A Heuristic Trajectory Decision Method to Enhance the Tracking Performance of Multiple Honeybees on a Flat Laboratory Arena*

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In recent ethological studies, the behaviors and interactions of animals have been recorded by digital video cameras and webcams, which provide high functionality at reasonable cost. However, extracting the behavioral data from these videos is a laborious and time-consuming manual task. We recently proposed a novel method for tracking unmarked multiple honeybees in a flat arena, and developed a prototype software named “K-Track”. The K-Track algorithm successfully resolved nearly 90% of cases involving overlapped or interacted insects, but failed when such events happened near an edge of a circular arena, which is commonly employed in experiments. In the present study, we improved our K-Track algorithm by comparing the interaction trajectories obtained from forward and backward playing of video episodes. If the tracking results differed between the forward and backward episodes, the trajectory with lower maximum moving distance per frame is chosen. Based on this concept, we developed a new software, “K-Track-kai”, and compared the performances of K-Track and K-Track-kai in honeybee tracking experiments. In the cases of 6 and 16 honeybees, K-Track-kai improved the tracking accuracy from 91.7% to 96.4% and from 94.4% to 96.7%, respectively.

1. Introduction

Most ethological studies rely on accurate observations of animal behavior. Social mechanisms in animals are popularly observed in insects such as bees[1] and ants. The social behaviors of honeybees, such as the waggle dance discovered by Karl von Frisch in 1967[2], are of great interest to ethologists. Consequently, the honeybee has become a popular model animal for studying social and swarm behaviors. By monitoring and analyzing the individual honeybee behaviors alongside their social interactions, we could better understand the social structure and performance of a honeybee colony. In recent experiments, behavioral information was gathered by recording the trajectories and variation of animal movements within a circular arena [3,4]. Recording equipment such as digital video cameras and webcams, which provide high functionality at reasonable cost, acquire important ethological data on animal sociality. Video record-
this method, we developed a prototype software named “K-Track”[11]. The tracking performance of K-Track was evaluated on videos of 16 bees walking around a circular arena. The algorithm successfully tracked nearly 90% of all recorded interaction events, but failed when interactions occurred near a wall (edge) of the circular arena. Most of failure are caused by the honeybees’ nonlinear movement and the sudden directional changes of their bent bodies as they follow the curvature of the arena wall.

In the present study, we improve the existing K-Track algorithm by acquiring additional information on the motion properties of the tracking target. To this end, interaction events are extracted from both normal forward tracking and by time-inverted picture streaming. Based on this idea, we developed a new software named “K-Track-kai”, and validated its performance in tracking 6 and 16 honeybees on a circular arena. The novel results were compared with those obtained from the existing original K-Track algorithm as a reference.

2. Materials and Methods

2.1 Proposed tracking method using both forward and backward movies

The researchers had developed automatic tracking methods for target animals. In previous work, we had already proposed the automatic tracking algorithm for unmarked honeybees, named K-Track[11] (see Section 2.2).

Most of tracking methods including K-Track can track and predict movements of animals using forward-play movies(see “forward” in Fig.1). For example, there is the interaction situation of two bees such as “original” in Fig.2. Our K-Track processes it and gets tracking result of “forward” in Fig.2. On the other hand, the software can easily process it using backward-play movie(see “backward” in Fig.1) without changing the method and get tracking result of “backward” in Fig.2. Two tracking results are sometimes different such as this case, one of them had resulted from incorrect tracking, characterized by sud-
den changes in the moving distance and direction of the target between two continuous frames. Therefore, when comparing the forward and backward trajectories of the same interaction, we assumed that the maximum moving distance per frame in the incorrect tracking is larger than the distance in the correct tracking. In other words, we adopted this concept as the method to select one trajectory of two that has a lower change of moving distance per frame. The concept was adopted in our new program “K-Track-kai”, which improves our original K-Track algorithm (see Fig. 3). Our new program generates the sequential image data in the forward-time and backward-time directions from one original video. In interaction frames (see Fig. 1) around each overlap or merger event of two individuals, the K-Track-kai algorithm extracts the both individuals’ trajectories from both streams of sequential images. We defined each interaction frame by interval from start and end time of each interaction. Depending on the interaction situation, the frames extended from a few seconds to tens of seconds in our movies. In each case, whether the correct trajectory was the forward or backward tracking result (see Fig. 2) was decided by the following steps:

