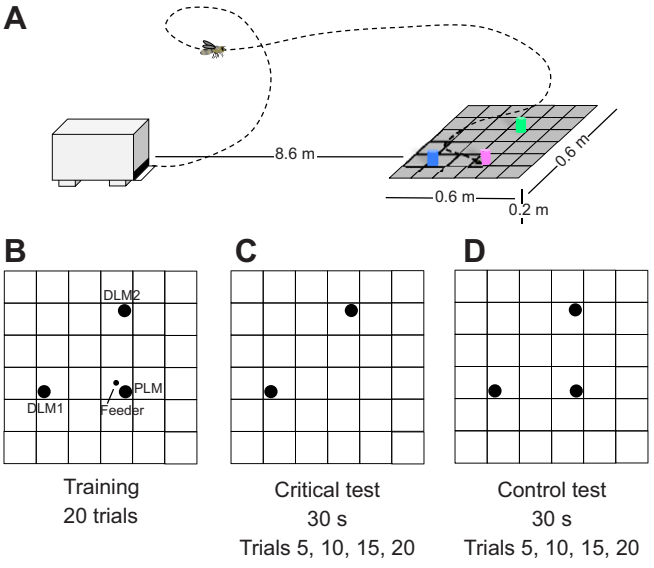

Fig. 2. Spatial learning task set up and paradigm. (A) Bees were trained to find a feeder located on a platform 8.6 m from the hive within an array of landmarks (blue, pink and green). DLM, distal landmark; PLM, proximal landmark. (B) Example position of each trial. (C) Example of landmark position in a critical test in which the feeder and proximal landmark were removed. (D) Example of landmark position in a control test in which the feeder was removed.

