RESEARCH ARTICLE

Waggle dance effect: dancing in autumn reduces the mass loss of a honeybee colony

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SUMMARY

A honeybee informs her nestmates about the location of a profitable food source that she has visited by means of a waggle dance: a round dance and a figure-of-eight dance for a short- and long-distance food source, respectively. Consequently, the colony achieves an effective collection of food. However, it is still not fully understood how much effect the dance behavior has on the food collection, because most of the relevant experiments have been performed only in limited locations under limited experimental conditions. Here, we examined the efficacy of the waggle dances by physically preventing bees from dancing and then analyzing the changes in daily mass of the hive as an index of daily food collection. To eliminate place- and year-specific effects, the experiments were performed under fully natural conditions in three different cities in Japan from mid September to early October in three different years. Because the experiments were performed in autumn, all six of the tested colonies lost mass on most of the experimental days. When the dance was prevented, the daily reduction in mass change was greater than when the dance was allowed, i.e. the dance inhibited the reduction of the hive mass. This indicates that dance is effective for food collection. Furthermore, clear inhibition was observed on the first two days of the experiments; after that, inhibition was no longer evident. This result suggests that the bee colony adapted to the new environment.

Key words: insect, social behavior, waggle dance, food collection, adaptability.

INTRODUCTION

The honeybee *Apis mellifera*, a social insect species, has the ability to communicate with its nestmates using a waggle dance (they perform a round dance and a figure-of-eight dance for a short- and long-distance food source, respectively) to convey the location of profitable food sources she has visited (von Frisch, 1993). Once a scout bee has found a profitable food source and returned to the hive, she will perform a waggle dance to recruit numerous nestmates to visit the food source. Bees surrounding a dancing bee might be recruited to visit the food source that the dancer is advertising (Judd, 1995; Okada et al., 2008a; Okada et al., 2008b). After their successful forages, some of the recruited bees will perform a dance to recruit new bees. Consequently, the colony achieves an effective collection of food (Srinivasan, 2010), although it is well established that the outcomes of bee activities, including food collection, rate of successful recruitments and dance occurrence rate, vary widely among colonies (von Frisch, 1993).

Compared with the huge number of studies in which the dance efficiency has been assessed by counting the number of correctly recruited bees or estimating ecologically the energy intake (e.g. von Frisch, 1993; Seeley, 1995), there have been relatively few straightforward experiments performed to determine the effects of dance efficacy on food collection. In a notable exception, based on the knowledge that the directional information is lost when diffused light is applied to bees on a horizontal comb (von Frisch, 1993),

Sherman and Visscher (Sherman and Visscher, 2002) compared foraging success at natural food sources by measuring the mass of two colonies housed in California. One colony was subjected to a diffuse light treatment and the other to an oriented light treatment. They found that bees that were allowed to perform a dance with the directional information intact (an oriented dance) could collect more food than those who performed a dance in which the directional information was disrupted (a disoriented dance). Interestingly, this difference was statistically significant only in winter (late December to early March), and not in summer or autumn (late July to early September or late September to late December). Ideally, to identify the general features of dance effects, experiments should be performed many times. In addition, experiments should be performed in different locations and in different years to eliminate place- and year-specific effects. However, it is often difficult to carry out such experiments. Dornhaus and Chittka (Dornhaus and Chittka, 2004) performed disoriented dance experiments by using diffuse light in India, Germany and Spain within a year and found that foraging efficiency was substantially impaired in an Indian tropical forest, but not significantly impaired in temperate habitats in Germany or Spain, when dance information was lost. These two studies suggest that the efficacy of dance language communication in regard to food source location may depend on the habitat bees live in, and thus the authors proposed that dance helps a bee colony to find food, particularly in habitats in which food is scarce.

In addition to dance communication, Thom et al. (Thom et al., 2007) recently identified four substances – tricosane, pentacosane, Z-(9)-tricosene and Z-(9)-pentacosene – that bees emit during dancing, and found that a compound containing three of the four substances encouraged bees to fly out from the hive. This implies that the effect of dancing may have two different functions: to transfer the food location information and to increase the number of bees that fly out to forage. In the Dornhaus and Chittka (Dornhaus and Chittka, 2004) and Sherman and Visscher (Sherman and Visscher, 2002) studies, bees were allowed to perform the dance. This means that substances were released from the dancer under the experimental conditions, i.e. the effects of the substances were not removed. If these substances substantially improve the foraging efficiency, then food collection would be severely impaired by preventing the bees from dancing and thus from releasing the dance-related substances.

In the present study, we physically prevented bees from dancing to eliminate both the transfer of dance information regarding food location and the release of dance-related substances. Additionally, to remove any place- and/or year-specific effects, we performed our experiments in three different cities in Japan in mid September and early October in three different years and examined the efficacy of the dance behavior under fully natural conditions.