1. Generate sequential images in the forward- and backward-time directions.
2. Apply K-Track’s tracking process on the forward and backward image sequences. The method detects the time and position of two overlapped animals (Fig. 4).
3. Decide the routes of overlapped or interacted situations (Fig. 5). If the two tracking results differ, the method calculates all moving distances of both objects from the forward sequence $F_s(k)$, and from the backward image sequence $B_s(k)$, in a frame by frame manner. Here, $F_s(k)$ and $B_s(k)$ are the functions of moving distance per frame in forward time $k$. The moving distances of both $F_s(k)$ and $B_s(k)$ is measured as the following Euclidean distance.

$$F_s(k) = \sqrt{(x_{fk} - x_{fk-1})^2 + (y_{fk} - y_{fk-1})^2}$$

$$B_s(k) = \sqrt{(x_{bk-1} - x_{bk})^2 + (y_{bk-1} - y_{bk})^2}$$

where $x_{fk}$ and $y_{fk}$ are location data of forward movie at time $k$, and $x_{bk}$ and $y_{bk}$ are location data of backward movie at time $k$. The $B_s(k)$ is defined in the above function to use same interval of the $F_s(k)$. If the maximum value $\max B_s$ of $B_s(k)$ is lower than the maximum value $\max F_s$ of $F_s(k)$, the tracking result from the backward sequence is adopted. Otherwise, the tracking result from the forward sequence is discarded. In the case of Fig. 5, the honeybees were backtracked without sudden changes in distance and direction, and the $\max B_s$ of the backward trajectory was lower than the $\max F_s$ of the forward trajectory.

### 2.2 K-Track algorithm

Our proposed method based on K-Track algorithm. In this algorithm, we describe a summarization of the tracking process. The outline of the process is shown at Fig. 4. This process consists of two main steps for...
tracking bees; (A) Extraction of bee candidate regions from each frame, and (B) Identification of each individuals frame by frame.

In first step, the algorithm gets a background image before recording of target’s behavior or by generating from the movie. A series of foreground images is obtained by subtracting the background image from each frame of the movie. To extract the sizes and shapes of bees, all foreground images are converted to binary images using a predetermined threshold.

Second step aims to detect and identify all target bees from binary images using size, shape and spatiotemporal overlap relation. First, our algorithm calculates the number of pixels in a single bee region from the initial movie frames without the identification of bees, which also reveal the valid size range of single honey bees (RSS: Range of Single-bee Size). Next, each bee candidate region in each frame is classified into three categories based on region size; 1) SBS: the region fits within the RSS, 2) PBS: the region is bigger than the maximum RSS and 3) NBS: the region is smaller than the minimum RSS. Then, the number of regions in previous frame overlapped on each current region is divided into three classes; 1) NBR: No overlapping bee regions, 2) OBR: One overlapping bee region, and 3) TBR: Two or more overlapping bee regions. Using two-type categories (size and overlap), individual bees can be identified by applying following method to all candidate regions of all frames.

1. SBS and NBR: A new unique number is assigned to this region.
2. SBS and OBR: The bee region is assigned the ID number of the previous overlapping region.
3. SBS and TBR: The region contains two or more individuals. The correct region are delineated by the linear motion assumption.
4. PBS and TBR: The region PBS is divided into two or more SBSs by regional matching, using spatiotemporal contextual information between the current region and previous SBSs.
5. Other cases: No processing is executed.

2.3 Image data and computer

The videos were recorded at the laboratory of Karl-Franzens University Graz, Austria, and were also analyzed in our previous study[11]. Under the same conditions, we acquired several new videos for evaluating our new algorithm. In those videos, 6 or 16 juvenile female worker honeybees were entered into the flat circular arena (60 cm diameter) in a dark-room. A temperature gradient from 32°C on the right to 36°C on the left was imposed on the arena floor. The animal behaviors under infrared overhead lighting were recorded by an infrared-sensitive CCTV (Closed-Circuit TeleVision) camera. The videos were recorded in PAL format, generating (720 x 576)-pixel digital image sequences.

