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## A new approach for the simultaneous tracking of multiple honeybees for analysis of hive behavior

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**Abstract** – Social activities are among the most striking of animal behaviors, and the clarification of their mechanisms is a major subject in ethology. Honeybees are a good model for revealing these mechanisms because they display various social behaviors, such as division of labor, in their colonies. Image processing is a precise and convenient tool for obtaining animal behavior data, but even recent methods are inadequate for the identification or description of honeybee behavior. This is because of the difficulty distinguishing between the large number of individuals in a small hive and their multiple movements. The present study developed a new computer-aided system, using a vector quantization method, for the identification and behavioral tracking of individual honeybees. The vector quantization method enabled separation of honeybee bodies in photographs recorded as a movie. This system succeeded in analyzing a huge number of frames quickly and can thus save both time and labor. Moreover, the system identified more than 72% of the bees in a hive and found and determined the active areas in the hive by extracting the trajectories of walking bees. In addition, useful behavioral data on the honeybee waggle dance were obtained using the present system.

behavioral tracking / honeybee / Apis mellifera / image processing

## 1. INTRODUCTION

Social activities are among the most striking of animal behaviors, and the clarification of their mechanisms is a major subject in ethology (Manning and Dawkins 1998). Honeybees are a good model for revealing these mechanisms because they display various social behaviors such as division of labor (Gould and Gould 1988; Tautz and Hubland 2008). A honeybee colony consists of tens of thousands of worker bees, one queen, and some hundreds of drones. While the queen expends her energy laying eggs, worker bees, depending on their age,

Corresponding author: T. Kimura, kimura@shse.u-hyogo.ac.jp Manuscript editor: Monique Gauthier clean and build the hive, take care of the brood and the queen, defend the hive from enemies, and leave the nest to search around the hive and collect food (Frisch 1967; Gould and Gould 1988; Tautz and Hubland 2008). Thus, individual bees exhibit different behaviors within the hive.

A unique communication behavior in honeybees, to share information regarding profitable food sources, was discovered by von Frisch (Frisch 1967). After a forager bee has returned to the hive from a successful foraging flight, it often performs a waggle dance to transfer information regarding the direction and distance of the discovered food source to other worker bees. Some workers (followers) surround the dancing bee (dancer), receive the information and then visit the food source that the dancer



has indicated (Dyer 2002; Esch et al. 2001; Frisch 1967; Gould and Gould 1988; Seeley 1995; Seeley and Visscher 1988).

To understand the mechanisms involved in such social behaviors, we must be able to precisely identify individual bees and provide a detailed description of their behaviors. These tasks have generally been achieved by manual analysis of film or video footage. For example, thousands of bees would be marked for an observation of a hive by means of numbers and/ or color combinations (Abramson and Bozic 2004; Gould and Gould 1988; Kirchner and Lindauer 1998; Seeley 1995; Seeley and Visscher 1988; Tautz 1996; Visscher and Seeley 1982). Some of the dancers and followers might then be tracked (Seeley 1994; Judd 1995) and various dance positions plotted (Seeley and Towne 1992; Tautz and Lindauer 1997). Thus, the dance precision was calculated in many behavioral experiments (Beekman et al. 2005; De Marco et al. 2008; Okada et al. 2008a, b; Towne and Gould 1988; Weidenmuller and Seeley 1999). However, such manual analyses require large amounts of time and labor.

Image processing is a useful method for the identification and tracking of multiple moving elements (Bodor et al. 2003; Bose et al. 2007; Zhao and Tao 2005). Some methods have been developed for studying sequential photographs of these movements, for example, tracking ants by body color and movement information (Balch et al. 2001); automatic identification of bees using a combination of k-nearest neighbor classification and a hidden Markov model (Feldman and Balch 2003); and tracking of unmarked bees using a Rao-Blackwellized particle filter and several principal images of bees (eigen-bees) (Khan et al. 2003). However, it is difficult to directly apply these methods for analyzing the social behavior of the animal group because they are developed for processing independent behaviors of a few individuals. One problem is that a background image with no tracking object is required for the extraction of tracking objects (Balch et al. 2001; Bodor et al. 2003; Bose et al. 2007; Feldman and Balch 2003; Khan et al. 2003; Zhao and Tao 2005).