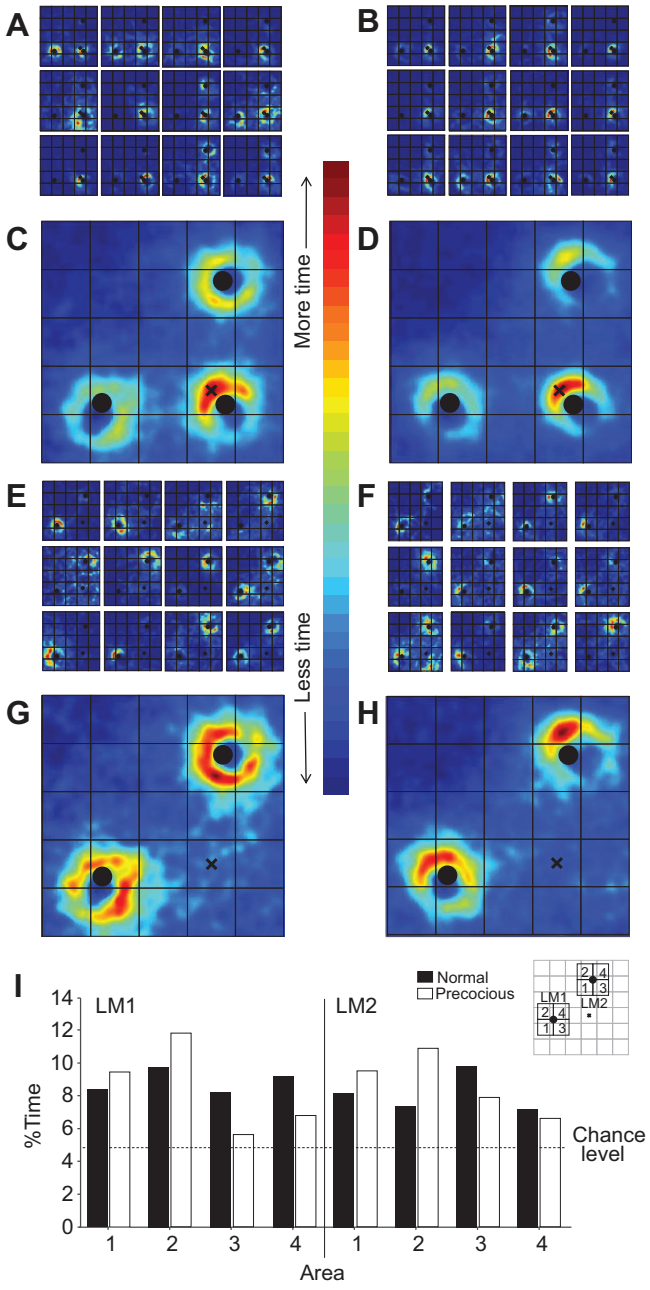

Fig. 3. Visualisation of time spent in areas around landmarks. (A) Data density plots for control tests for each normal-aged bee (4 trials pooled for each image for each bee). Red represents the greatest amount of time (see scale bar). (B) Data density plots for control tests for each precocious bee. (C) Data density plot pooling all control tests for all 12 normal-aged bees shown in A. (D) Data density plot pooling all control tests for all 12 precocious bees shown in B. (E,F) Data density plots for critical tests for each bee (E, normal-aged; F, precocious; 4 trials pooled for each image for each bee). (G) Data density plot pooling all critical tests for all normal-aged bees. (H) Data density plot pooling all critical tests for all precocious bees. (I) Percentage of time that all normal-aged bees and precocious bees spent in each quadrant around distal landmarks (LM; inset) in the critical test.

Journal of Experimental Biology

Journal of Experimental Biology (2016) 219, 412-418 doi:10.1242/jeb.126920

Table 1. Comparison of precocious and normal-aged forager performance in Experiment 1

Fixed effect	$d.f._N$	$d.f._D$	F	P
Bee type	1	64	0.47	0.4952
Block	1	1224	60.70	<0.0001
Learning phase	1	29	9.33	0.0048
Bee type×block	1	1224	0.13	0.7166
Bee type×phase	1	29	4.26	0.0481
Block×phase	1	1224	0.06	0.8075
Type×block×phase	1	1224	3.14	0.0769

Results of a generalised linear mixed model ANOVA comparing the proportion of choices for the rewarded colour of precocious and normal-aged forager bees (bee type) in successive blocks of learning trials (block) and initial and reversal learning phases of the training (phase).
$d.f._N$, numerator degrees of freedom; $d.f._D$, denominator degrees of freedom.

Table 2. Comparison of the spatial distributions of precocious and normal-aged bees in the critical test in Experiment 2

Fixed effect	$d.f._N$	$d.f._D$	F	P
Bee type	1	22	<0.01	0.9582
LM	1	22	0.01	0.9117
Area	3	66	3.04	0.0350
Bee type×LM	1	22	0.07	0.8007
Bee type×area	3	66	3.38	0.0234
LM×area	3	66	1.71	0.1733
Bee type×LM×area	3	66	0.09	0.9663

Results of a three-way repeated measures ANOVA comparing time spent within each of the four areas (area) around each of the two distal landmarks (LM) by precocious and normal-aged forager bees (bee type).

The increase in performance by both types of bees with successive learning trials indicated the success of the colour learning (Fig. 1C). The sudden fall in performance immediately after the change of the rewarded colour (21st to 25th trial) indicated that initially bees persisted with the colour learned in the first phase, but were able to learn a new preference and recovered their performance level over the next 15 learning trials.

To compare the performance of precocious and normal-aged foragers in the test, we employed a generalised linear mixed-model analysis of variance (ANOVA) for binary data (Proc GLIMMIX, SAS 9.4) with a binomial error distribution and a logit link function, and a maximum likelihood procedure based on a Gauss–Hermite quadrature rule to estimate variables. As noted above, only eight normal-aged and 12 precocious foragers completed the full 40 trials: the test we chose is able to cope with missing values caused by bees dropping out partway through the test. The test model included bee type (normal/precocious), block (1st to 4th) and learning phase (initial/reversal) and their interactions between these factors as fixed factors and bee as a random factor. Table 1 summarises the outcome of the model. There was no significant difference between normal-aged and precocious foragers. Learning phase explained a significant amount of variation in the model, suggesting that performance in the first phase (initial learning) differed from that in the second phase (reversal learning) and perhaps that the initial learning slowed the reversal learning. The interaction between bee type and learning phase was of borderline significance (Table 1, P=0.048). We conducted *post hoc* contrast tests to examine the simple main effect of learning phase in each bee type. The effect of learning phase was significant in the precocious foragers ($F_{1,29}$=16.70, P<0.001) but not in the normal-aged foragers ($F_{1,29}$=0.40, P=0.532). Inspection of Fig. 1C suggests this difference may be due to the relatively smaller change in performance level of the precocious foragers in the first phase compared with the second phase, whereas normal-aged foragers showed a similarly strong change in performance in the two phases. The smaller change in performance of precocious foragers in the first phase in turn stemmed from their higher performance in the first block of training.