MATERIALS AND METHODS
Experimental animals

Honeybees *Apis mellifera* Linnaeus 1758 were kept in a beekeeping box (10 combs inside) in each experimental location under fully natural conditions for at least 1 month (in most cases, more than 3 months). Approximately 1500 bees on one comb in the mother hive were then taken up and transferred with their queen to a single-frame observation box. The observation box was immediately placed in the same experimental location with the mother colony. The transferred colony was left in the observation box for at least 10 days after being transferred, except in the case of colony 5 (see the next section), to familiarize the bees with their new circumstances. Thus, bees in the observation box had sufficient opportunities for foraging before the experiment. Both sides of the box were covered with a removable plastic transparent plate for easy observation. Because we did not serve any food or pollen, the bees had to forage and collect food for their survival. The transferred comb was completely finished, i.e. there was no space to build new cells. Broods were

contained, a sufficient amount of nectar was stored and, importantly, enough space for depositing nectar was maintained during the experiment, suggesting that bees were not released from the pressure for food collection. All castes, i.e. allocations, were normally contained. We have previously reared many colonies under these conditions (e.g. Okada et al., 2008a; Okada et al., 2008b; Okada et al., 2010a; Okada et al., 2010b), and the colonies can survive for more than several months. Thus, we are sure that the transferred colonies can be regarded as perfectly normal, except in terms of colony size. If we found any abnormalities during the periods between the transfer and the experiment, e.g. no foraging, failure to care for the broods, returning to the original hive or moving from the original hive, we did not use the colony. No developmentally abnormal bees were found under these conditions during the experiments. We chose such a frame suited for the experiment. Because honeybees could collect and use honey storage freely before the experiments began, initial hive masses were different among colonies (2732–5054 g). At least, no prominent abnormal honeybee behavior at the individual or the colony level was observed. Hence, each colony was healthy enough to be used for the purposes of the present study. Indeed, we found no clear relationship between the results and the initial hive mass (see Results).

Experimental setup

The experiments were performed in three different cities in Japan in mid September to early October in three different years. The experimental locations and conditions are summarized in Fig. 1 and Table 1. Colonies 1 and 2 were reared and used on the campus of Hokkaido University (Sapporo, Japan; 43°4′28″N, 141°20′35″E) from 13 to 22 September 2007 (6 days), colonies 3–5 were reared and used at University of Hyogo (Himeji, Japan; 34°50′55″N, 134°41′15″E) from 27 September to 4 October 2008 (6 days), and colony 6 was reared and used at Tokushima Bunri University (Sanuki, Japan; 34°19′36″N, 134°11′30″E) from 23 to 24 September 2009 (2 days). Because Sapporo is in the northern part of Japan and gets cold earlier in the year than the other cities, the experiments in Sapporo began 10–14 days earlier than those in the other locations so that the experiments would be conducted under similar weather conditions. In Japan, late September is the beginning of autumn. Most of the experiments were carried out from 08:30 to 16:30 h (temperature: 25–36°C).
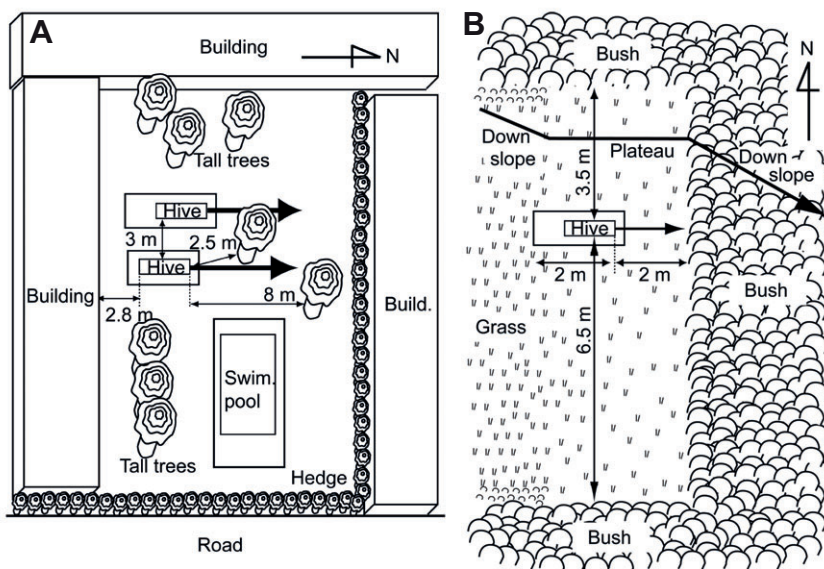

Fig. 1. Experimental environments. (A) Experimental location in Himeji. (B) Experimental location in Sanuki. The experimental environment in Sapporo is not shown because the experiment was performed on the roof of a building without plants, grasses or remarkable landmarks. For ease of interpretation, drawings are not to scale.

Table 1. Colony conditions

Location	Experimental dates	Colony ID	Treatment	No. of disturbances
Sapporo	13–22 September 2007	1, 2	One side	Not counted
Himeji	27 September–4 October 2008	3, 4, 5	Both sides	Counted
Sanuki	23–24 September 2009	6	Both sides	Counted

Sapporo

Bees (colonies 1 and 2) were kept on the roof of a two-story building at Hokkaido University in Sapporo. The campus of Hokkaido University is rich in vegetation, but the plants are distributed in patches rather than randomly. Although our bees needed to work to find food patches, they succeeded in this search and did not face a starvation crisis. Bee observation hives were placed on the roof under a permanent shadow at a distance of 4 m from each other. The entrances of two hives, set 70 cm from the floor, were directed to the east and west so that they faced each other. The floor of the roof was concrete, and devoid of trees or flowers. Other honeybee colonies were near the experimental location but we never saw typical guarding behavior, such as attacks against bees approaching the experimental comb or the alert posture with head and forelegs raised. However, we often observed bees that were already on a comb allow approaching bees to land on it. These observations suggested that bees that came to the experimental colonies were judged to belong to the same colony, rather than to other colonies that were trying to steal food.