Another is that marks on and/or numbers of tracking objects to be analyzed may also be required (Balch et al. 2001; Bodor et al. 2003; Khan et al. 2003). Methods developed for tracking a few individuals are generally inadequate for simultaneously tracking large numbers of individuals (Bodor et al. 2003; Bose et al. 2007; Feldman and Balch 2003). There are no methods that can simultaneously track hundreds of unmarked targets in a changing background. Thus, the development of suitable methods for analyzing animal behaviors is still worthy of considerable attention.

In the present study, a computer-aided system was developed for the identification and behavioral tracking of individual honeybees within a hive. The system was based on vector quantization and temporal contextual information. The vector quantization method (VQ) allowed honeybee bodies in time series photographs to be separated from one another without any preconditions. Individual bees were extracted from images using their body size and locomotion. This new system successfully tracked hundreds of bees simultaneously without the need for marking. The system identified more than 72% of the bees in the hive and successfully traced the trajectory of the waggle dance.

### 2. METHODS

## 2.1 Procedure

Despite widespread interest in the social behavior of honeybees, a number of factors, such as their small body size, the large population within a hive and the lack of pronounced external features on individual bees, make behavioral analyses problematic. To overcome these problems the following three steps were taken in the development of the analysis system: (a) extraction of a honeybee-code image from the whole hive image using the VQ; (b) separation of single and plural regions of honeybees from the honeybee-code image using an average honeybee body size and shape; (c) tracking of honeybee movement from temporal contextual information in sequential image data or movies.

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# 2.2 Extraction of honeybee body regions using VQ

VQ is used to separate many elements into several groups with approximately the same characteristics. Each group is represented by a centroid vector, which is called the "code vector" (CV) (Chen et al. 2000). This method has been applied to various fields, such as data compression, signal processing, and data clustering.

Normally, a color image consists of three color components: red (R), green (G), and blue (B). From quantization of the R, G, and B components of images, R was found to be the best. It seems likely that this reflects the warm colors such as yellow and orange which predominate in the honeybee body. Thus, the highest contrast image was obtained from the R image (Figure 1a). This image was split into small regions of 2×2 pixels, and each region was categorized according to spread and the sharpness of the image. Based on the 256 levels of each pixel each  $2 \times 2$  pixel region mapped a point (training vector (TV)) in four-dimensional space. Analyses were investigated using regions of different pixel numbers  $(3 \times 3, 4 \times 4, \text{ and more})$ . Increasing the pixel number within each region increases the space mapped but also increases distance between TVs and thus reduces the resolution of the image. The  $2 \times 2$  pixel regions were found to be the best for the separation of the honeybee individuals from their background.

In VQ applications the generation of good CV values is critical. Previously, it was extremely difficult to calculate CV values from the image data. However, the Linde–Buzo–Gray algorithm (Gray 1984; Linde et al. 1980) used in the current study automatically generated CV values from TV values using the following iterative process.

- 1. An initial CV (e.g., CV<sub>0</sub>) value was calculated using the average of all TV values.
- 2. A first CV (e.g.,  $CV_1$  and  $CV_2$ ) value was generated from the initial CV ( $CV_0$ ) value by the addition and subtraction of a small value to and from the initial CV value, respectively.
- The TV values were separated into two groups (TV<sub>1</sub> and TV<sub>2</sub>) based upon the new CV values (CV<sub>1</sub> and CV<sub>2</sub>).
- 4. The two CV values  $(CV_1 \text{ and } CV_2)$  were then each recalculated using the average of the

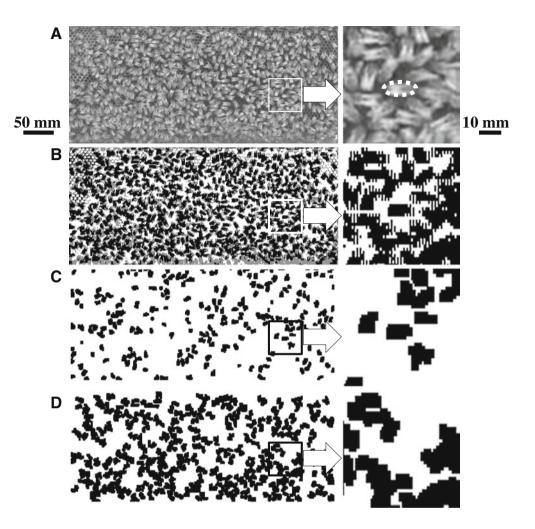
relevant reclassified TV values (i.e., from each  $TV_1$  and  $TV_2$ ).

