

Adjustment of honey load by honeybee pollen foragers departing from the hive: the effect of pollen load size

K. Harano¹ · M. Sasaki¹

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Abstract Honeybee foragers load a small amount of honey into the crop when they leave the hive. This “honey at departure” is used as a material to build pollen loads (glue honey) as well as fuel during flight in pollen foragers. We investigated the relationship between the size of pollen loads that a forager collected and the amount of honey at departure in the Western honeybee, *Apis mellifera*. Dancing pollen foragers increased honey at departure with the size of collected pollen loads. Analysis of the waggle-run duration revealed that the size of pollen load affected the rate of increase of honey at departure with food source distance in dancers. The increase rate was significantly higher in dancers that had returned to the hive with large pollen loads versus nectar-collecting dancers, but not in those with smaller pollen loads. The higher increase rate may reflect additional fuel carried for the return trip in pollen-foraging specialists that do not use collected nectar as fuel. Although honey carried by departing dance followers also increased significantly with the size of pollen load carried by dancers, the adjustment was different from that of dancers. These results suggest that recruited bees adjust the amount of honey at departure, including glue honey, based on communicated information and modify the amount of honey load subsequently based on their own experience.

Keywords Communication · Foraging · Pollen collection · Social learning · Waggle dance

Introduction

Bees show various adaptations for transporting pollen from flowers to the nest. While some bees in basal clades use the crop to carry pollen by swelling it with nectar, most bees have external structures for carrying pollen, such as scopae and corbiculae (pollen baskets) on the hind legs or the ventral side of the abdomen (Thorp 1979, 2000; Michener 2007). In addition to the morphological adaptations, they facilitate pollen collection behaviorally. When pollen is packed into the scopae or corbiculae, some species of Panurginae (Andrenidae), Melittidae, and Apidae mix nectar with collected pollen (Thorp 1979). This nectar serves as glue to stick pollen grains together and allows the bees to use various pollen sources with different sizes of pollen grain (Thorp 1979). In some social bees, the glue for the pollen load is carried from the nest (Parker 1926; Beutler 1950, 1951; Leonhardt et al. 2007). For example, honeybee pollen foragers leave the hive with a crop partially filled with honey (this honey is referred to as “honey at departure” hereafter) and mix the honey with collected pollen during foraging (referred to as “glue honey”) (Beutler 1950; Harano et al. 2013, 2014). Some of the honey at departure is used as fuel for flight (Beutler 1950; Gmeinbauer and Crailsheim 1993). This behavior is well described in the honeybee *Apis mellifera* (Parker 1926; Beutler 1950; Hodges 1952; Harano et al. 2013, 2014) and is suggested in stingless bees (Leonhardt et al. 2007).

Carrying glue honey from the nest seems to facilitate pollen collection. When bees carry glue honey from the nest, they can probably start pollen collection soon after

✉ K. Harano
kharano@lab.tamagawa.ac.jp

¹ Honeybee Science Research Center, Tamagawa University, Machida, Tokyo 194-8610, Japan

arriving at a pollen source, whereas if they use nectar collected at flowers for building the pollen load, they must collect it before starting pollen collection. In addition, pollen-source plants do not necessarily have nectar (Parker 1926; Shuel 1992). The facilitation of pollen collection in this way may be one advantage emerging from sociality because a member of the colony can use nectar collected by other members. On the other hand, carrying honey from the nest was suggested to incur mass-dependent costs, such as increasing the energy expenditure for flight (Schmid-Hempel et al. 1985; Wolf et al. 1989), decreasing flight speed, and increasing predation risk due to increased body weight (Harano et al. 2013). Thus, it is expected that bees optimize the amount of honey at departure. Honeybee workers may have stretch receptors on the crop which they could use to monitor the amount of food in the crop (Brosch and Schneider 1985). The amount of honey at departure depends on the distance to a food source (Beutler 1950; Harano et al. 2013, p 3014), suggesting that foragers minimize costs incurred by carrying fuel honey, but it is not understood whether pollen foragers regulate the amount of glue honey that is loaded in the hive.

One of the possible factors influencing the need for glue honey to carry from the hive is the amount of pollen that bees will collect. Honeybee foragers return with pollen loads of various sizes. Large pollen loads probably require more glue honey than smaller ones. If a bee returns with similar sizes of pollen loads in a series of foraging trips, she could predict the amount of glue required in the following trip and adjust the amount of honey at departure to the need.