Himeji

Bees (colonies 3–5) were kept in a courtyard on the campus of University of Hyogo in Himeji. The campus is located near the middle of the city but there are low mountains near the campus. Thus, we do not think that the bees at this site were in any danger of starvation, but they did need to scout to find their food. Bee observation hives were placed on separate tables (Fig. 1A) at a distance of 3 m from each other. The entrance was 70 cm from the ground and directed towards the north. Some tall trees stood in the court but we did not observe any instance in which these trees disturbed the bee behaviors related to flight. Neither the trees nor the hedges in the courtyard had flowers at the time of the experiment. There was a swimming pool nearby but it was empty, and thus the pool had no influence on, for example, water collection by the bees. The ground consisted of soil with low grasses. All buildings around the courtyard were relatively short (none rose higher than a single story). Other Western and Japanese honeybee colonies resided near the courtyard, thus we needed to pay attention to keep them away from the experimental observation hives.

Sanuki

Bees (colony 6) were kept on the campus of Tokushima Bunri University in Sanuki. The campus is located in a hilly area with tall trees. Near the campus there is a large area occupied by rice and vegetable fields. Thus, bees could find food without a several kilometer journey. Because most of the vegetable fields did not have any flowers in this season, bees had to search for their food source. The bee observation hive was placed on a table (Fig. 1B). The entrance was at 70 cm from the ground and directed towards the east. Many small trees and bushes surrounded the 10×4 m experiment field but they did not disturb the bee behavior because they were situated on a downward slope. The experimental field itself was flat and consisted of soil and grasses. Although we did not find flowers near the experimental hive, we could not exclude the possibility of blossoming flowers because we did not make an effort to rule this out. No bees from other colonies came to the hive during the experiments; those bees that did approach were assumed to be from the same colony (see above, Sapporo).

Experimental procedure

Basically, except for Sanuki, two beehives used simultaneously in each experiment were placed close to each other (Fig. 1), and thus no local location-specific effects were expected. In the morning, we removed the transparent covers from both sides of each beehive and measured the mass of the whole hive with a digital scale (EK-12Ki, A&D Company, Ltd, Tokyo, Japan). After the experiment, we measured the mass again and replaced the covers. We then subtracted the mass in the evening from that in the morning as an index of food collection, because the main factor affecting the change in hive mass over the course of a single day is the change in nectar storage in the hive (Meikle et al., 2008). Finally, all the observation hives except for colonies 1 and 2 were covered with a non-transparent box to keep them in darkness until the next day of the experiment.

Disturbance of waggle runs

Each colony was subjected to both a day with a disturbance treatment and a day without a disturbance treatment. The day when the disturbance was performed was referred to as the disturbance day, and the day when disturbance was not performed was referred to as the control day. On the disturbance day, we disturbed all observed waggle runs by touching the body of the waggling bee with a paintbrush. Because the paintbrush was fine (smaller than the body size of an individual bee) and small enough to touch only a single insect, no bees other than the targeted dancing bee were disturbed by this procedure, although we cannot exclude the possibility of some form of unobservable disturbance. In cases in which the bee tried to keep waggling under the brush, we picked the bee up and threw it away from the hive, although in such cases it soon flew back to the hive and began waggling again. We repeated this procedure until the bee gave up waggling. In our experiments, to persuade bees to stop dancing we had to throw 20–25% of dancing bees away from the hive. The remaining 75–80% of bees stopped dancing at the first touch of the paintbrush. Our preliminary observations showed that disturbed bees walked on the comb and later flew out from the hive as normal. The number of disturbances (touching and picking up) was counted and totaled every 30 min. On the control day, we did nothing but video the observation hive.

Analysis of bee behavior

On the control days, bee behavior on the vertical comb of both sides of the hive was recorded using GR-HD1 (JVC, Tokyo, Japan) and HDR-HC9 (Sony, Tokyo, Japan) video camcorders for 5 min every 30 min (30 frames s^{-1}), and the movies were stored digitally. Additional striking behaviors were noted, such as playing flight, forming clusters, building living chains, fanning, mood of the colony (active or static), etc., to facilitate data analysis. All video data were transferred onto the hard drive of a computer for further analysis. Videoed bee behaviors were analyzed offline frame by frame. Because both the figure-of-eight dance and the round dance contain a waggling run phase where a dancing bee runs while wagging her

body, the numbers of all waggle runs observed during 5 min every 30 min were counted. For the disturbance day, we counted the number of incidences of touching (disturbing) the waggling bees. The experimental durations were not the same throughout all the experiments because of the weather conditions. For this reason, we normalized the change in hive mass by dividing the measured mass by the recording time (8 h). The normalized numbers of disturbances and waggle runs (per hour) were also calculated by dividing each of them by the experimental time. For colonies 1 and 2, the number of waggle runs was doubled because only one side was videoed (Table 1).

Data grouping

To eliminate experimental sequence-specific effects, we divided the six colonies into two groups. The first group underwent an experimental sequence in which the first day was the disturbance day and the second day was the control day (DC group), whereas the other group was subjected to the opposite sequence (CD group). The experiments with colonies 1–5 were continued for more than 2 days. During each experiment, the bees on a comb gathered, hung down form the top of the observation hive and formed a 'living chain', which is typically seen when new combs are being built (Fig. 2) (Tautz, 2008). Although honeybees were walking on the comb behind the chain, we could not observe their behavior behind the chain, and we did not want to break up the chain because we wanted to maintain their social environment. In general, the chain was built every day after the removal of the plastic cover, and the time required to build the chain became shorter and shorter. Therefore, two experimental periods were assigned for further analysis: an early period and a later period. The early period included only the first two experimental days, and the later period included all the days after the second day. Each data point was thus characterized by the combination of three factors: colony ID, group (CD or DC) and period (early or late).