- The process was repeated, adding further CV values and TV groups until a minimum Euclidean distance between the CV values and TV values was reached.
- Each CV was split into two vectors again. The same operation was carried out in a target vector space.

In a preliminary experiment, the number of CV values for classification was varied. It was found that eight CV values produced the best result for the separation of objects within the observation hive. These vectors correspond to the honeybee body, the dark and light of the honeybee wing, the hive, the hive frame, and the background and noise. Thus, eight CVs were needed to represent all of the objects. The vector distribution and quantization results for each region are shown in Figure 2. Each vector consists of four dimensions (v1, v2, v3, v4). The distribution of CV values was examined in 2 twodimensional graphs (v1-v2 plane, Figure 2a and v3v4, Figure 2b). The CV values, cv3 and cv5 representing the hive and honeybee body regions, respectively, are approximately at the center of those regions. In most cases, TV values could classify the central CV values for each region. The system employed in this study generated eight CV values from all of the TV values and extracted an image for honeybee body regions, called a "honeybee-code image," from each frame.

## 2.3 Separation of a honeybee-code image into single honeybee regions and plural honeybee regions

Two different types of regions in the honeybeecode image were extracted from the hive image. The first type was a single honeybee region (SHR) which consisted of a single honeybee, and the second was a plural honeybee region (PHR) which consisted of a group of two or more honeybees. First all of the SHRs were extracted from the hive image based upon the morphological characteristics of honeybees (Figure 1c). Subsequently, the PHRs were processed to separate individuals (Figure 1d). Morphological information, including the body size and shape of



**Figure 1.** The honeybee-code image and honeybee region. **a** A whole hive image. **b** A honeybee-code image obtained using VQ and the whole hive image **a**. The detected honeybee regions are indicated in black in **b**. **c**, **d** After repeating our procedure, a single honeybee region image **c** was obtained, which consisted of single honeybee regions alone. By subtraction of the single honeybee region image **f** on the honeybee-code image, the remaining image was defined as a plural honeybee region image **d**. The *boxed areas* to the right of each main image **a**–**d** are enlarged for comparison with the original images. The *dotted oval* in the enlarged area of **a** shows the approximation of the honeybee body to an ellipse shape.

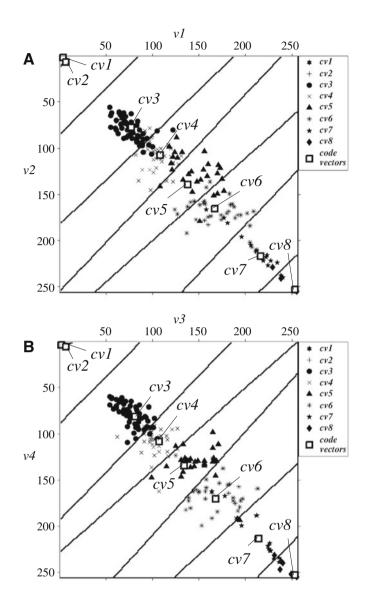
single honeybees from the images, was used for detection of the SHR. The shape of a honeybee was approximated visually as an ellipse in Figure 1a, and the size of the SHR estimated as  $100\pm18$  pixels from 20 manually selected ellipses. Similar sized regions (from 80 to 120 pixels) were extracted as the other SHRs from Figure 1b.

After the SHRs were processed, the individual honeybees in these regions were identified. Over-

lapping areas between the honeybee regions were noted. There were two possible overlapping areas in the SHR. The first type consisted of a temporal overlap between two SHRs that were in fact the same bee (Figure 3a). That is where the SHR for a bee #1 at time t is  $S^{1}_{t}$  and at time t+1 the SHR is  $S^{1}_{t+1}$  and the regions  $S^{1}_{t}$  and  $S^{1}_{t+1}$  overlap. The two images needed to be superimposed to identify the two honeybees as identical.