Our software was developed and evaluated on a personal computer (Intel Core i7-3970X, 3.50 GHz, 64 GB memory, 1TB SSD). The software was developed on a Windows 8 Enterprise 64-bit operating system, Visual Studio 2013 Ultimate (C++ 2013). We used OpenCV 2.4.10 as the image processing library.

3. Results

3.1 Tracking of 16 honeybees

Applying the above methods, we evolved our original algorithm K-track into K-Track-kai. To confirm the performance of our improved method, we applied K-Track-kai to three videos (Movie16-1, Movie16-2 and Movie16-3) of 16 moving honeybees. The same
Fig. 6 Five patterns of interaction between two bees. (A) Crossing, (B) Touching, (C) Passing, (D) Overlapping and (E) Waiting.

Table 1 Success rates of tracking 16 honeybees by K-Track-kai, K-Track and Ctrax software. The asterisk (*) marks indicate the improved items.

| Movie16-1 | K-Track-kai | K-Track | Ctrax[11] |
|-----------|-------------|---------|-----------|
| Occurrence | Success rate(%) | Success rate(%) | Success rate(%) |
| Touching  | 2            | 100.0    | 100.0     | 100.0    |
| Passing   | 3            | 100.0    | 100.0     | 66.7     |
| Waiting   | 10           | 100.0    | 100.0     | 40.0     |
| Movie16-2 | Crossing    | 1        | 100.0    | 100.0    |
|           | Passing      | 5        | 100.0    | 100.0    |
|           | Overlapping  | 1        | 100.0    | 100.0    |
|           | Waiting      | 15       | 100.0    | * 93.3   |
|           | Multiple     | 1        | 100.0    | 100.0    |
| Movie16-3 | Crossing    | 3        | 100.0    | 100.0    |
|           | Passing      | 18       | 100.0    | * 94.4   |
|           | Overlapping  | 2        | 50.0     | * 0.0    |
|           | Waiting      | 14       | 92.9     | * 85.7   |
|           | Multiple     | 9        | 88.9     | 88.9     |
| Total     | 84           | 96.4     | * 91.7   | 71.4     |

Videos had been previously applied in the evaluation of K-Track (Table 1) using five interaction categories ("Crossing", "Touching", "Passing", "Overlapping" and "Waiting") (see Fig. 6)[11].

We defined five categories as follows: (a) Crossing: Two bees touch at time \( T_1 \) and do not change their moving directions before and after the time \( T_1 \), (b) Touching: Two bees come from same directions. They touch at time \( T_1 \) and change their moving directions before and after the time \( T_1 \), (c) Passing: Two bees come from different directions. They touch at time \( T_1 \) and change their moving directions before and after the time \( T_1 \), (d) Overlapping: Two bees overlap at time \( T_1 \) and do not change their moving directions before and after the time \( T_1 \), (e) Waiting: After they touch at time \( T_1 \), one bee keeps stopping until another bee pass by.

By analyzing the backward results, K-Track-kai successfully tracked the honeybees in four out of seven trackings that had failed in K-Track. The total success rate was improved from 91.7% in K-Track to 96.4% in K-Track-kai (Table 1). The success rate of "waiting", a situation in which one bee remains still until passed by another bee, increased from 93.3% to 100.0% in ‘Movie16-2’ and from 85.7% to 92.9% in ‘Movie 16-3’. In both videos, K-Track-kai correctly tracked the two interaction events that were erroneously treated by K-Track. The success rate of the ‘passing’ and ‘overlapping’ situations also increased from 94.4% to 100.0% and from 0.0% to 50.0% respectively, because K-Track-kai successfully tracked the interactions that failed in the original K-Track. Moreover, K-Track-kai can improve two items in table 1 that K-Track has lower success rate than the current tracking algorithm Ctrax[11]. K-Track-kai has better performance in all items than Ctrax.

