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Return of drones: flight experience improves returning performance in honeybee drones

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Abstract

The effect of experience on the behavior of worker bees has been extensively investigated; however, few such studies have been conducted on male bees. Honeybee (*Apis mellifera*) males (drones), unlike the males of other social hymenopterans, return to their nest after performing a mating flight and have, therefore, an opportunity to learn from their experiences. This provides a chance to understand the significance of experience in social hymenopteran males. Here, we investigated whether experience improves the returning performance in drones (rate and time of return to the hive). We compared the returning performance of "Experienced" drones that were allowed to fly freely and thus had an opportunity to learn the position of the hive before the experiment with "Naive" drones that were not allowed to fly and therefore, had no opportunity to learn. We found that Experienced drones returned to the hive after a displacement, whereas Naive drones did not. Furthermore, time to return decreased with the age of drones. These results suggest that flight experience improves the returning performance, which should increase the possibility of mating success and overall colony fitness.

Keywords: Honeybee; drone; social hymenoptera; age; flight experience

Introduction

Based on prior experiences, organisms can alter and improve their behavior such that it is better suited in a given context. Behavioral changes also increase the robustness, survival and reproduction of an individual (Shettleworth 2001; Dukas 2004; Dukas 2013). These effects indicate that behavioral alteration due to prior experience may be crucial for these organisms.

Honeybees, *Apis mellifera*, have frequently been a subject for investigation of the effect of prior experience on behavior. Most of these studies have been conducted on honeybee workers, and have shown that prior experience improves the performance of tasks, including foraging, orientation and nest-mate recognition (Gould and Gould 1988; Richter and Waddington 1993; Menzel and Müller 1996; Capaldi et al. 2000). Studies have shown that honeybee males (drones) possess strong learning abilities under laboratory conditions (Bitterman et al. 1983; Benatar et al. 1995), as well as bumblebee males (Wolf and Chittka 2016). These studies imply that drones can reflect prior experience in their behavior. However, few investigations have been performed to assess whether the experiences affect the behavioral changes in drones because males of social hymenopterans generally do not return once they leave their nest (Hölldobler and Wilson 1990; Goulson 2003; O'Donnell and Beshers 2004).

Honeybee drones, unlike the males of other social hymenopterans, fly many times in their lives (Oertel 1956; Witherell 1971). Drones initiate flight 8 days after emergence (Howell and Usinger 1933; Ruttner 1966; Rueppell et al. 2006), and initiate mating flights when they are sexually mature 4 days later (Ruttner 1966; Winston 1987). Some tens of thousands of drones assemble in a drone congregation area (DCA) during the mating flight, where they compete for mating opportunities (Ruttner 1966; Koeniger et al. 2005). Drones die immediately after mating, or return to their nest if they fail to mate. The drones that return fuel up in the nest, because they cannot survive for long in the field, and then perform another mating flight.

It is likely that drones memorize the location of the nest, including that of the surrounding environment, during the flight experience, as previously demonstrated for workers (Becker 1958; Capaldi and Dyer 1999; Menzel et al. 2006; Degen et al. 2016). However, it has not been determined whether the flight experiences affect the rate and time of return to the nest (returning performance) in drones. In the present study, we investigated the returning performance of drones that had flight experience versus those that did not, to examine the effects of experience. Furthermore, we investigated how the returning performance changes with age because drones repeatedly fly. Thus, we hypothesized that as flight experience increases with age, drones would return to the nest more efficiently (more often and faster), as reported for workers (Becker 1958; Capaldi and Dyer 1999; Capaldi et al. 2000).