Another possible factor affecting the need to carry glue honey from the hive is the degree of specialization in pollen collection. Although many foragers collect both nectar and pollen, they tend to specialize in either pollen or nectar collection (Free 1960). The sizes of pollen and nectar loads collected by an individual forager are negatively correlated (Page 2013). When non-specialist foragers collect pollen together with much nectar, they may reduce their honey at departure by using collected nectar for building pollen loads (Parker 1926), whereas pollen specialists characterized by large pollen loads perhaps have to carry almost the entire amount of glue honey that they would use during a trip.

Based on the two reasons mentioned above, we assumed that the size of the pollen load is associated with the need for carrying glue honey from the hive and that larger pollen loads indicate the greater need. The main purpose of the present study is to examine whether bees adjust honey at departure depending on the need for glue honey.

The present study also addresses a question whether the information about the need for glue honey is transmitted from successful foragers to potential recruits. Honeybees communicate location of food sources using the waggle dance (von Frisch 1967). Potential recruits leave the hive

with an amount of fuel corresponding to the food source distance indicated by waggle dances (Harano et al. 2013, 2014). Previous study showed that followers have more honey at departure when they followed dances of pollen foragers than when following nectar foragers, suggesting that dancers convey information about the type of food material available at the indicated site and followers can use this information (Harano et al. 2013). The presence of pollen loads on dancers was suggested as a cue to indicate the presence of pollen at the site. However, it remains unknown whether the size of the pollen loads on dancers affects the amount of honey at departure in followers.

To examine these questions, the amounts of honey at departure were determined in dancing pollen foragers and their followers and the effect of size of pollen loads presented by dancers was examined.

Materials and methods

Bees

Colonies of the Western honeybee (*A. mellifera* L.) with two frames of comb, 3000–4000 workers, various stages of brood, and abundant food storage were used. The colony was held in an observation hive fitted with a wedge-shaped wooden block in the entrance to guide returning foragers to one side of the comb where they performed waggle dances. The area adjacent to the entrance (“dance floor”) was covered with nylon screen (18 × 20 cm; 4.2 mesh) for marking dancers and followers (Harano et al. 2013). The hive was placed in an air-conditioned room at ca. 26 °C. Workers were allowed to forage freely in the field.

Measurements of crop content at departure

We used the same methods and procedures as in previous studies (Harano et al. 2013, 2014) unless otherwise stated. Briefly, waggle dancers and their followers were classified by the size of pollen load presented by dancers and the amount of honey at departure was compared among groups with a different size of pollen load. Waggle-run duration was also recorded because honey at departure is affected by the distance to the food source to which they will fly (Beutler 1950; Harano et al. 2013, 2014).

Dancers and followers were marked with chalk powder using a fine paint brush (no. 0; Sakura Co. Ltd, Tokyo, Japan) through the nylon screen covering the dance floor upon performing or following a dance. Among a number of followers chasing a dancer, the most intensive one was selected to sample. They were then captured using sampling apparatus (Harano et al. 2013) at the corridor connecting to the field on leaving the hive. Immediately after capture,

these bees were fixed on a dissection dish with an insect pin. Each bee's abdomen was gently pulled from the thorax using tweezers to expose the crop. The crop content was measured using a 10- or 50- μL microsyringe (Hamilton, Reno, NE) which enabled us to measure the content with a limit of 0.1 μL . The crop was torn with the tip of a microsyringe needle on a dissection dish, and liquid from the crop was collected by the syringe. The volume of liquid was measured using the syringe scale. When the content was too small to be collected although a small amount of liquid could be seen in the crop, it was regarded as 0.1 μL .

Durations of waggle runs in waggle dances were recorded 3–6 times per dance using a digital voice recorder (ICR-PS401RM; Sanyo, Tokyo, Japan) before capture. The durations were averaged and used as an indicator of distance to food source (von Frisch 1967).

The experiment was carried out from April 14, to June 27, 2014 when various food sources were available in the field.