Small sample sizes, in general, increase the chance of Type II error and may favour a false null hypothesis; hence, we performed a Bayesian analysis to evaluate the likelihood of a false-negative result. The Bayesian information criterion (BIC) of the model adopted for the ANOVA (Table 1) was 1674.92. We created a new generalised linear mixed-model ANOVA for the same data set but implementing a model in which only block and learning phase and their interaction were included and bee type was excluded. The BIC of this model was 1664.90. The smaller BIC value of this model suggests that excluding the effect of bee type from the model explained the current data set better. Consequently, this analysis supports the conclusion that there was no actual difference in learning performance between the normal and the precocious foragers.

Experiment 2: comparison of performance in a spatial learning task

Normal-aged and precocious foragers were trained to find a small sucrose feeder placed within an array of three small landmarks (Fig. 2A,B). In tests, either the feeder was removed (control test; Fig. 2D) or the feeder and its proximal landmark were removed (critical test; Fig. 2C). In the critical test, it appeared as though precocious foragers were concentrating their time in the area of the

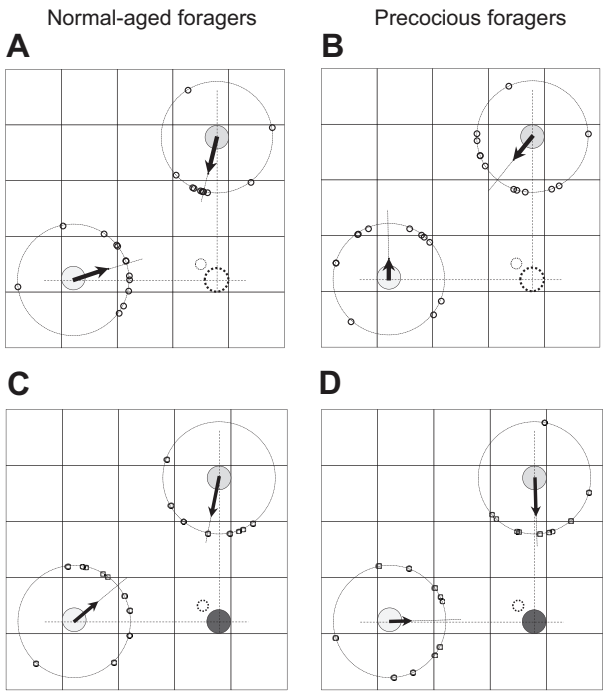

Fig. 4. Direction of flight in distal landmark areas during tests. The mean vector on departure from the landmark (large dotted circle circumscribing 90 mm distance from each landmark) for each bee (small open circles) and average vector when exiting the area (arrow) for: (A) all normal-aged bees during critical tests; (B) precocious bees during critical tests; (C) normal-aged bees during control tests; and (D) precocious bees during control tests. Arrows indicate the mean direction of all the mean vectors for each data set, and their length indicates the variance of the directions (the longer arrow, the less variable the direction).

Journal of Experimental Biology (2016) 219, 412-418 doi:10.1242/jeb.126920

distal landmarks at the point where the feeder might have been located relative to each landmark (Fig. 3H), whereas normal-aged foragers were spending their time more uniformly searching around the distal feeders (Fig. 3G).