Materials and methods

Experimental Setup

Honeybee (*A. mellifera*) colonies reared at Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan were used. Empty drone combs were introduced into queenright colonies with 20,000–32,000 workers after which, the queens laid male eggs in the combs. The drone combs were removed from the colonies 3 days before the drones emerged, and were placed in an incubator at 33 °C until emergence. To age the drones, we gave individuals markings on the thorax and abdomen using paint (Mitsubishi Co. Ltd., Tokyo, Japan) within 24 h of emergence. Drones were introduced into genetically unrelated queenright colonies. The hive that drones were introduced into was two-storied with horizontal queen excluders. Drones that were introduced into the hive above the queen excluders could not fly out of the hive and were classified as the Naive group. The other drones were introduced below the queen excluders, and could fly out freely, and were classified as the Experienced group. Drones were kept in the colonies until they were used in the experiments described below. Brood, pollen and honey were provided equally to both the groups. Queens were placed below the excluders.

Experiment 1. Effect of Flight Experience and age on the Return of Drones to the Hive

Drones were sampled from the colonies at the age of 8–10 days, 12 days and 15 days. We used 3 colonies for the experiments. Sample sizes are shown in Table 1. Drones were provided with 50% sucrose solution before the experiment. A numbered tag was attached to the thorax of each drone for identification. We placed queen excluders to catch drones that reached entrance of the hive. Then, the drones were released 200 m away from the hives. We measured the return rate and time to return to their hives. The drones that returned were removed from the entrance of the hive. The experiments were performed between 11:00 and 13:00. Naive and Experienced drones were released separately on the same days. This experiment was conducted over several days (3–4 days) for each age group.

Experiment 2. Effect of Flight Experience and age on the Duration of Orientation

Drones were collected on day 12 or 15 post-emergence (12 days: $N = 21$, $N = 23$; 15 days: $N = 18$, $N = 22$, Naive and Experienced groups, respectively). We used 2 colonies for this experiment. Bees make an arc in the air after a displacement. The bees gradually increase the size of the arc to determine the direction to their hive, and disappear from sight (Capaldi and Dyer 1999). In this experiment, we measured the duration that drones spent making arcs (orientation) in the air at the release point. A piece of colored cellophane tape (3.0 cm × 0.5 cm) was attached to the dorsal surface of the abdomen of each drone to help an observer to see the flight path within a distance of about 70 m. We released the drones from the same point as in Experiment 1. We measured time to return as showed in experiment1. Naive and Experienced drones were released separately on the same day. This experiment was conducted over several days (2–3 days) for each age group.

Statistical Analysis

We analyzed the combined effects of flight experience and age on the return rate using a generalized linear mixed model (GLMM). The success or failure of return, which we treated the family assuming binomial distribution with a logit link function, were assigned as response variables; flight experience and age were assigned as explanatory variables. The colony origin of the drones and date of the experiment were treated as random factors. Multiple comparisons were performed for each age category in the Experienced group using GLMM, and the *P*-values were corrected using the Bonferroni method.

Similar analyses were conducted to understand the effect of flight experience on the return rate in each age category, the effect of age on time to return, and the effects of flight experience and age on the duration of orientation using GLMM. We treated the family assuming Gamma distribution with an identity link function to analyze time to return, and a Gamma distribution with a log link function to analyze the duration of orientation.

We also used a GLMM to analyze how the duration of orientation and age affected the time to return. We treated the family assuming Gamma distribution with a log link function in this analysis. The colony origin of the drones and date of experiment were treated as random factors. The approximate curve was predicted based on the fitted model.

We used R 3.2.3 with the Lme4 package to perform the GLMM. *P*-values were calculated using likelihood ratio tests.

Results

Experiment 1. Effect of Flight Experience and age on the Return of Drones to the Nest

In the Experienced group, 12 out of the 39 individuals (31%) that were 8–10 days, 34 out of the 36 individuals (94%) that were 12 days, and 24 out of the 25 individuals (96%) that were 15 days returned to their hives (Fig. 1, Table 1). None from the Naive group returned to their hives (Table 1). The return rate in the Experienced group was, therefore, significantly higher than in the Naive group (GLMM: flight experience: $df = 1, \chi^2 = 169.830, P < 0.001$; results of analyses for each age are summarized in Table 1). In the Experienced group, the return rate significantly increased with age (GLMM: age: $df = 1, \chi^2 = 6.229, P = 0.012$; Fig. 1, see Table 2 for details). The return rates of drones that were 12 and 15 days were not significantly different to each other (see Table 2 for details). The time taken for drones to return to the hive significantly decreased with age in the Experienced group (GLMM: age: $df = 1, \chi^2 = 9.814, P = 0.002$, Fig. 2; see Table 3 for details).