Classification of size of pollen load

The sizes of pollen loads on dancers were visually classified into the classes using the criteria of Harano et al. (2014). Pollen loads with a diameter as short as or shorter than the width of dancer's corbicula were classified as small (*S*), two times larger than corbicula in width as large (*L*), and loads between *S* and *L* as medium (*M*) (Fig. 1a). Dancers with *S*-, *M*-, and *L*-pollen loads and those without pollen loads were referred to as *S*-, *M*-, *L*-, and *N*-dancers, respectively. We regarded *N*-dancers as nectar foragers because the waggle dance by water foragers is rare. Bees following waggle dances of *S*-, *M*-, *L*-, and *N*-dancers were referred to as *S*-, *M*-, *L*- and *N*-followers.

Measurement of crop load on arrival at hive

We assumed that the size of pollen load served as an indicator of specialization to pollen foraging (Page 2013) and was positively correlated with the need for carrying glue honey from the hive. To confirm this assumption, the relationship between sizes of pollen and nectar load was investigated in returning foragers. Bees returning from a foraging trip with pollen loads were captured at the hive entrance from May 20 to May 21, 2012. The classification of pollen load by size was carried out as described above. The pollen loads were collected and weighed after drying at 100 °C for 2 h.

Consistency of pollen load size for individual forager

If individual foragers tend to collect a similar amount of pollen in a series of foraging trips, they could use their

experience as a reference for the amount of glue honey to carry in following trips. To investigate how the size of pollen load was consistent, its change was recorded in two consecutive trips on an individual basis. The observation was carried out from November 9, to December 5, 2011. Foragers were individually marked with acrylic paints (Acryla Gouache; Holbein Works Ltd., Osaka, Japan) on their thorax through a screen that covered the dance floor when they returned with pollen loads. The size of the pollen load was visually classified as described above. A total of 22 bees were observed for more than two consecutive trips, and the size of the pollen load was recorded for each trip. For all pairs of trips in a sequence, the degree of change in the pollen load size was obtained as follows: One-size change, a change of pollen load size from *S* to *M*, from *M* to *L* or vice versa; Two-size change, a change from *S* to *L* or vice versa.

Departing load in dancers collecting both pollen and nectar

It is possible that pollen foragers omit glue honey loaded in the hive when nectar is available at pollen sources, because they are likely to use the nectar as glue for pollen loads. If this is true, pollen foragers would carry only fuel for pollen sources with nectar, and the amount of honey at departure should be the same irrespective of the size of their pollen loads. To examine this possibility, we took advantage of a group of pollen foragers indicating a site by waggle dances (waggle runs lasted approximately 2 s in the south direction from the hive), which was found in a period from the end of June to mid-July, 2011. In the preliminary observation, they were found to carry yellowish pollen loads of various sizes together with nectar. Pollen was sampled from these bees to determine the morphological characteristics under a microscope. Some pollen samples were sent to Palynosurvey Co. (Tokyo, Japan) for pollen analysis to identify the plant.