Statistics

The Wilcoxon matched-pairs test was applied to examine significant changes in daily mass between the disturbance and control days using StatView 5.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Because the net mass of each hive was different (2732–5054 g), the efficacy of food collection in each colony was evaluated separately as the daily change in hive mass between morning and evening. Throughout the experiments in all three cities, most daily mass changes showed negative values, indicating that the hive mass decreased during the day. This could be a normal phenomenon associated with seasonality in Japan (see Discussion). The daily mass changes varied highly with both experimental day and colony. For example, the net daily mass changes of colony 4 varied from approximately +56 g (27 September) to –61 g (3 October). In contrast, those of colony 1 ranged from –2 g (14 September) to –19 g (18 September).

In the early period, five (two CD colonies and three DC colonies) out of six colonies we tested showed that daily mass reduction of the disturbance day was greater than that of the control day irrespective of the experimental sequence (Fig. 3, Table 2). Both the median and the mean of the disturbance days were much lower than those of the control days (median: –38.1 and –4.8 g 8 h^{-1} for the disturbance and control days, respectively; mean: –40.0 and +7.9 g 8 h^{-1} for the disturbance and control days, respectively), suggesting that mass loss was enhanced by the prevention of waggle
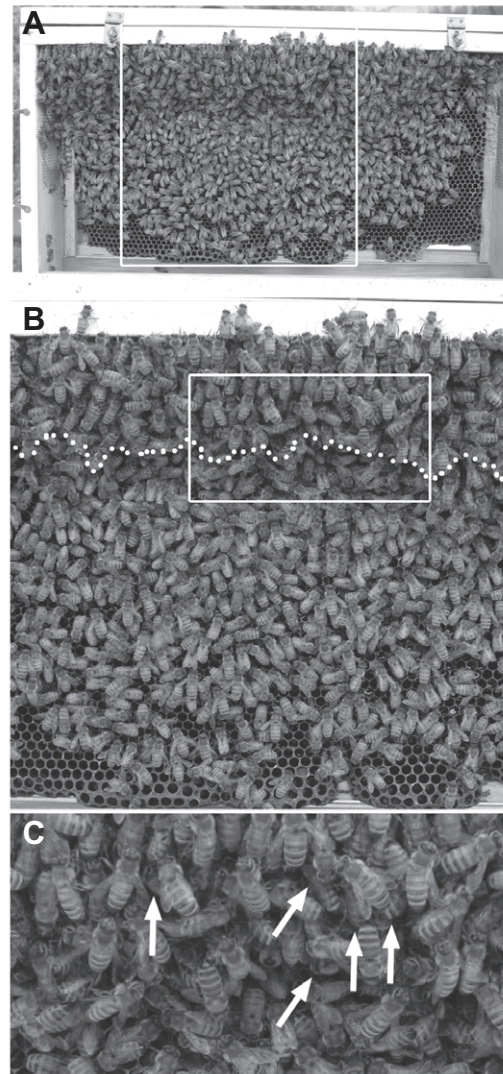

Fig. 2. The living chain. (A) An entire side of a comb. A huge number of bees gathered, hung down from the top of the observation hive and formed a living chain. (B) Close-up of the area surrounded by the rectangle in A at high magnification. The dotted line indicates the boundary of the living chain. Bees appeared to build a new comb parallel to the original comb. (C) Bee behavior behind the living chain, showing a close-up of the area surrounded by the rectangle in B at high magnification. Although honeybees were walking on the comb behind the chain (arrows), unfortunately we could not observe their behavior because we wanted to maintain their social environment.

dances, except for colony 2, where the reduction of mass on the disturbance day was slightly smaller than that on the control day (disturbance –10.5 g 8 h^{-1}, control –17.5 g 8 h^{-1}). This difference, however, between the disturbance day and the control day (7.0 g 8 h^{-1}) was smaller than in the other five colonies. In the later period, two out of six colonies showed a greater decrease in hive mass when disturbed (Fig. 4). The medians were –14.0 and –19.2 g 8 h^{-1} for the disturbance and control days, respectively, and the means were –20.1 and –26.1 g 8 h^{-1}, respectively. Notably, in the later period, both the median and mean on the disturbance days were smaller than those on the control days, in contrast to the early period. Taking these results together, we conclude that there was a trend for dancing behavior to enhance the efficacy of food collection in the early period, although the differences between the disturbance
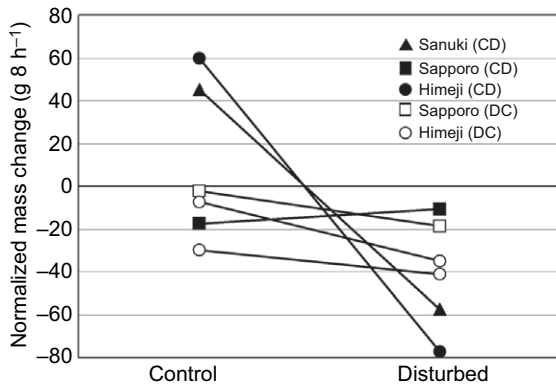
Fig. 3. Daily changes in hive mass during the early period (the first two experimental days). Six colonies were divided into two groups: DC (colonies that received disturbance treatments on the first day and no disturbance treatments on the second day; open symbols) and CD (colonies that received no disturbance on the first day and disturbance treatments on the second day; filled symbols). In five out of six colonies, daily mass reduction on the disturbance day was greater than that on the control day, but there was no significant difference between the disturbance days and the control days (Wilcoxon matched-pairs test, P=0.063).

and control days were not statistically significant (Wilcoxon matched-pairs test, P=0.063). In contrast, there was clearly no significant difference for the later period (Wilcoxon matched-pairs test, P=0.844). No prominent behavioral differences between control days and disturbance days were found.