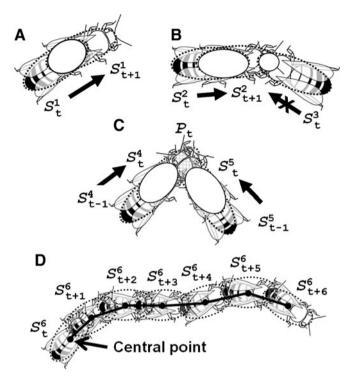
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Figure 2. The vector distribution and quantization results for each region. The position of each CV is shown as a *box* and is labeled. Other *symbols* represent vectors generated using VQ. The *solid lines* show the borders of each region. Four dimensions were used to generate these twodimensional graphs. In most cases, the training vectors could determine the central CVs for each region.



The second type of SHR consisted of an overlap among two or more SHRs (Figure 3b). When SHR  $(S_{t+1})$  overlapped with two or more SHRs at time *t*, the sizes of the overlapping regions were compared. For example, the size of the overlap between  $S_{t+1}$  and  $S_t^2$  were compared that between  $S_{t+1}$  and  $S_t^3$ . It was assumed that the largest overlap occurred between the same bee at different times. That is, the overlapping area between  $S_{t+1}$  and  $S_t^2$  was larger than that between  $S_{t+1}$  and  $S_t^3$  thus  $S_{t+1}$  and  $S_t^2$  represent the same bee and  $S_{t+1}$  can be renamed  $S_{t+1}^2$ . When PHRs were divided into SHRs using before and after frames, the identification of the honeybees was more complicated. When a PHR was separated into two or more SHRs, the honeybees were identified as follows (Figure 3c). A PHR at time *t* was named  $P_t$ . If this PHR overlaps with two SHRs at time *t*-1, i.e.,  $S^4_{t-1}$ and  $S^5_{t-1}$  then it is likely that the PHR  $P_t$  actually consisted of two SHRs  $S^4_t$  and  $S^5_t$ . Thus, the PHR is separated and identified and from then on is processed as an SHR. This process was repeated until no more PHRs existed which could be processed.





**Figure 3.** Numbering and tracking of individual honeybees. *Open circles* show the temporary areas of overlap between bees or bee images. The *dotted ovals* around the bees show the approximation of the honeybee body as an ellipse. **a** When a single honeybee region  $S_{t+1}$  at a given time t+1 was overlapped with one in the preceding image  $(S^{1}_{t})$ , we assigned the same individual number to this region, i.e., bee number #1 in this case. **b** When a single honeybee region was temporally overlapped with more than one single honeybee region, the individual number belonging to the largest overlapped region was assigned, i.e., bee number #2 in this case. **c** When two honeybee regions were merged into one region, the region was separated into two single honeybee regions by examining movement in previous and subsequent frames in this region. **d** The trajectory of a honeybee. The central points of an individual that was assigned the same number were connected along a temporal sequence.

Individual movements were tracked by calculating central points by averaging the coordinates of each extracted area. Central points calculated for the same honeybee for different times were connected sequentially. The honeybee trajectory was thus extracted (Figure 2d).

### 3. EXPERIMENTS AND RESULTS

#### 3.1 Experimental setup

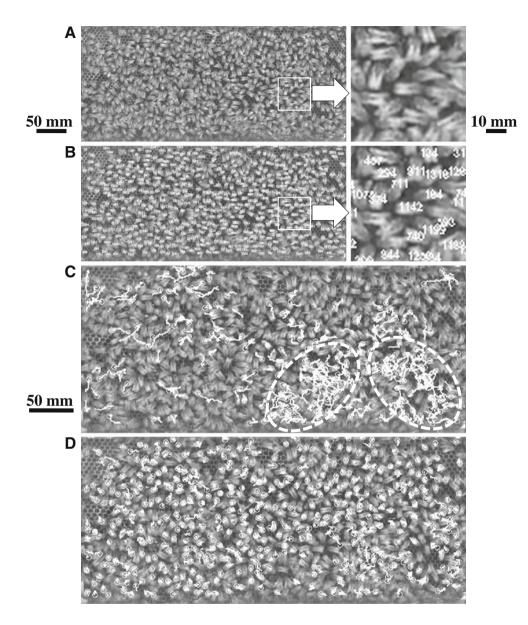
The behavior of honeybees, *Apis mellifera*, in an observation hive was recorded as a movie using a digital video camera (GR-HD1; JVC, Yokohama, Japan). The resolution was 720×

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480 pixels; the video frame rate was 29.97 frames per second, and the movie was recorded for a bee hive frame (width, 44.0 cm×height, 19.6 cm) (Figure 4a). In the movie, there were more than 700 honeybees. They had not been marked and could walk freely in the hive.