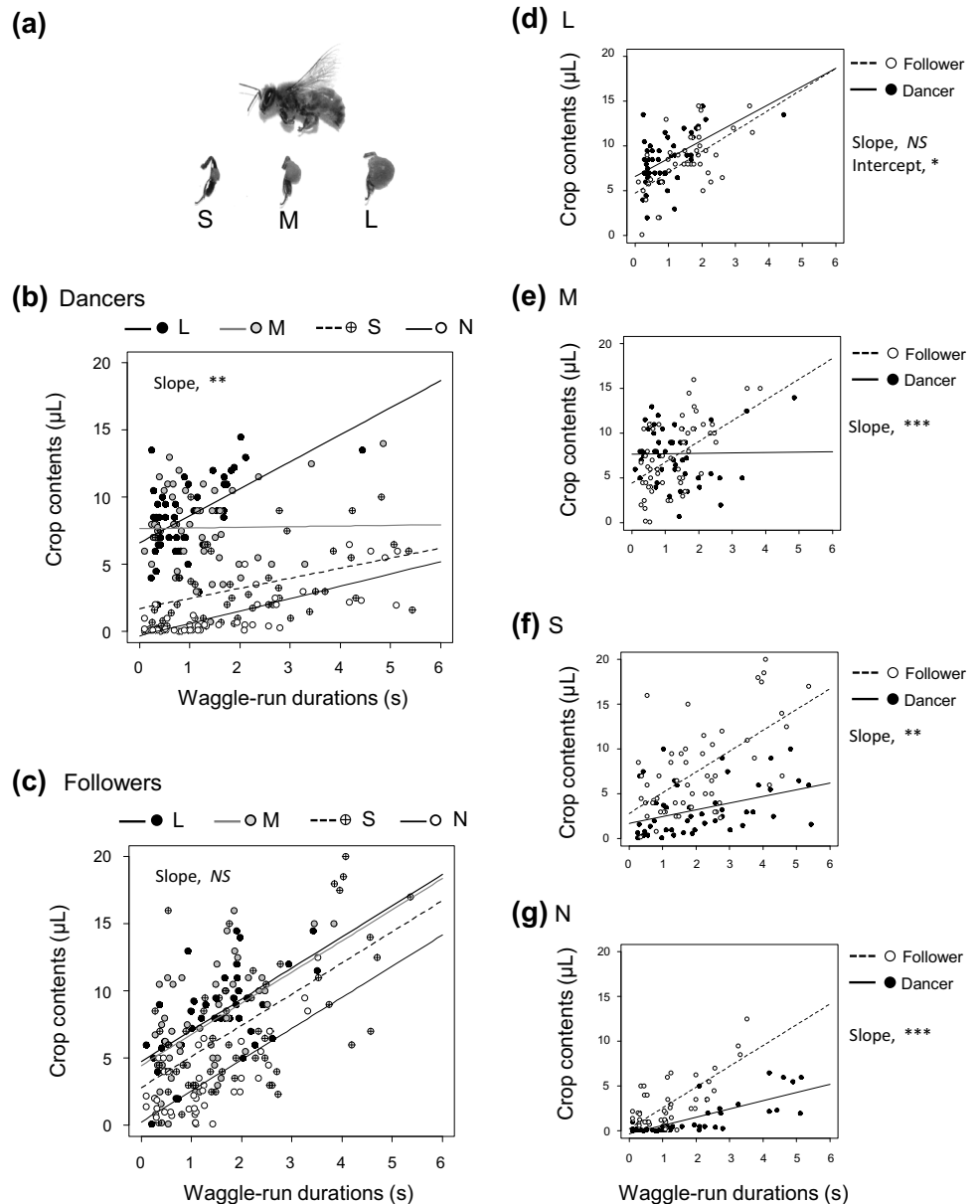
To confirm nectar collection, returning pollen foragers with yellowish pollen loads were captured at the nest entrance and the sizes of pollen and nectar loads were measured as described above. To confirm visits to the focal plant, pollen was collected from the pollen brushes on hind legs and was inspected under a light microscope. The data were used only when the pollen was morphologically identical to that of the focal plant.

To investigate the effects of pollen load size on honey at departure when departing to the focal plant, dancers were captured at the nest entrance on departure. Durations of waggle runs and pollen load size were recorded before capture as described above. The hind legs were collected to confirm the visit to the focal plant by inspecting remaining pollen grains on their pollen brushes. These experiments were carried out from July 8, to July 18, 2011.

Fig. 1 The effect of pollen load size on the amount of honey at departure when bees forage at different source plants.

Classification of pollen load size (a). The relationship between the amount of honey at departure and waggle-run duration in waggle dancers (b) and followers (c). Comparison between dancers and followers in each pollen load size class (d–g). Solid or dashed lines represent regressions between the amount of honey at departure and waggle-run durations.

L large pollen load, *M* medium pollen load, *S* small pollen load, *N* no pollen load. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, *NS* not significant. See text for detail. See also Tables 1



Statistical analysis

To understand the effects of waggle-run duration, pollen load size, and type of bees (dancers/followers) on the amount of honey at departure, we first performed linear regression using these three factors as explanatory variables in the generalized linear model (GLM). The data were then analyzed separately for dancers and followers; models were constructed with two explanatory variables, “waggle-run duration” and “pollen load size” for each type of bee. Using the models, the significance of the interaction between the two variables was tested by a likelihood ratio test. When a significant interaction was detected, indicating that the slopes of the regression lines were significantly different

among pollen load size groups, the slopes were compared between each group by multiple comparisons using likelihood ratio tests. In the multiple comparison, Holm’s corrected significance levels were used to avoid the problem of multiplicity. When no significant interaction was detected between “waggle-run duration” and “pollen load size”, models that do not contain an interaction were adopted and their intercepts were compared between groups by likelihood ratio tests. The comparison of intercepts was carried out only when a significant interaction was not detected because this comparison is possible only for regression lines aligned linearly. The effect of waggle-run duration on the amount of honey at departure was tested for each group by a likelihood ratio test.