Next, we examined whether the waggle runs were related to the daily mass changes. A positive relationship between the number of the waggle runs and the daily mass change in the early period was found; this relationship became weaker in the later period (R^2=0.214, P=0.588 for the early period; R^2=0.157, P=0.671 for the later period; Tables 2, 3). We also examined the relationship between the number of disturbances and the daily mass change, but no relationship was found in the early period (R^2=0.07, P=0.800; Table 2), and we did not calculate this relationship for the later period because of the small number of samples.

Although the hive mass decreased steadily during this experimental season, we found that the daily mass reduction was enhanced by preventing dancing in only the first two days and that this enhancement was no longer found during the subsequent experimental days. The correlation coefficient between the number

of waggle runs and the daily mass change decreased over time (R^2=0.214 and 0.157 for the early and later periods, respectively; Tables 2, 3). These results suggest that dancing plays an important role in food collection and that bees are highly adaptable to a new artificial environment in which the information provided by the dance has become unavailable. The results of our study suggest that information transfer by dance communication plays a more dominant role in effective food collection than the release of scents, because we obtained results similar to those of previous studies (Sherman and Visscher, 2002; Dornhaus and Chittka, 2004) in which only spatial information was removed from the dance communication.

DISCUSSION
Daily changes in colony mass

All six colonies reduced their mass during the experimental period. This was an expected seasonal change for the species. In Japan, vigorous foraging flights are observed from March to early September. In autumn, however, the bees begin preparing for winter and the frequency of foraging flights gradually decreases. Thus, hive mass continuously decreases until early spring. We have observed this annual rhythmic change previously in a normal colony in Japan. Additionally, Sherman and Visscher (Sherman and Visscher, 2002) reported that colonies they tested in California lost hive mass in autumn and gained it in summer. These studies indicate that the permanent reduction in colony mass observed on the days of our experiment was not an artificial effect or the result of any abnormality of the colonies but rather the normal state for a healthy colony in this region. Interestingly, in the present study, colonies 4 and 6 in the CD group showed a large difference in the daily mass change between the control and disturbance days, i.e. the hive mass increased on the control day and decreased on the disturbance day. This suggests that the experiments in these colonies were performed under weather, hive and/or other conditions in which the dance was extremely effective for food collection. Autumn must be a good season for examining the efficiency of dance on food collection because colony mass increased under some conditions and decreased under others, with the result that we obtained a good contrast of results with different experimental procedures.

The daily mass change was extremely different among colonies and experimental days, as reported in previous studies (Seeley, 1995; Dornhaus and Chittka, 2004). Although bee loss is one of a number of factors in daily mass change, only 10–20 dead bees were found around the observation hive every day. Our preliminary data indicate that the mean body mass of a single bee is 0.07–0.08 g,

Table 2. Numbers of waggle runs and disturbances, and daily mass changes in the early period

	Waggle runs		Disturbances			
Colony	Runs (no. h^{-1})	Daily mass change (g 8 h^{-1})	Disturbances (no. h^{-1})	Daily mass change (g 8 h^{-1})	Group	Date
1	335.8	−2.3	n.d.	−18.6	DC	13–14 September 2007
2	427.6	−17.5	n.d.	−10.5	CD	13–14 September 2007
3	5.5	−7.3	14.7	−35.2	DC	27–28 September 2008
4	196.0	59.7	20.0	−77.1	CD	27–28 September 2008
5	21.0	−30.0	24.0	−41.0	DC	3–4 October 2008
6	826.9	44.8	25.6	−57.6	CD	23–24 September 2009

CD group, colonies that received no disturbance on the first day and disturbance treatments on the second day; DC group, colonies that received disturbance treatments on the first day and no disturbance treatments on the second day.

The median daily mass change for disturbance and control days was −38.1 and −4.8 g 8 h^{-1}, respectively; the mean daily mass change was −40.0 and +7.9 g 8 h^{-1}, respectively.

n.d., No data.

P-values for the correlation between daily mass change and the numbers of runs/disturbances were 0.588 and 0.800, respectively.
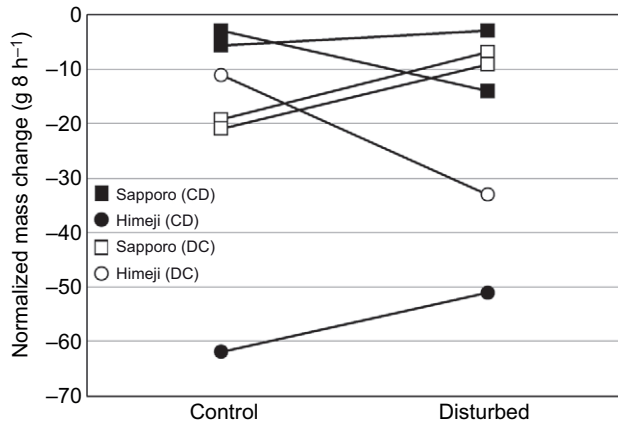
Fig. 4. Daily changes in hive mass during the later period (all experimental days after the second day). Six cases were divided into two groups: DC (open symbols) and CD (filled symbols). There was clearly no significant difference between the disturbance days and the control days (P=0.844, Wilcoxon matched pair test).