#### 3.2 Identification of honeybees

A 10-s movie (300 frames) was processed. The system was able to automatically number more than 500 honeybees in each frame (Figure 4b). Three frames were selected at random, and we calculated the percentage of correct identification by our software to the total



**Figure 4.** The identification and trajectories of bees. **a** A whole hive image. Only the *red* component of a color movie was used to create the hive image. **b** Identification of honeybees. The *number* on each honeybee indicates the individual number assigned using the study method. Enlarged images for **a** and **b** are shown on the *right hand side*. Most bees were identified, and the system only failed to detect a small number. **c**, **d** Trajectories of bees walking a long distance (**c**) or short distance (**d**). *Open circles* superimposed on the relevant individuals indicate starting positions. The method identified two areas of high activity in **c**. These are indicated using a *dotted line* on the image. The area to the *bottom right* was particularly active; this area was near the entrance of the hive and is well-known as the site of the waggle dances performed by bees returning from foraging. Indeed, dancing behavior was found in this area at this time.



number of individuals in each frame. The total number was a number of honeybees extracted and identified manually. More than 72% of the bees manually identified were also identified using the analysis system (510 of 704, 522 of 718, 516 of 700). This result indicates that most bees in a hive can be identified. The only limitation was that bees which only walk only a short distance overlap with other bees and these individuals were difficult to identify.

## 3.3 Tracking of honeybee movement

Individual trajectories were plotted from sequential temporal identification of individuals. The system succeeded in simultaneously tracking hundreds of honeybees in a single hive, resulting in the production of trajectories for more than 50% of all of the bees. As expected from the analysis of overlapping areas between single and plural honeybee regions, the tracking was easy for honeybees displaying characteristic movements, such as long distance walks and the waggle dance, but the tracking was difficult for bees that did not walk over a long distance. Thus, long distance walkers (Figure 4c) and short distance walkers (Figure 4d) were defined according to whether or not a honeybee moved more than 30 mm in 10 s.

The trajectories of long distance walkers determined in this study revealed the presence of active areas and static areas within the hive. Figure 4c shows the location of two active areas in the lower right part of the hive frame. As these areas were near the entrance of the hive, they corresponded to the site of dances performed by workers returned from foraging (Seeley 1994; Seeley and Towne 1992).

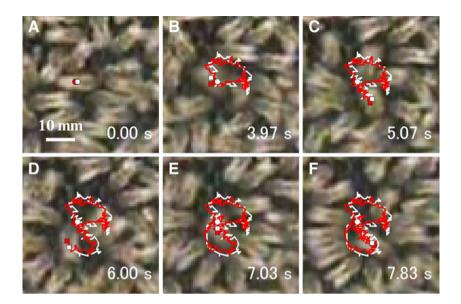
## 3.4 Extraction of waggle dance

In a hive, some bees have specific behaviors. The system employed in this study succeeded in extracting the trajectories of some of these, such as the waggle dance. The waggle dance consists of a combination of straight waggling walking and right- or left-turn walking to generate a "dance" in a figure eight. The white lines of Figure 5 show an example of the waggle dance in one of the active areas extracted using the system. Several parameters, such as the timing of, the duration (coding the distance of the food source from the hive), and the direction (indicating the direction of the food source from the hive) of the straight waggling walk can be calculated from this extraction. These data could be very valuable for ethological studies of honeybees. In Figure 5, the honeybee performed two waggle walks within 10 s. The analysis system developed and employed in this study obtained the behavioral parameters of the waggle dance as obtained by manual tracking. From the system, the durations of these walks were 1.10 and 0.80 s (1.10, 0.87 s from manual). The directions were 138.3° and 143.1° (131.6° and 145.0° in manual).