However, K-Track-kai failed to track the honeybees in three interaction events (‘overlapping’, ‘waiting’ and ‘multiple’) in ‘Movie16-3’ (Table 1). In the ‘overlapping’ event, K-Track-kai incorrectly selected the forward trajectory rather than the backward trajectory (Fig. 7). In the ‘waiting’ event, both the forward and backward trajectories were incorrect; consequently, K-Track-kai yielded the wrong results irrespective of the selection. In this study, K-Track-kai selected the forward trajectory because the maximum moving distance was identical in the forward and backward videos (Fig. 8). K-Track-kai did not im-
Fig. 7 Erroneous K-Track-kai results when the trajectories extracted from both forward and backward videos are incorrect. The white and black diamonds are start locations in tracking and two triangles are end locations. The start and end locations between forward and backward locate opposite positions. In this case, the forward trajectory is selected (The square with dashed line).

Fig. 8 Erroneous K-Track-kai results when the wrong trajectory is selected from the trajectories yielded by the backward and forward analyses. (A) Temporal changes of the tracking trajectories. The white and black diamonds are start locations in tracking and two triangles are end locations. The start and end locations between forward and backward locate opposite positions. (B) Graphs of moving distance between forward and backward results. In this case, the $\text{max} F_s$ and $\text{max} B_s$ are 2.24, respectively. In this case, the forward trajectory is selected (The square with dashed line in A).

prove the tracking results of ‘multiple’ events because the decision process operates only when two honeybees interact. Honeybee tracking by the original K-Track failed in one of 9 ‘multiple’ event cases. Because this failure was not corrected by K-Track-kai’s decision process, it appeared when the same video was
Table 2  Success rates of tracking 6 honeybees by K-Track-kai and K-Track software. The asterisk (*) marks indicate improved items.

| Movie, Duration      | Touching | Waiting |
|----------------------|----------|---------|
| K-Track-kai          | Success rate(%) | Success rate(%) |
| Movie6-1             | 4        | 100.0   | 100.0 |
| Movie6-2             | 3        | 100.0   | 100.0 |
| Movie6-3             | 8        | 87.5    | * 75.0 |
| Movie6-4             | 8        | 100.0   | 100.0 |
| Movie6-5             | 18       | 100.0   | 100.0 |
| Total                | 90       | 96.7    | * 94.4 |

analyzed by K-Track-kai.

3.2 Tracking of 6 honeybees

Both variants of our tracking software (K-Track and K-Track-kai) were applied to five videos (Movie6-1, Movie6-2, Movie6-3, Movie6-4 and Movie6-5) recording the motions of six honeybees. In interaction cases, the total success rate of K-Track-kai was 96.7%, versus 94.4% for K-Track. K-Track failed to track two out of eight ‘waiting’ cases in ‘Movie6-2’ and two out of 13 ‘waiting’ cases in ‘Movie6-4’. K-Track-kai provided the correct results in the one ‘waiting’ case that were failed by K-Track in ‘Movie6-2’ and the one in ‘Movie6-4’ (Table 2).

4. Discussion

K-Track-kai improved the tracking accuracy of our previous tracking software K-Track. In our previous study, K-Track failed a total of seven interaction events. All of those events occurred near the wall of the circular arena in the video tagged ‘Movie 16’[11]. In contrast, our present K-Track-kai algorithm successfully tracked four out of seven cases, and two out of five cases in ‘Movie 6’ that were failed by K-Track. This means that K-Track-kai corrected 40-70 % of the interacting events that were failed by K-Track. The K-Track-kai algorithm compares the two trajectories obtained from the forward- and backward-played image sequences of a video in interaction frames around an interaction event, then selects the trajectory with the lower maximum moving distance of the honeybees in each frame. The maximum distance was superior to other parameters (such as the average or sum of the moving distances) in the trajectory-selection process.