To examine the effect of bee type (dancers/followers) on the determination of amount of honey at departure, regression equations obtained by GLM were compared between dancers and followers belonging to the same pollen load size class. Their slopes were first compared using a likelihood ratio test, and when they were not significantly different, intercepts were compared using models without interaction.

The relationship between the dry weight of pollen load and crop load upon arrival at the hive was examined using Spearman's correlation coefficient. In addition, data for nectar load were averaged for each pollen load size category (*S*, *M*, and *L*) and compared using Steel–Dwass multiple comparisons after a Kruskal–Wallis test.

The consistency in the size of pollen loads between two consecutive trips was tested by comparing the degree of change between the observed and expected values. The expected values were calculated based on an assumption that the size of pollen load was determined by chance for each trip, independently of its size in the previous trip. A goodness-of-fit test was used for the statistical test. When the observed data was significantly different from values expected by chance, it was concluded that there was some consistency in the pollen load size.

All statistical tests were performed at the 5 % significance level using Excel plug-in software (Ekuseru-tokei 2012; SSRI, Tokyo, Japan) or R (R Core Team 2015).

Results

Effects of waggle-run duration, pollen load size, and foraging role

We constructed a model with three explanatory variables, “waggle-run duration,” “pollen load size,” and “type of

bee (dancers/followers)” to understand the effect of these variables on the amount of honey at departure. The GLM showed that there was a significant interaction among the three variables (residual $df = 371$, 374 , $df = 3$, deviance = 64.55, $P < 0.05$), indicating that the effects of each explanatory variable depend on other variables.

To investigate the effects of these variables further, we analyzed data separately for dancers and followers. In dancers, all pollen load size groups, except M-dancers, showed significant increases in the amount of honey at departure with waggle-run duration (Table 1; Fig. 1b). The interaction between the explanatory variables “waggle-run duration” and “pollen load size” (difference in slopes of regression lines) was significant (residual $df = 193$, 196 , $df = 3$, deviance = 66.51, $P < 0.01$), indicating that the size of pollen load affected the rate of increase of honey at departure with waggle-run duration. L-dancers showed the largest slope, which was significantly larger than that of M- and N-dancers (Table 1).

In followers, all pollen load size groups showed significant increases in honey at departure with waggle-run durations (Fig. 1c; Table 1). Since no significant interaction was found between the explanatory variables “waggle-run duration” and “pollen load size” on the amount of honey at departure (residual $df = 178$, 181 , $df = 3$, deviance = 20.30, $P = 0.57$, *NS*), models were constructed without the interaction (Table 1). The intercept of regression lines was significantly larger in L-followers than in S- and N-followers (Fig. 1c; Table 1).

The relationship between dancers and followers for the amount of honey at departure depended on the size of pollen loads carried by dancers (Fig. 1d–g). The slope of regression lines was not different in L-dancers and their followers (residual $df = 85$, 86 , $df = 1$, deviance = 1.00, $P = 0.69$), but its intercept was significantly larger in the former than

Table 1 Models estimated by GLMs for different pollen load size groups in dancers and followers

Type of bees and pollen load size	<i>N</i>	Estimated models (regression equations)	Significance of waggle-run duration ^a	Difference in slope ^b	Difference in intercept ^b
Dancers					
<i>L</i>	50	$y = 6.61 + 2.01x$	<0.001	a	Not analyzed
<i>M</i>	52	$y = 7.66 + 0.05x$	0.92	b	
<i>S</i>	51	$y = 1.72 + 0.75x$	<0.01	ab	
<i>N</i>	52	$y = -0.31 + 0.92x$	<0.001	b	
Followers					
<i>L</i>	41	$y = 4.75 + 2.32x$	<0.001	<i>NS</i>	a
<i>M</i>	53	$y = 4.45 + 2.32x$	<0.001		ab
<i>S</i>	53	$y = 2.81 + 2.32x$	<0.001		b
<i>N</i>	42	$y = 0.25 + 2.32x$	<0.001		c

y amount of honey at departure (μL), *x* waggle-run duration (s)