which indicates a 0.7–1.6 g loss in hive mass per day due to bee loss. We consider that bee loss had a negligible contribution to daily mass loss because this value is quite small compared with our daily mass change values. Another possible factor is water loss due to the removal of plastic covers during experiments. However, all plastic covers were removed during the experiments on both control and disturbance days. Thus, it is unlikely that water loss would have been consistently greater on only control or disturbance days under such experimental conditions. Furthermore, Meikle et al. (Meikle et al., 2008) reported that the main factor in the daily changes of hive mass is the change in the amount of stored honey. For these reasons, we regard our experimental data, i.e. the daily change in hive mass, as largely representative of the nectar store in the hive, i.e. food collection/loss. We cannot make any conclusions regarding the reasons for the variability in the change in hive mass because we did not control any bee behaviors, e.g. the number of foraging bees or the number of bees visiting a feeder. Clearly, more experiments are needed to reveal the environmental and colony-specific factors that produce a high variety in mass change among colonies as well as the factors affect the efficacy of waggle dances.

Adaptation of the honeybee colony

Differences in the mass change both between CD and DC colonies and between the early period and the later period suggest the highly adaptive capabilities of honeybee colonies when exposed to a new environment (Tables 2, 3). In the early period, the two CD colonies increased their hive mass on the control days and decreased their hive mass on the disturbance days, resulting in a large difference in the daily mass change between the control and disturbance days. In contrast, the three DC colonies showed smaller differences in the daily mass change between the control and disturbance days. This can be interpreted from the standpoint of the bees' adaptability. For the CD colonies, the disturbance occurred for the first time on the second day, and on the first day they could behave in the usual manner. Thus, we speculate that the CD colonies foraged and collected a large amount of food on the first day, the control day. In contrast, the DC colonies had already undergone disturbance on the first day. Thus, an adaptation at the colony level, e.g. reallocation of labor, had already begun and resulted in a reduction in collection efficiency on the next day, the control day.

During the experiments, the transparent covers were removed. This apparently provided potential space for building combs in parallel with the original comb plane, i.e. building a double- or triple-layered comb, although this apparent space vanished after the covers were replaced. Nevertheless, the honeybees gathered, hung down from the top of the observation box (Fig. 2), and formed a 'living chain' (Tautz, 2008) every experimental day. Although we did not confirm that the bees in the chain will start building the honeycomb afterward, we have called this a living chain here because the formation was extremely similar. It is well known that individual honeybees change their labors flexibly depending on the colony situation (Gould and Gould, 1988; Robinson, 1992; Seeley, 1995). It was clear in our experiments that labor had been reallocated for building a living chain. This would have led to a temporary change in the resources allocated to food collection. A smaller population of forager bees would of course translate into a smaller amount of collected food. In the later period, the disturbance no longer had this effect on the food collection (Fig. 4). This might have been attributable to the bees' adapting to their new foraging environment. As a honeybee colony is capable of adapting to a changing environment (Seeley, 1995), we speculate that the bees in the later period found a good way to simultaneously handle both maintaining a chain and collecting food.

Table 3. Numbers of waggle runs and disturbances, and daily mass changes in the later period

| Colony | Waggle runs | | Disturbances | | Group | Date |
	Runs (no. h^{-1})	Daily mass change (g 8 h^{-1})	Disturbances (no. h^{-1})	Daily mass change (g 8 h^{-1})		
1	105.6	−19.2	n.d.	−6.8	DC	17–18 September 2007
1	n.d.	−3.0	n.d.	−14.0	CD	21–22 September 2007
2	32.9	−5.7	n.d.	−3.0	CD	17–18 September 2007
2	1.5	−21.0	n.d.	−9.0	DC	21–22 September 2007
3	372.8	−11.0	12.5	−33.1	DC	1–2 October 2008
4	9.6	−61.9	9.4	−51.0	CD	1–2 October 2008

CD group, colonies that received no disturbance on the first day and disturbance treatments on the second day; DC group, colonies that received disturbance treatments on the first day and no disturbance treatments on the second day.

The median daily mass change for disturbance and control days was −14.0 and −19.2 g 8 h^{-1}, respectively; the mean daily mass change was −20.1 and −26.1 g 8 h^{-1}, respectively.

n.d.: No data.

The P-value for the correlation between daily mass change and the number of runs was 0.671. The P-value between daily mass change and the number of disturbances was not calculated because of the small number of data points.

Effects of dance on food collection

Sherman and Visscher (Sherman and Visscher, 2002) found that oriented dances resulted in significantly more successful recruits that arrived at the artificial feeder than disoriented dances, and that the colony lost mass while under a diffuse light treatment and gained mass while under an oriented light treatment. Interestingly, the efficacy of the dance communication was found only in winter (late December–early March), not in summer (late July–early September) or autumn (late September–early December), indicating that the effect of dance changes seasonally. We observed a dance effect by performing our experiments in late September–early October, although Sherman and Visscher (Sherman and Visscher, 2002) did not find such an effect during the same period, i.e. autumn. This was probably due to other differences between the studies, such as differences in the food distribution between Japan and the west coast of the US.