To compare the spatial distributions of precocious and normal-aged bees in the critical test, we conducted a three-way repeated-measures ANOVA. Landmark and area (the quadrants surrounding each landmark; Fig. 3I) were within-subject factors and bee type was a between-subject factor. The results are summarised in Table 2. Only the main effect of area and the interaction between bee type and area were statistically significant (Table 2). A subsequent test for the simple main effects of area for each bee type revealed that the effect of the area was significant only for the precocious group ($F_{3,66}$=6.24, P<0.001), but not for the normal group ($F_{3,66}$=0.18, P=0.913). Thus, we conducted multiple comparisons with Bonferroni correction of the significance level (α=0.008) to compare between every pair of four areas (six pairs in total), revealing that the time spent in area 2 (hypothetical feeder position) was significantly longer than that in areas 3 and 4 (t_{66}=3.59, 3.63, respectively, P<0.001) for the precocious foragers (Fig. 3I). In summary, these statistics support the inference drawn from inspection of Fig. 3G,H that in critical tests, normal-aged foragers spent equal time searching around the distal feeders, whereas precocious foragers concentrated their search at the point at each distal feeder equivalent to where the feeder had been located relative to the proximal feeder.

We used circular statistics to further analyse the movements of bees in the critical and control tests. We analysed the vectors of bees on departure from (passing >90 mm away) one of the distal landmarks. For each bee, we calculated the mean vector from all vectors across all tests. Fig. 4 shows the results of the critical test for normal-aged foragers (Fig. 4A) and precocious foragers (Fig. 4B). Note that several bees from each group did not approach the distal landmarks at all, and so the number of open circles is less than 12. In Fig. 4, the arrows indicate the mean directions for the precocious and normal-aged forager groups, and their length indicates the variance of the directions (the longer the arrow, the less variable the direction).

In the critical test condition, the proximal landmark was removed (Fig. 4A) and normal-aged foragers appeared to leave the distal landmarks on a vector that would have taken them closer to the location of the proximal landmark than for precocious foragers in the same test (Fig. 4B). A Rayleigh V-test for the directional uniformity revealed that the concentration of the flight directions with respect to the proximal landmark direction was statistically significant for normal-aged foragers for both LM1 (u=3.05, P<0.001) and LM2 (u=3.02, P<0.001) and for precocious forager for LM2 (u=2.03, P=0.020), but not significant (i.e. uniform around the landmark) for precocious foragers for LM1 (u=0.02, P=0.507). Because these Rayleigh V-tests suggested that both normal-aged and precocious foragers were statistically significantly heading for the proximal landmark direction from LM2, we conducted a Var-test (Julle-Daniere et al., 2014) to examine differences in directional spread between groups, and found that the two groups differed significantly in the uniformity of the flight direction from LM2 (P=0.037). The distribution of flight direction was more scattered in precocious foragers than in normal-aged foragers (Fig. 4A,B). In summary, these analyses showed that normal-aged foragers departed from the distal landmarks more closely to the direction of the removed proximal landmarks than did the precocious foragers.

In the control test condition, the proximal landmark was present, but the feeder was not. In this condition, there was no difference between the mean vectors of normal-aged and precocious foragers on departure from the distal landmarks (Fig. 4C,D). A Rayleigh V-test revealed that the concentration of the flight direction with the respect to the proximal landmark direction was statistically significant for both normal and precocious foragers for both LM1 (normal: u=1.87, P=0.031; precocious: u=1.80, P=0.036) and LM2 (normal: u=2.77, P=0.002; precocious: u=3.08, P<0.001). This suggests that when the proximal landmark was still visible in the control test in the same way as in the training trials, both precocious and normal-aged foragers oriented towards the proximal landmark from the distal landmarks with similar accuracy and precision.

DISCUSSION

As far as we know, this is the first evidence for a difference in cognitive ability between precocious and normal-aged forager bees, suggesting a subtle deficit in the capacity of precocious foragers to adapt to changes in learned landmark information.

Honeybees learn readily to search in lab set ups proffering an array of landmarks (Cartwright and Collett, 1983), and can cope with various transformations of the landmark array, including the removal of a landmark from the training array. The performance of precocious bees in this study shows that they too can learn to search with respect to an array. It is when the array was transformed by having the proximal landmark removed that differences in performance from those of normal-aged bees were found. In the absence of the proximal landmark, precocious foragers focused their search around the distal landmarks (Fig. 3H). Their lack of directed flights from distal landmarks to the goal may indicate a deficit in coding sensorimotor vectors (Collett and Baron, 1995; Collett and Rees, 1997) that connect different locations in space.