^a *P* values by likelihood ratio test with GLM; *NS*, not significant ($P > 0.05$)

^b Different letters indicate a significant difference between the groups by likelihood ratio tests

the latter (residual $df = 85, 87, df = 1$, deviance = 42.47, $P < 0.05$). Significant differences in slopes were found between dancers and followers in M-, S-, and N-classes (M, residual $df = 101, 102, df = 1$, deviance = 169.78, $P < 0.001$; S, residual $df = 99, 100, df = 1$, deviance = 97.88, $P < 0.01$; N, residual $df = 86, 87, df = 1$, deviance = 38.80, $P < 0.001$).

Relationship between nectar and pollen loads in returning foragers

The volume of crop content upon returning to the hive was negatively correlated with the size of pollen loads that the bee carried ($N = 83, r_s = -0.36, P < 0.001$; Fig. 2). When bees were classified into three classes in terms of pollen load size, the crop contents were significantly larger in S-foragers than in M- and L-foragers (mean \pm SD: S, $19.7 \pm 12.3 \mu\text{L}$; M, $5.8 \pm 8.4 \mu\text{L}$; L, $5.2 \pm 5.6 \mu\text{L}$; Kruskal–

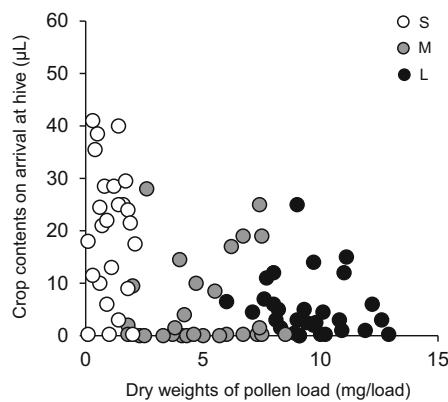


Fig. 2 The relationships between the sizes of nectar and pollen loads in bees upon arrival to the hive. L large pollen load, M medium pollen load, S small pollen load

Wallis test, $df = 2, \chi^2 = 23.55, P < 0.001$; Steel–Dwass test, $P < 0.001$).

Consistency of pollen load size

Sixty-four sets of two consecutive trips were observed for 22 pollen foragers. Of these, the size of pollen load changed by one size (from S to M, from M to L, or vice versa) in 22 cases (34.4 %) and two sizes (from S to L or vice versa) in one case (1.6 %). The other 41 cases (64.1 %) showed no changes in the size. The proportions were significantly different from those expected by chance (goodness-of-fit test, $N = 64, \chi^2 = 31.75, P < 0.001$; Table 2), indicating that the size of pollen load tended to be constant at least in two consecutive trips.

Honey at departure for flowers with nectar and pollen

The effect of pollen load size on the amount of honey at departure was also found in bees collecting pollen together with nectar. Analyzed pollen foragers danced for 2.20 ± 0.23 s (mean \pm SD) ($N = 41$) in waggle runs directing the south of hive. The pollen remaining on pollen brushes was morphologically identical. Pictures of the pollen are shown in Fig. 3a. Based on the pollen morphology, the family of the source plant was identified as Fabaceae but the species was not identified. Foragers returning from this food source carried a considerable amount of nectar as well as pollen (Fig. 3b). Although the size of nectar load was negatively correlated with the size of pollen load ($N = 30, r_s = -0.51, P < 0.01$; Fig. 3b), bees returning with L-pollen loads still had relatively large nectar loads (mean \pm SD = $21.7 \pm 12.5 \mu\text{L}$, $N = 11$). Their honey at departure was found to increase with the size of pollen loads that they carried in the previous trip; honey at

Table 2 Observed and expected values for changes in size of pollen load between two consecutive trips

Degree of change	Size of pollen loads in previous trip			Total
	S	M	L	
Observed				
No change	7	10	24	41
1 size (S–M, M–L)	5	12	5	22
2 sizes (S–L)	1	0	0	1
N	13	22	29	64
Expected by chance ^a				
No change	4.3	7.3	9.7	21.3
1 size (S–M, M–L)	4.3	14.7	9.7	28.7
2 sizes (S–L)	4.3	0.0	9.7	14.0
N	13.0	22.0	29.0	64.0

^a Values were calculated based on the assumption that the size of the pollen load is determined by chance and independent for each trip