Experiment 2. Effect of Flight Experience and age on the Duration of Orientation

In the Experienced group, 16 out of the 23 drones that were 12 days (70%) and 19 out of the 22 drones that were 15 days (86%) returned to the hive. None from the Naive group returned to the hive, as in Experiment 1. Drones made an arc in the air after a displacement. In the Experienced group, drones made an arc in the air, using the release point as the center, and then they immediately departed from the release point. Naive drones made an arc in the air, initially using the release point as the center, but then the center of the arc gradually shifted from the release point.

The duration of orientation in the Experienced group was shorter than that in the Naive group. Both age and flight experience had significant effects on the duration of orientation (GLMM: flight experience: $df = 1, \chi^2 = 81.884, P < 0.001$; GLMM: age: $df = 1, \chi^2 = 15.139, P < 0.001$; Fig. 3). There was a significant interaction between these 2 parameters (GLMM: interaction: $df = 1, \chi^2 = 5.657, P = 0.017$). Thus, we separately analyzed the effect of age and flight experience in relation to the duration of orientation. The duration of orientation showed a marginally significant decline with age in the Experienced group (GLMM: age: $\text{df} = 1, \chi^2 = 3.735, P = 0.053$), although there was no significant in Naive group (GLMM: age: $\text{df} = 1, \chi^2 = 0.056, P = 0.813$). The duration of orientation in the Experienced group was significantly shorter than in the Naive group for each age (GLMM: 12 day: df = 1, χ^2 = 28.907, P < 0.001; 15 day: df = 1, χ^2 = 36.809, P < 0.001).

Effect of age on time taken to return to the hive was not significant in Experienced group (GLMM: age df = 1, χ^2 = 0, P = 0.996; 12 days: 222 ± 57 s; 15 days: 151 ± 17 s, mean ± SE). Time to return increased with duration of orientation (GLMM: df = 1, χ^2 = 4.406, P = 0.036; Fig. 4).

Discussion

To understand how experience affects the returning performance of honeybee drones, we investigated whether flight experience affects the duration of orientation until drones leave the release point and returning performance. We found that orientation and returning performance were enhanced by flight experience, with Naive drones failing to return to their hives and spending significantly more time in orientation. Orientation and returning behavior also improved with age in the Experienced group, Because flight experience increases with age (Witherell 1971), these improvements were probably correlated with age. Our findings suggest that flight experience is crucial for honeybee drones to return to their hives from an unexpected release site.

The effects of flight experience on the proportion of drones that returned to the nest and how long they take are consistent with previous results for workers. The effective returning requires memorizing the geographic features and landscapes around the hive during repeated flights in workers (Capaldi and Dyer 1999; Capaldi et al. 2000), which increases the return rate and decreases the time to return, although an innate response is also used (Dyer and Dickinson 1994). Furthermore, the returning performance improves with age in workers (Becker 1958). In this study, drones improved their ability to return to the nest after flight experience, indicating that drones, like workers, may need to memorize their surroundings to return to their hives.

A study using radar clearly showed that workers search the area surrounding the release point after a displacement, and then fly toward their hives (Menzel et al. 2005). Probably, bees need to orientate themselves after a displacement to identify environmental cues, such as landmarks, to travel in the correct direction from the release point to the hive. Such cues are acquired during flight experience (Menzel et al. 2000; Menzel et al. 2005; Degen et al. 2016). In this study, the duration of orientation was shorter for Experienced drones than for Naive drones, with duration also decreasing with age. Naive drones might need more time to orientate because they were not able to find the necessary environmental cues to return after a displacement. Similarly, younger drones required longer for orientation after a displacement than older drones, which might also be because older drones have had the opportunity to assimilate more information about their surroundings. Thus, a combination of flight experience and age improve orientation, due to greater familiarity (or experience) with the surroundings at the release point.