If this difference were also seen at the typical foraging ranges of honey bees (rather than the reduced spatial scale of our assay), it would suggest precocious foragers may not perform well in estimating locations of forage sites or their home nest if their learned navigational cues have changed. Environments are rarely stable, and this is especially true of modern agricultural environments in which locations and features of bee yards are frequently changed and even landscape-scale landmarks such as fields of crops can be suddenly removed. With the rich knowledge gathered so far on honey bee navigation (Srinivasan, 2011), it is certainly worth comparing precocious and normal-aged foragers on more navigational tasks, and at more realistic spatial scales.

Several prior studies have identified differences in learning ability between young and old bees, but the results have been inconsistent. Ray and Ferneyhough (1997) reported bees less than 6 days old as adults performed poorly in simple olfactory learning with the classic proboscis extension response training, and performance levels did not stabilise until bees were 10 days old. During the first 10 days of adult life the olfactory system of the honey bee is still maturing, which may certainly influence performance in learning assays (Allan et al., 1987; Masson and Arnold, 1984; Morgan et al., 1998), and the rate of maturation can be influenced by several factors including the condition of the colony and the behavioural state of the individual (Morgan et al., 1998; Ray and Ferneyhough, 1999). Whereas young nurses showed poorer learning performance than older foragers, these differences were attributed more to behavioural state than to age because in simple olfactory associative learning, forager bees performed better than same-aged nurse bees regardless of age (Ray and Ferneyhough, 1999). This may in part be explained by the lower responsiveness to sucrose of young nurses compared with older foragers (Pankiw and Page, 1999), as sucrose responsiveness is related to learning

performance (Scheiner et al., 2005). We note that Ben-Shahar et al. (2000) found faster extinction of an association between odour and sucrose reward in nurses than in foragers, but this may reflect weaker learning of the initial contingency in nurses than in foragers.

Age-related changes in cognitive performance of foragers have been reported in terms of a decline in the cognitive performance in older foragers that have accumulated more than 2 weeks of foraging experience (Behrends and Scheiner, 2010; Behrends et al., 2007; Scheiner and Amdam, 2009). This is interpreted as senescence caused more by the accumulated demands of foraging than chronological age, and is evidence that prolonged foraging causes cognitive ageing in a bee (Behrends and Scheiner, 2010; Behrends et al., 2007; Scheiner and Amdam, 2009). In our experiments, foraging experience was not precisely controlled and therefore could cognitive decline explain the difference in performance between precocious and normal-aged foragers? We cannot fully exclude this possibility, but we consider it unlikely. Cognitive decline has only been reported in bees that have accumulated more than 2 weeks of foraging experience (Behrends et al., 2007; Scheiner and Amdam, 2009), which is longer than the average life expectancy for most forager bees (Dukas, 2008; Woyciechowski and Moron, 2009). In experiment 1, however, all bees were sampled within their first 3 days of foraging. In experiment 2, precocious foragers had $\leq$3 days foraging experience, but we did not know how much foraging experience bees from the normal demography colony had had before they were tested. In this experiment, the bees from the normal demography colony outperformed the precocious foragers, which is opposite to the predicted outcome assuming cognitive decline. As far as we know, this is the first evidence for lower cognitive performance in young and precocious foragers compared with normal-aged foragers.

Research suggests several phases to the cognitive life of a honey bee: an early phase during which sensory systems are still maturing (Allan et al., 1987; Masson and Arnold, 1984; Morgan et al., 1998), a middle phase when bees can perform at their cognitive best, and a later phase during which cognitive decline may occur (Behrends and Scheiner, 2010; Behrends et al., 2007; Scheiner and Amdam, 2009). Our data suggest that bees in the first 2 weeks of adult life have not developed the full cognitive capacity of normal-aged foragers, with the implication being that cognitive development is still ongoing during the first 2 weeks after adult emergence. It is possible the bee emerges from the pupal stage with its brain only developed to a stage of being able to navigate and operate in the safe, predictable and simple hive environment. The cognitive capacity of a bee on emergence may be more than sufficient for the safe, predictable, spatially limited and ordered environment inside the hive, and the bee would be able to contribute to the hive economy through nursing jobs. The brain may need a period of further development during adult life before being able to fully process the complex world outside the hive and cope with foraging. It is also possible that the bee brain may need sensory and/or spatial experience in order to complete cognitive development. Quite fine-scaled analyses of the impact of chronological age on cognitive ability would be needed to explore the true complexity of these cognitive phases.