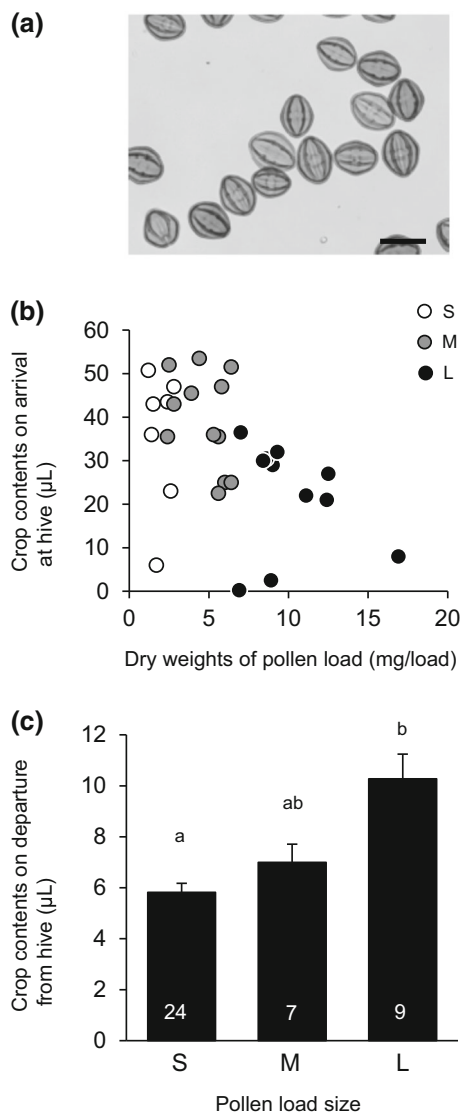


Fig. 3 The effect of pollen load size in foragers collecting pollen with nectar. A light microscope image of pollen derived from a papilionaceous plant from which foragers collected both nectar and pollen simultaneously (a) bar 50 μm . The relationship between the size of nectar load and that of pollen load in foragers that returned from the papilionaceous plant (b). The effect of the size of pollen load carried in the previous trip on the amount of honey at departure in dancers leaving to the papilionaceous plant (c): different letters indicate a statistical significance by Tukey–Kramer test ($P < 0.05$). Numbers in bars represent sample sizes

departure was significantly larger in L-dancers than S-dancers (ANOVA, $N = 40$, $F_{2,37} = 15.37$, $P < 0.001$; Tukey–Kramer test, $P < 0.01$; Fig. 3c).

Discussion

When the size of pollen and nectar loads was investigated in returning foragers, they were found to be negatively correlated (Fig. 2), supporting our argument that dancers

returning with small pollen loads have access to nectar at the pollen source. When nectar is available at the pollen source, bees may use collected nectar as glue for building pollen loads. In addition, building small pollen loads probably requires little glue honey. It is reasonable to postulate that foragers returning with small pollen loads have less need to carry glue honey from the hive than those with larger pollen loads.

We found a significant interaction between the three explanatory variables “waggle-run duration”, “pollen load size”, and “type of bee” on the amount of honey at departure but its meaning is not easy to clarify. To understand the effect of these variables, we analyzed data separately for dancers and followers. The effects of pollen load size on the amount of honey at departure were still complicated in dancers. We found significant interactions between the pollen load size and waggle-run duration, indicating that foragers modify the rate of increase of honey at departure with distance depending on the size of pollen loads collected. In other words, the effect of pollen load size was not simply additive on the amount of honey at departure in dancers.

L-dancers showed a significantly steeper regression line versus N-dancers but M- and S-dancers did not (Table 1). The variation in slopes of regression lines (Fig. 1b; Table 1) might be associated with specialization in pollen foraging. We confirmed previous findings (Free 1960; Page, 2013) that foragers with large pollen loads are more specialized for pollen foraging than those with small loads and collected little nectar (Fig. 2). Thus, it is reasonable to think that most L-dancers are pollen-foraging specialists. Since pollen-foraging specialists collect little nectar that can be used as fuel, they probably have to carry fuel for the return trip, causing their larger increase in honey at departure per unit distance than non-specialists and nectar specialists. Previous studies reported a larger increase rate in pollen specialists (Harano et al. 2014) versus nectar foragers (Harano et al. 2013). Increased energy expenditure per unit distance due to large honey load at departure and pollen loads (Wolf et al. 1989; Feuerbacher et al. 2003) may also cause the steeper regression line in L-dancers.