The improved tracking accuracy of K-Track-kai over K-Track means that when tracking interacting honeybees, a backward-running image sequence sometimes yields a more accurate trajectory than a forward-running sequence. The forward and backward trajectories may differ if the extracted shapes of the honeybees before their interaction differ in the forward and backward sequences. In this study, the shape of an interacting honeybee was estimated from its shape just before the interaction. Thereafter, the estimated shapes were used for estimating the honeybee locations. The accumulated locations produced the whole trajectories of the bees over time. Therefore, if a bee’s shape is distorted during the interaction and the altered shape remains in a few successive frames until the undistorted shape is recovered, the extracted shape immediately after interaction will differ from that before the interaction. In this scenario, the extracted shapes of the honeybees (used for estimating the shapes during the interaction) will differ in the forward- and backward-running sequences, generating different trajectories in the image sequences. In some cases, the honeybees’ shapes and positions during the interaction are more accurately estimated from the backward videos, which trace the distorted animal images after an interaction.

The failure results of tracking two-bee interactions by K-Track-kai were categorized into two types: (1) incorrect tracking in both forward and backward directions and (2) incorrect selection of the trajectory. The first type of failure occurs when the bee’s shape distorts only during the interaction, and recovers after the interaction. In this case, the trajectories from both the forward and backward image sequences can lead to incorrect tracking. In our present study, the second type of failure occurs only in overlapping interactions. If one bee moves over another bee to avoid being pushed aside, no unnatural changes in moving distance will appear. Therefore, the maximum mov-
ing distance informs an incorrect decision for choosing trajectories.

Our new software K-Track-kai can obtain the movement trajectories of plural animals from video data, enabling new ethological analysis and quantitative evaluation of animal behavior. Such studies will improve our understanding of the complex interaction-based self-regulation of social insect colonies, which have led to significant bio-inspired algorithms and robotic applications in past studies [13–15].

Animal tracking can reveal various behavioral properties, such as aggregation, scattering and avoiding. Such behaviors can be further quantified by considering factors such as the number of animals in an aggregation or the moving directions preferred by animals. Moreover, the energy consumptions of such behaviors could be estimated from the measured moving distance, speed and observed acceleration. Our software was developed for analyzing honeybee videos, but can be adapted to the analysis of various other animals by changing the possible target object shapes.

In K-Track-kai, the target is assumed as a solid object with a linear movement. Our experiments were performed on videos with a standard frame rate, but higher frame rates should improve the tracking accuracy by providing a smaller, more linear movement in each time step. As infrared light is a necessary requisite of biological honeybee experiments, we recorded the bees’ motions by an infrared-sensitive camera. However, infrared light degrades the image quality, rendering the tracking effort significantly more difficult. For example, if animals are recordable under normal light conditions, the animals in the images can easily be separated by considering the subtle differences in their color-shades. The resolution of the used infrared camera is not high and the contrast in the produced images is poor. Improving the contrast and resolution of the images would enable more precise segmentation of the target individuals. More advanced image acquisition equipment would yield finer images for extracting precise data on animal behavior. However, many video data have been already acquired under poor conditions. Quantitative measurements from these image data are important for utilizing the existing scientific resources.

Even in our current situation, our tracking software could be improved in several ways. We assumed that individuals are solid objects; in reality, an object’s shape can change during and after collisions. An elastic object model would further improve the estimations of shape and center of gravity of each object[16]. To improve the tracking accuracy, we could consider the irregularity of movement near the edge of a flat arena. However, these improvements would not entirely remove tracking errors in complex interactions and collision cases. Thus, in practical applications of our software, an additional manual tracking function, in which automatic tracking results can be edited and corrected by human operators, could be developed in future. To achieve this functionality, questionable tracking estimates must be automatically identified in the videos, then presented to the human operator. By admitting a human into the loop, K-Track-kai might achieve perfect tracking results (100% tracking accuracy) with minimum involvement of human handwork.

5. Conclusions

In this study, we improved our K-Track algorithm by comparing the interaction trajectories obtained from forward and backward playing of video episodes. In the case of tracking results are different between the forward and backward, we chose the trajectory with the smaller maximum moving distance per frame in our new software, K-Track-kai. In the cases of 6 and 16 honeybees, K-Track-kai improved the tracking accuracy from 91.7% to 96.4% and from 94.4% to 96.7%, respectively.

An automatic tracking is useful for various behavioral experiments. Our software has been already in use in analyzing not only bees but also other insects, such as ants and crickets. In future work, we will improve our software to adopt other types of animals.

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