The nature of this brain development is presently unclear. It is certainly the case that the honey bee mushroom body calyx continues growth and development throughout adult life, and accelerates in growth during foraging (Farris et al., 2001; Withers et al., 1993). The honey bee mushroom body receives visual input (Mobbs, 1982), but it is not clear that the visual input to the mushroom body preserves spatial structure. It is more likely that allocentric and egocentric visual motion and celestial navigational cues are processed by the central complex (Pfeiffer and Homberg, 2014; Plath and Barron, 2015; Seelig and Jayaraman, 2015).

The behavioural differences we have seen could contribute to the poor performance of precocious foragers while foraging (Chang et al., 2015; Perry et al., 2015). Reduced performance in spatial memory could be a factor leading to the very high loss rates seen in precocious foragers (Chang et al., 2015; Perry et al., 2015). Performance in a short-range (660 m) navigation task was found not to differ between precocious and normal-age foragers (Chang et al., 2015), but at this short distance bees may have been able to see landmarks indicating the position of the hive and use these 'beacons' as stable navigational references by which to return home. In our study, precocious foragers performed as well as normal-aged foragers in visual reversal learning (Fig. 2) and learning landmarks (Fig. 4C,D), but it was in the more complex task of compensating for a change in the spatial arrangement of landmarks that a difference between precocious foragers and normal-aged foragers was seen (Fig. 4A,B). Learning spatial arrangements between familiar landmarks would aid bees in reacting to changes in their environment. Our data suggest that it is under these conditions that precocious foragers may be more prone to becoming lost. But further comparisons are needed on the large scale of travel that forager honey bees naturally undertake.

Acknowledgements
This work was supported by an Endeavour Scholarship awarded to C.J.P.

Competing interests
The authors declare no competing or financial interests.

Author contributions
T.U., C.J.P., K.C. and A.B.B. designed the experiment. T.U. and C.J.P. carried out data collection and analysis. T.U., C.J.P., K.C. and A.B.B. wrote the paper.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References
Allan, S. A., Slessor, K. N., Winston, M. L. and King, G. G. S. (1987). The influence of age and task specialization on the production and perception of honey bee pheromones. *J. Insect Physiol.* **33**, 917-922.
Becker, L. (1958). Untersuchungen über das Hemifindevermogen der bienen. *Z. Vgl. Physiol.* **41**, 1-25.
Behrends, A. and Scheiner, R. (2010). Learning at old age: a study on winter bees. *Front. Behav. Neurosci.* **4**, 15.
Behrends, A., Scheiner, R., Baker, N. and Amdam, G. V. (2007). Cognitive aging is linked to social role in honey bees (*Apis mellifera*). *Exp. Gerontol.* **42**, 1146-1153.
Ben-Shahar, Y., Thompson, C. K., Hartz, S. M., Smith, B. H. and Robinson, G. E. (2000). Differences in performance on a reversal learning test and division of labor in honey bee colonies. *Anim. Cogn.* **3**, 119-125.
Bloch, G., Toma, D. P. and Robinson, G. E. (2001). Behavioral rhythmicity, age, division of labor and period expression in the honey bee brain. *J. Biol. Rhythms* **16**, 444-456.
Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., Edwards, A. S., Martin, A., Robinson, G. E., Poppy, G. M. et al. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature* **403**, 537-540.
Cartwright, B. A. and Collett, T. S. (1983). Landmark learning in bees. *J. Comp. Physiol. A* **151**, 521-543.
Chang, L.-H., Barron, A. B. and Cheng, K. (2015). Effects of the juvenile hormone analogue methoprene on rate of behavioural development, foraging performance and navigation in honey bees (*Apis mellifera*). *J. Exp. Biol.* **218**, 1715-1724.
Collett, T. S. and Baron, J. (1995). Learnt sensori-motor mappings in honeybees: interpolation and its possible relevance to navigation. *J. Comp. Physiol. A* **117**, 287-298.
Collett, T. S. and Rees, J. A. (1997). View-based navigation in Hymenoptera: multiple strategies of landmark guidance in the approach to a feeder. *J. Comp. Physiol. A* **181**, 47-58.