Because of the interaction, the effect of pollen load size alone cannot be tested statistically in dancers. However, when comparing L- and S-dancers, not only the intercept of regression line but also its slope was larger in the former than the latter, suggesting that the amount of honey at departure would be larger in L-dancers than S-dancers when they use food sources at the same distance (Fig. 1b). Plots of M-dancers appeared to be distributed between the two groups. This argument is supported by another observation with a papilionaceous pollen source. In this case, bees foraged at the same food source but the amount of honey at departure

varied depending on the size of the pollen load (Fig. 3c). Based on these results, we suggest that pollen foragers change the amount of honey at departure in response to the amount of pollen collected or the need for glue honey assessed during previous trips. Since bees tended to continue to collect similar amounts of pollen (Table 2), it may be beneficial to determine the amount of honey at departure based on previous experience.

Information transfer from dancers to followers

The size of pollen load carried by dancers also affected the amount of honey at departure in dance followers. However, the effect was different from that in dancers. The size of pollen load did not significantly affect the slope of the regression line but altered its intercept. In other words, the effect of pollen load size was additive in followers, unlike in dancers.

The dependence of honey at departure on pollen load size in followers indicates the transfer of information from dancers to followers. Although the mechanism remains unknown, dancers seem to provide several stimuli representing the need for honey at departure. Since the size of pollen loads carried by dancers is likely to reflect the need for glue honey, followers might reference it to determine the amount of honey at departure.

Some followers might use their memory to adjust the amount of honey at departure because unemployed but experienced foragers often follow dances indicating a familiar food source that they have previously visited (von Frisch 1968). Flower scents brought into the hive have been demonstrated to recall memory of food source location in experienced foragers (Reinhard et al. 2004). It might also recall other memories associated with the food source, such as the need for glue honey.

Change in honey at departure through foraging experience

We previously showed a difference in the effect of foraging experience on the amount of honey at departure between nectar foragers and pollen foragers (Harano et al. 2013, 2014). In nectar foragers, the amount of honey at departure was larger in followers than in dancers and decreased as they made successful foraging trips (Harano et al. 2013). It was argued that more fuel honey may benefit inexperienced bees in finding the communicated food source. On the other hand, Harano et al. (2014) showed that pollen foragers had less honey at departure in followers than dancers, suggesting that they increased the honey at departure after making successful foraging trips.

The present study suggests that the effect of foraging experience depends not only on the type of forage but

also the size of pollen load. The honey at departure of an L-dancer is significantly more than that of L-followers (Fig. 1d), which showed the largest amount of honey at departure among follower groups, suggesting that bees increase honey at departure after successful foraging experience with large pollen loads. This change is consistent with the previous observation in pollen foragers visiting crape myrtle (Harano et al. 2014). On the other hand, the regression line for S-dancers was lower than for S-followers (Fig. 1f), which showed the lowest amount of honey at departure among pollen dance followers (Fig. 1c). The lower regression line for S-dancers suggests that successful foraging experience with small pollen loads (probably with a large nectar load) reduces the amount of honey at departure. This response is similar to that observed in nectar foragers (Harano et al. 2013). Recruited bees probably gather various information including the need for glue honey when they find a target food source, and reflect it in the amount of honey at departure in following trips.

Effect of pollen load size in the presence of nectar

We observed a significant variation in the amount of honey at departure depending on the pollen load size in bees collecting nectar as well (Fig. 3). This observation confirmed the importance of pollen load size on determination of honey at departure. The results also suggest that pollen-biased foragers carry some glue honey from the hive even when they can access nectar at flowers. However, it is still possible that the amount of glue honey carried from the hive is reduced when nectar is available at pollen sources. The effect of the presence of nectar at the pollen source on the honey at departure should be examined further.

In summary, the present study demonstrated the effect of pollen load size on the amount of honey at departure and its difference between dancers and followers. Although followers adjust the amount of honey at departure according to the size of pollen loads presented, the adjustment is different from the amount of honey at departure in dancers. These results suggest that bees determine the amount of honey at departure by taking advantage of communicated information and modify it subsequently based on experience.

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