The experiments in all three cities, Sapporo, Himeji and Sanuki, showed similar results in that disturbance of the waggle dance reduced the mass of the hive. As Dornhaus and Chittka (Dornhaus and Chittka, 2004) discussed, the distribution of the potential food (flowers, in the case of their paper) has a major influence on the foraging strategy. They showed that obscuring dance information was insignificant for foraging in temperate habitats in Germany or Spain. However, foraging efficiency was significantly impaired in a tropical forest in India. These results indicate that dance behavior is effective in some habitats but not in others. In addition, they estimated, by calculating a patchiness coefficient for food distribution, that the food sites the bees indicated by dances in the tropical forest in India were more clustered than in the other two experimental sites (Dornhaus and Chittka, 2004). Although we do not know the detailed spatial distribution of food in the three cities used in the present study, in the experimental season flowers were obviously not randomly distributed and there was no markedly concentrated area of flowers around the experimental site. Thus, it should have been difficult, or at least not easy, for bees to find food during the experiment, suggesting that our experimental conditions in the three cities should be similar to those in the experiments in India by Dornhaus and Chittka (Dornhaus and Chittka, 2004). Considering our findings together with those of Dornhaus and Chittka (Dornhaus and Chittka, 2004), we conclude that dance information must help bees to find a food source when the food distribution is aggregated in a small area, i.e. when there is a distribution with a high deviation from random. If the food is randomly distributed, random searching might be sufficient for food collection. Computer simulations about bee foraging behavior essentially support this opinion that dance is beneficial when the food patch is hard to find (Beekman and Lew, 2008; Okada et al., 2010a; Okada et al., 2010b).

Thom et al. (Thom et al., 2007) collected air surrounding dancing bees and found four conspicuous substances – tricosane, pentacosane, Z-(9)-tricosene and Z-(9)-pentacosene – by solid-phase microextraction and gas chromatography coupled with mass spectrometry. The injection of a compound containing three odorant scents, tricosane, pentacosane and Z-(9)-tricosene, into the hive increased the number of worker bees exiting the hive, presumably foraging. Reinhard et al. (Reinhard et al., 2004a; Reinhard et al., 2004b) showed that odors can be a trigger leading honeybees to recall the feeders they have visited. Therefore, dance scent might influence mass change as a result of forages of bees that were activated by dance scent. Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004) removed only orientation information from the waggle dance by using disoriented light but allowing bees to dance. Therefore, in the studies by Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004), dancing bees would presumably have produced a scent that promoted worker bees to go out. Although it remains unknown whether the dance scent includes any information about food location, it is unlikely that scent contains specific geographical information. If so, the ratio of unsuccessful foraging flights would increase under conditions that allowed scent alone, because the bees would be activated to go foraging without spatial information. Hence, the colony might waste more energy when foraging is triggered by a dance scent alone because the flight requires 15- to 26-fold more energy than simply staying in the hive (Nachtigall et al., 1989; Rothe and Nachtigall, 1989). The main energy source for honeybees is nectar, and nectar is a main factor in mass change (Meikle et al., 2008). Therefore, some portion of the reduction in hive mass observed in these two studies (Sherman and Visscher, 2002; Dornhaus and Chittka, 2004) might reflect this unrewarded activity. In contrast, in the present study, we physically prevented bees from dancing and/or threw the dancing bees away from the colony in order to remove dance communication itself in the colony. This procedure would remove the potentially large chance that scent was released by dancers. Therefore, the dance-scent effect in our study should be much lower (maybe not absolutely removed) than in Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004). If the dance scent has a crucial function in food collection, it would be expected that the results of these two studies and those of our study would be quite different. However, because our study showed essentially the same results as the studies of Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004), we can conclude that dance scent does not greatly contribute to the promotion of food collection, although we cannot exclude the possibility that scent has an influence on effective food collection under some conditions. But in general, information transfer by dance communication is probably a more dominant factor for effective food collection compared with scent.

A positive relationship between daily mass change and the number of waggle runs was found in the early period and there was a weak relationship in the later period. Such a positive relationship was consistent with many previous studies, including the study by von Frisch (von Frisch, 1993). When bees were prevented from communicating the food location in that study, no correlation was found, just as in our study. In the present study, bees formed a living chain during the experiments and we often observed walking bees and sometimes dancing bees behind the living chain. To keep the colony behavior as natural as possible, the living chain was left in place. Thus, we could prevent runs and measure the number of runs only when possible without destroying the chain. If the behaviors occurred deep below the surface of the chain or if they could only be observed by destroying the chain, we did not observe them or count the number of invisible waggle runs. Therefore, we would have been able to obtain more detail about the relationships if we could have found a better way to disturb the dancing bees and count the waggle runs while leaving the chain intact. Additionally, we do not exclude the possibility that the number of disturbances may not have indicated the real number of runs that bees initially wanted to perform because we persuaded bees to stop dancing before they completed their runs. Thus, we realize that the number of the disturbances may have been underestimated. This might be the reason why the number of waggle runs on the control days was larger than that on the disturbance days. We also cannot exclude the possibility that observation error was caused by different sampling manners between disturbances and waggling runs. Although we counted the number

of disturbances for the entire day, we only counted waggle runs for 5 min every 30 min and the results were normalized in order to obtain an hourly value. It might be possible to find a relationship between daily mass change and the real number of runs when bees were disturbed, if the number could be estimated accurately.