Experience and development related to age might cause physiological changes to the navigational abilities used for returning as previously shown for workers (Meinertzhagen 2001). The mushroom bodies are the major brain centers for learning and memory. These bodies noticeably change during the behavioral development of adult workers (Withers et al. 1993). More learning and memory are required when the workers shift from nursing to foraging, with the volume of mushroom body neuropils increasing during this transformation; thus, these changes are probably associated with the cognitive demands (Brandon and Coss 1982; Withers et al. 1993; Durst et al. 1994). Drones also exhibit an increase in neuropils in the mushroom bodies, with this change largely coinciding with the onset of flying activity (they initiate flight at 8 days); thus, drones have the ability to meet the cognitive demands of their life history requirements (Fahrbach et al. 1997). These changes to the brain might help to improve the return rate as drones gain experience as they age, especially for 8–10- and 12-days individuals.

Flight experience may increase the fitness of drones. Honeybees have an extremely male-biased sex ratio in the DCA, with about 20,000 males present per female (Page and Metcalf 1984). Consequently, drones are exposed to intense intra-sexual selection (Baer 2005; Jaffé and Moritz 2010). Individual drones could increase the possibility of successful mating via repeated attendance at mating flights. Unlike the males of other social hymenopterans, honeybee drones return to their hives when they fail to mate, and perform other mating flights later (Galindo-Cardona et al. 2015). Our study showed that drones need flight experience to return to their hives after a displacement. Therefore, flight experience increases their opportunity to attend mating flights by returning to the hive between mating flights, which ultimately increases their chances for mating success. Artificial displacement might reflect the natural displacement of drones followed by an unexpected event, such as wind; thus, the ability to relocate the hive following displacement is important. Improved returning may also increase their fitness for two additional reasons. First, the shorter time taken to return as drones age might minimize the energy consumption associated with returning, which may allow the drones to stay longer in the DCA, and thus, provide them with more opportunities to mate. Second, a prompt return to the hive can contribute to reduced predation risk and increase the opportunities for drones to mate. Drones spend 20–30 min in the air during mating flight (Winston 1987), and are exposed to predation risk during this period. Indeed, we observed that drones were preyed upon by swallows and robber flies during this study.

Our results may indicate that experience increases the opportunities for encounters with potential mates in honeybee drones, which do return to the nest between mating events, supporting that suggested for bumblebees, *Bombus terrestris* (Wolf and Chittka 2016). Based on the above-mentioned findings, we propose that experience may increase the possibility of mating success in drones. Our results suggest that experience is important not only for workers but also for honeybee drones, and it enhances the colony fitness by improving their behavioral ability to navigate to the nest.

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Ethics declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

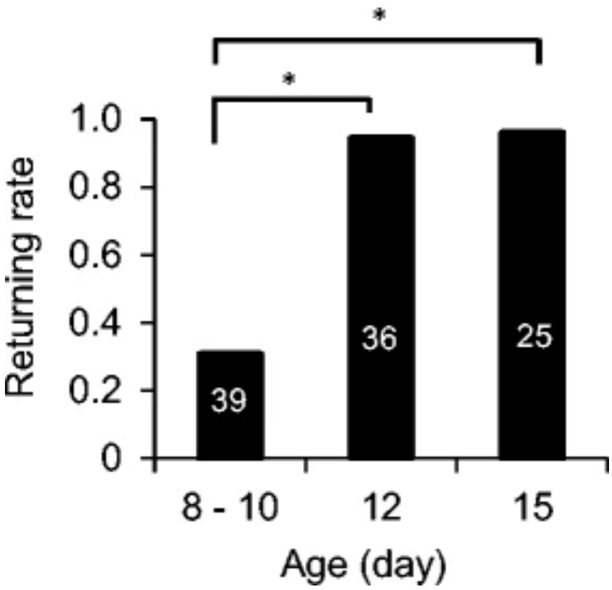

Figure 1. Return rates of the Experienced group. Numbers in bars indicate sample sizes. * $P < 0.05$