Correa-Fernandez, F. and Cruz-Landim, C. (2010). Differential flight muscle development in workers, queens and males of the eusocial bees, *Apis mellifera* and *Scaptotrigona postica*. *J. Insect Sci.* **10**, 85.
Dukas, R. (2008). Mortality rates of honey bees in the wild. *Insectes Soc.* **55**, 252-255.
Fahrbach, S. E., Giray, T. and Robinson, G. E. (1995). Volume changes in the mushroom bodies of adult honey bee queens. *Neurobiol. Learn. Mem.* **63**, 181-191.
Farris, S. M., Robinson, G. E. and Fahrbach, S. E. (2001). Experience-and age-related outgrowth of the intrinsic neurons in the mushroom bodies of the adult worker honeybee. *J. Neurosci.* **21**, 6395-6404.
Free, J. B. (1961). Hypopharyngeal gland development and division of labor in honey-bee (*Apis mellifera*) colonies. *Proc. R. Entomol. Soc. Lond. A* **36**, 5-8.
Fukuda, H. and Sakagami, S. (1968). Worker brood survival in honeybees. *Res. Popul. Ecol.* **10**, 31-39.
Goblirsch, M., Huang, Z. Y. and Spivak, M. (2013). Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. *PLoS ONE* **8**, e58165.
Groh, C., Lu, Z., Meinertzhagen, I. A. and Rössler, W. (2012). Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. *J. Comp. Neurol.* **520**, 3509-3527.
Gronenberg, W., Heeren, S. and Holldobler, B. (1996). Age-dependent and task-related morphological changes in the brain and the mushroom bodies of the ant *Camponotus floridanus*. *J. Exp. Biol.* **199**, 2011-2019.
Harrison, J. M. (1986). Caste-specific changes in honeybee flight capacity. *Physiol. Zool.* **59**, 175-187.
Herold, R. C. (1965). Development and ultrastructural changes of sarcosomes during honey bee flight muscle development. *Dev. Biol.* **12**, 269-286.
Higes, M., Martín-Hernández, R., Botías, C., Bailón, E. G., González-Porto, A. V., Barrios, L., del Nozal, M. J., Bernal, J. L., Jiménez, J. J., Palencia, P. G. et al. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environ. Microbiol.* **10**, 2659-2669.
Huang, Z.-Y. and Robinson, G. E. (1996). Regulation of honey bee division of labor by colony age demography. *Behav. Ecol. Sociobiol.* **39**, 147-158.
Ismail, N., Robinson, G. E. and Fahrbach, S. E. (2006). Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proc. Natl. Acad. Sci. USA* **103**, 207-211.
Janmaat, A. F. and Winston, M. L. (2000). The influence of pollen storage area and *Varroa jacobsoni* Oudemans parasitism on temporal caste structure in honey bees (*Apis mellifera* L.). *Insectes Soc.* **47**, 177-182.
Jeanne, R. L. (1986). The evolution of the organisation of work in social insects. *Monit. Zool. Ital.* **20**, 119-133.
Julle-Daniere, E., Schulthesis, P., Wystrach, A., Schwarz, S., Nooten, S.S., Bibost, A.-U. and Cheng, K. (2014). Visual matching in the orientation of desert ants (*Melophorus bagoti*): the effect of changing skyline height. *Ethology* **120**, 783-792.
Masson, C. and Arnold, G. (1984). Ontogeny, maturation and plasticity of the olfactory system in the workerbee. *J. Insect Physiol.* **30**, 7-14.
Menzel, R. (2001). Searching for the memory trace in a mini-brain, the honeybee. *Learn. Mem.* **8**, 53-62.
Menzel, R. and Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends Cogn. Sci.* **5**, 62-71.
Mobbs, P. G. (1982). The brain of the honeybee *Apis mellifera*. L. The connections and spatial organization of the mushroom bodies. *Philos. Trans. R. Soc. B Biol. Sci.* **298**, 309-354.
Morgan, S. M., Butz Huryn, V. M., Downes, S. R. and Mercer, A. R. (1998). The effects of queenlessness on the maturation of the honey bee olfactory system. *Behav. Brain Res.* **91**, 115-126.