Experimental design

Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004) found that the colony mass was reduced substantially more when hive mass was compared between oriented and disoriented dance colonies by using oriented and diffuse light treatments in natural foraging situations. Our results are consistent with their results when the dances were physically disturbed. Because our experiments were performed with only six colonies, the generality of our results is not clear, i.e. they may be specific to our particular experimental conditions. Indeed, we cannot fully exclude this possibility and realize that we would obtain clearer results if we made our experiments under well-organized, but artificial, conditions with sufficient repetition. However, in field studies like the honeybee dance study, sample sizes tend to be small because it is often very hard to prepare a large number of experimental colonies that are under similar conditions; for instance, it is difficult to standardize the number of bees in the hive, the amount of stored honey/water/pollen, and the foraging environment, and then to actually execute experiments in this large number of colonies. Many studies have thus used small numbers of colonies; e.g. Sherman and Visscher (Sherman and Visscher, 2002) reached their results from experiments in four colonies, and Dornhaus and Chittka (Dornhaus and Chittka, 2002) used only two colonies for each country. To overcome this problem of small sample size, we prepared colonies in both treated days and control days the same, as in previous works (Dornhaus and Chittka, 2002; Sherman and Visscher, 2002) and performed our experiments in the same manner in different cities, in different climatic regions, under different environments for foraging, in different years, and with colonies generated from different queens. If, under these conditions, a common result (or a certain tendency) could be obtained from even the small number of samples in each condition, then this result could be considered representative of a natural phenomenon, in the same way that a result from a large population of samples under a single experimental condition would be. Five out of six colonies showed a reduction in hive mass after disturbance. Therefore, we believe that our result was not akin to case studies but a general dance effect.

It is not likely that the change in hive mass in a negative direction resulted from the decrease in the number of forages of the dancers. In the present experiments, we disturbed only waggling behavior. Thus, disturbed bees could behave without any differences from other bees except for waggling, i.e. they were allowed to re-forage. Although we did not follow the details of the behavior of bees that had been disturbed (stressed), our preliminary observation showed that disturbed bees walked on the comb and later flew out from the hive as normal. Thus it is not likely that a decrease in the number of forages of disturbed dancers caused our results. Many studies have shown that many bees will visit a food source advertised by dancers even if the number of dancers is small at the start time of the experiment. For example, Seeley et al. (Seeley et al., 1991) began their experiment with 10–15 foragers in a colony (the colony contained approximately 4000 bees) that had been trained to visit a feeder (these bees were expected to perform dancing); they found that 4 h later, approximately 100 bees were recruited (10 times the number of initial bees), possibly in response to the dances of the initially trained foragers. That is, the number of dancers did not reflect the number of visitors or foragers. In a natural setting, the recruited bees would recruit novel potential foragers to a flower. This phenomenon is a kind of positive feedback system. The disturbed bees in our experiments might be similar to the initially trained bees in Seeley's experiments. If so, the number of disturbed bees must be sufficient to examine dance effects. In addition, the technique we used to prevent dancing should not have had any affect on bees other than dancers, because we touched only dancing bees with a fine, small paintbrush (smaller than one body size of a single bee). Thus, it is not likely that our results were caused by stopping the foraging of any other type of bees, such as potential followers. Therefore, we believe that our results were caused by disturbing dancing and thereby inhibiting an increase in the number of recruits (breaking the positive feedback loop), i.e. our results showed a dance effect, although we realize that we cannot fully exclude the possibility of other effects arising from our treatment.

As the control experiment, we left the colony without any relevant disturbances, e.g. disturbing non-dancing bees. This type of control experiment was also performed in Sherman and Visscher (Sherman and Visscher, 2002) and Dornhaus and Chittka (Dornhaus and Chittka, 2004). Neither of these papers stated any reasons for the application of their control experiments, but we speculate that they had the same reasons as we did. If we disturbed (administered a stressor to) followers, clearly a reduction in food collection would occur because far fewer foragers would fly out than when the followers were not disturbed. Even if we disturbed other bees in the hive, i.e. non-dancers or non-followers, this would not strictly be a control experiment because we did not disturb such bees on the disturbance days. If we obtained a similar result under this control experiment as our original result (i.e. the mass of the dancer-disturbed colony was reduced more than that of the other-disturbed colony), we cannot exclude the possibility that a degree of the reduction in mass of the other-disturbed colony was inhibited by administering a stressor. That is, disturbing dancers has no effect but administering a stressor to non-dancers has a positive effect on food collection. In the present study, we left the colony untreated on the control day with the assumption that the mass change in the control colony would indicate a putative mass change if the treatment had not been added. Therefore, we decided not to disturb any other bees. We believe that our control experiment is sufficiently functional for a first step, although we realize the importance of additional types of control experiments in future experiments.

We evaluated the dance effect using hive mass as an indicator. Because the change in hive mass reflects mainly nectar storage (Meikle et al., 2008), we regarded usage of hive mass as an index of food collection as appropriate. However, we do not exclude the possibility that the reduction in the net number of foraging bees by a reallocation of labor due to the experimental manipulations might impair the net food collection at the colony level. In the present study, we did not control the number or distribution of the food sources. Thus, we could not estimate the number of successfully visiting foragers that were recruited by dance communication. Clearer evidence for waggle dance effects on food collection might be obtained if the number of bees that leave the colony to forage as well as the number of successful visitors could be counted together with the measurement of hive mass.

Despite some disadvantages, the use of an uncontrolled food source gave the present study the distinct advantage of replicating natural bee behavior. In addition, the fact that we obtained similar results in three different cities in three different years means that our results were not location, colony or year specific, but rather reflected the natural behavior of the species.

LIST OF ABBREVIATIONS

CD group   colonies that received no disturbance on the first day and disturbance treatments on the second day

DC group   colonies that received disturbance treatments on the first day and no disturbance treatments on the second day

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