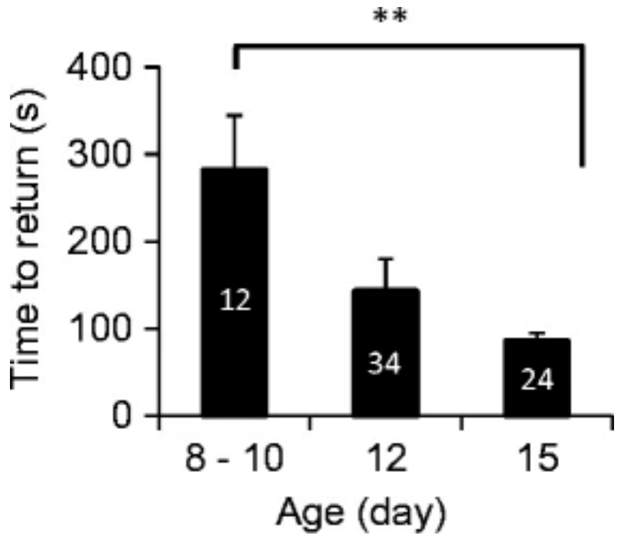

Figure 2 Time to return in the Experienced group. Error bars indicate standard errors. Numbers in bars indicate sample sizes. ** $P < 0.01$

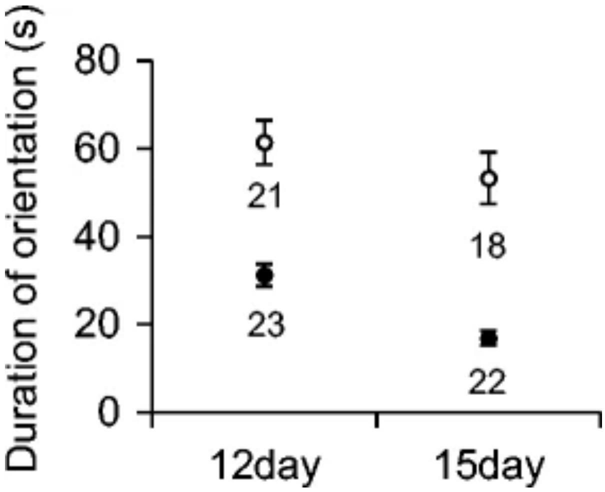

Figure 3. Mean duration of orientation in the Experienced and Naive groups. Experienced groups are indicated by filled circles; Naive groups are indicated by open circles. Error bars indicate standard errors. Numbers under symbols indicate sample sizes.

Table 1. Summary of the analysis for the return rate among Experienced and Naive groups for each age category in Experiment 1.

	Number of samples Released individuals (Returned individuals)				
	Experienced	Naive	*df*	$\chi 2$	*P*-value
8–10 day	39 (12)	38 (0)	1	26.606	< 0.001
12 day	36 (34)	24 (0)	1	67.24	< 0.001
15 day	25 (24)	28 (0)	1	64.604	< 0.001

Table 2. Summary of the multiple comparisons of the return rate among the age categories in the Experienced group.

	df	$\chi 2$	*P*-value
8–10 day vs 12 day	1	6.832	0.027
8–10 day vs 15 day	1	7.053	0.024
12 day vs 15 day	1	0.270	1

Table 3. Summary of the multiple comparisons of the time to return among the age categories in the Experienced group.

	df	$\chi 2$	*P*-value
8–10 day vs 12 day	1	2.639	0.313
8–10 day vs 15 day	1	13.721	< 0.001
12 day vs 15 day	1	4.543	0.099