## 4. **DISCUSSION**

A new tracking system for honeybee behavior was developed utilizing a VQ. This system had two distinct advantages for identification and tracking. First, the system successfully identified about 500 of more of the 700 bees crowded into a small hive, including overlapping bees. This number is substantially greater than numbers extracted in previous studies where only several tens of the target insects were separated in the image (Khan et al. 2003). Second, this system simultaneously tracked about 350 bees, 50% of the entire hive. This is much more than the number of individuals tracked in previous studies, e.g., one bee (Khan et al. 2004), seven ants (Balch et al. 2001), or 20 ants (Khan et al. 2003). Nonetheless, although this new method can track the behavior of most honeybees either extracted as single or plural regions, there are some circumstances where this method does not work. Such cases are when one bee stands directly on top of another bee or when bees hide in holes in the hive structure. Thus, there are additional improvements that can be made for tracking such complex behaviors.

Further evidence of the power of this new system was its ability to track hundreds of



**Figure 5.** The trajectory of a dancer during a waggle dance. The *white* and *red lines* indicate the trajectories described by automatic and manual tracking methods, respectively. The dancer indicated by the *open circle* (**a**) was tracked during one cycle of a waggle dance. The *number* in the *lower right* of each image shows the time elapsed from the start of tracking. First, the bee moved in a wide circle from and back to the initial position (**b**). Then the bee made a first waggle walk (**c**). Next, the bee turned right and walked in a straight line (**d**), then returned to her initial position (**e**). Lastly, the bee made a second waggle walk (**f**). Overall, the path taken follows a figure eight.

objects under the relatively poor conditions. For example, the resolution of the images was  $720 \times$ 480 pixels for 44×20 cm, which is close to those used by Balch et al. (Balch et al. 2001) (640×480 pixels, 40×20 cm), but lower than that used by Khan et al. (Khan et al. 2003) (720×480 pixels, 15×10 cm). Our analyses were performed using natural sunlight, which is much poorer and less controlled than the light sources generally used in these experiments. Furthermore, the complexity caused by dynamic change in images is compounded as the number of targets is increased. Thus, tracking hundreds or thousands of objects also is much more difficult than tracking small numbers.

Our system could process a movie up to 3 min due to memory limitation. For a longer time movie, it is necessary to split it into the series of processable time movies. The utilization of a computer with a high-capacity memory will solve this problem. We can also process the longer time movie by reducing the frame rate, but it is not recommended because some important information of movements, such as overlapping, might be lost by the deduction, which will decrease the accuracy of tracking.

It is expected that the accuracy of tracking would decrease with time due to the complex movements of individuals. However, this problem will be avoided by modifying the trajectories of bees manually and visually as necessary. Additionally, the following attentions are recommended during image recording: (1) the focus of the digital video camera is accurate on the whole hive during the record, (2) the lighting is uniform on the whole hive, (3) a homogenous background and new combs are used for the record.

This computer-aided system using VQ was found to be highly applicable for bee experiments. A single colony of social insects like honeybees contains a large number of individuals. Conventional computational methods are limited by CPU, memory, and other hardware ceilings and cannot meet the requirements of



complex processing to deal with data sets for such a large number of targets simultaneously. However, VQ can select the essential information and drop the unessential data, thereby reducing the workload for the computer (Gersho and Gray 1992) and enabling the analysis of hundreds of bees simultaneously. To identify and track 700 individual bees manually would require more than 50 days for 11 frames of a 10s movie. Using the present system, this required only 1 h for 300 frames of the same movie.

These results indicate that the developed system is an effective tool for behavioral analyses in honeybee experiments. In addition, the system was able to at least partly resolve the problem of overlapping honeybees. Making improvements in the accuracy of individual identification by using a high resolution digital video camera and a motion estimation algorithm could resolve this problem. This system should ultimately not only be applicable to honeybees but also to the social behavior of human beings.

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Analyse du comportement dans la ruche: une nouvelle approche pour le suivi simultané de plusieurs abeilles.

suivi comportemental / abeille / Apis mellifera / enregistrement du comportement / traitement de l'image

Eine neue Methode für die gleichzeitige Beobachtung von zahlreichen Bienen zur Verhaltensanalyse in einem Bienenvolk

Verhaltensbeobachtung / Honigbiene / Apis mellifera / Bildbearbeitung

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