Pankiw, T. and Page, R. E., Jr. (1999). The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A* **185**, 207-213.
Perry, C. J., Søvik, E., Myerscough, M. R. and Barron, A. B. (2015). Rapid behavioral maturation accelerates failure of stressed honey bee colonies. *Proc. Natl. Acad. Sci. USA* **112**, 3427-3432.
Pfeiffer, K. and Homberg, U. (2014). Organization and functional roles of the central complex in the insect brain. *Annu. Rev. Entomol.* **59**, 165-184.
Plath, J. A. and Barron, A. B. (2015). Current progress in understanding the functions of the insect central complex. *Curr. Opin. Insect Sci.* **12**, 11-18.
Ray, S. and Ferneyhough, B. (1997). The effects of age on olfactory learning and memory in the honey bee *Apis mellifera*. *Neuroreport* **8**, 789-793.
Ray, S. and Ferneyhough, B. (1999). Behavioral development and olfactory learning in the honeybee (*Apis mellifera*). *Dev. Psychobiol.* **34**, 21-27.
Robinson, G. E., Page, R. E. and Huang, Z.-Y. (1994). Temporal polyethism in social insects is a developmental process. *Anim. Behav.* **48**, 467-469.
Rueppell, O., Bachelier, C., Fondrk, M. and Page, R. E. Jr. (2007). Regulation of life history determines lifespan of worker honey bees (*Apis mellifera* L.). *Exp. Gerontol.* **42**, 1020-1032.
Sakagami, S. F. and Fukuda, H. (1968). Life tables for worker honeybees. *Res. Popul. Ecol.* **10**, 127-139.
Scheiner, R. and Amdam, G. V. (2009). Impaired tactile learning is related to social role in honeybees. *J. Exp. Biol.* **212**, 994-1002.
Scheiner, R., Kuritz-Kaiser, A., Menzel, R. and Erber, J. (2005). Sensory responsiveness and the effects of equal subjective rewards on tactile learning and memory of honeybees. *Learn. Mem.* **12**, 626-635.
Schulz, D. J., Huang, Z.-Y. and Robinson, G. E. (1998). Effects of colony food shortage on behavioral development in honey bees. *Behav. Ecol. Sociobiol.* **42**, 295-303.
Seeley, T. D. (1995). *The Wisdom of the Hive*. Cambridge: Harvard University Press.
Seelig, J. D. and Jayaraman, V. (2015). Neural dynamics for landmark orientation and angular path integration. *Nature* **521**, 186-191.
Søvik, E., Perry, C. J., LaMora, A., Barron, A. B. and Ben-Shahar, Y. (2015). Negative impact of manganese on honeybee foraging. *Biol. Lett.* **11**, 20140989.
Srinivasan, M. V. (2011). Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics. *Physiol. Rev.* **91**, 413-460.
Stieb, S. M., Hellwig, A., Wehner, R. and Rössler, W. (2012). Visual experience affects both behavioral and neuronal aspects in the individual life history of the desert ant Cataglyphis fortis. *Dev. Neurobiol.* **72**, 729-742.
Tofilski, A. (2002). Influence of age polyethism on longevity of workers in social insects. *Behav. Ecol. Sociobiol.* **51**, 234-237.
Toth, A. L. and Robinson, G. E. (2005). Worker nutrition and division of labour in honeybees. *Anim. Behav.* **69**, 427-435.
Vance, J. T., Williams, J. B., Elekonich, M. M. and Roberts, S. P. (2009). The effects of age and behavioral development on honey bee (*Apis mellifera*) flight performance. *J. Exp. Biol.* **212**, 2604-2611.
Visscher, P. K. and Dukas, R. (1997). Survivorship of foraging honey bees. *Insectes Soc.* **44**, 1-5.
Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge: Harvard University Press.
Withers, G. S., Fahrbach, S. E. and Robinson, G. E. (1993). Selective neuroanatomical plasticity and division of labour in the honeybee. *Nature* **364**, 238-240.
Woyciechowski, M. and Moroń, D. (2009). Life expectancy and onset of foraging in the honeybee (*Apis mellifera*). *Insectes Soc.* **56**, 193-201.