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Video tracking analysis of behavioral patterns during estrus in goats

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Abstract. Here, we report a new method for measuring behavioral patterns during estrus in goats based on video tracking analysis. Data were collected from cycling goats, which were in estrus (n = 8) or not in estrus (n = 8). An observation pen (2.5 m × 2.5 m) was set up in the corner of the female paddock with one side adjacent to a male paddock. The positions and movements of goats were tracked every 0.5 sec for 10 min by using a video tracking software, and the trajectory data were used for the analysis. There were no significant differences in the durations of standing and walking or the total length of movement. However, the number of approaches to a male and the duration of staying near the male were higher in goats in estrus than in goats not in estrus. The proposed evaluation method may be suitable for detailed monitoring of behavioral changes during estrus in goats.
Key words: Behavior, Estrus, Goat, Video tracking

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Behaviors exhibited during estrus have significant importance for the successful mating of females and males in all mammals. Estrus behavior is characterized by three components: attractivity, receptivity and proceptivity [1]. Proceptivity is any behavior exhibited by a female that initiates or maintains sexual interaction with a male, which in goats includes approaching males, sniffing, mounting and tail wagging [2–4]. These behaviors are generally monitored by a human observer because direct observation is currently regarded as the best method for obtaining detailed data regarding specific behaviors. However, in large-scale investigations or studies accompanied by long-term continuous observation of each animal's behavior, there is a limit to the number of observations that can be made including detailed analyses of behavioral changes during the estrus cycle. Furthermore, the data obtained may be subjective and can vary among observers because the accuracy of the data relies on the observer's skill. Therefore, a more objective and quantitative analytical method is required to aid observations of estrus, such as in goats.

Automated video tracking systems for studying animal behaviors were introduced in the early 1990s, and they have been increasingly incorporated into studies of laboratory mice [5–7] and other small animals. In addition to the development of digital image processing technology, there are now various types of specialized computer software programs for animal tracking, which allow the positions and movement of subjects to be tracked automatically and the coordinates of the target's positions to be generated as time series data. Vector analysis of the trajectory data can be used to assess the activity of the subject by calculating the movement distance and the speed of movement. More complex behaviors such as social interactions between male and female mice can be identified automatically using statistical models [8, 9]. However, the practical use of video tracking systems is still limited to laboratory mice [6, 8] and small insects [10, 11].

Therefore, to develop a new automated quantitative method for assessing behavioral changes during estrus in goats, we attempted to monitor the behavior of goats using an automated video tracking system. The effectiveness of this method was also examined through comparisons with conventional video-recorded observation by a human observer. We focused on the proximity to the male and two behavioral patterns, i.e., "approaching the male" and "staying near the male," as indicators of proceptivity.

An observation pen was set up in the corner of the paddock where the female goats were housed (Fig. 1), with one side adjacent to the male paddock. In this experimental setting, we could obtain video tracking data from all the goats employed in this study. Based on the trajectory data shown in Fig. 2, the proximity to the male goat was investigated by assessing the position of the female goat relative to the distance from the male paddock (Fig. 3). The duration spent within 60 cm of the male paddock was longer ($P < 0.01$) for goats in estrus than the goats not in estrus. By contrast, the durations spent in the areas 120–180-cm area and more than 180 cm from the male paddock were shorter ($P < 0.05$ for both) in goats in estrus than in goats not in estrus.

The activities of goats during the 10-min observational period did not differ between goats in and not in estrus in terms of the durations of standing and walking and the total length of movement (Table 1). However, we observed that goats in estrus frequently approached the male, stayed near the male and sometimes made contact with the male through the bars before going away and then approaching the male again. Based on these observations, the two characteristic behavioral patterns, i.e., "approaching the male" and "staying near

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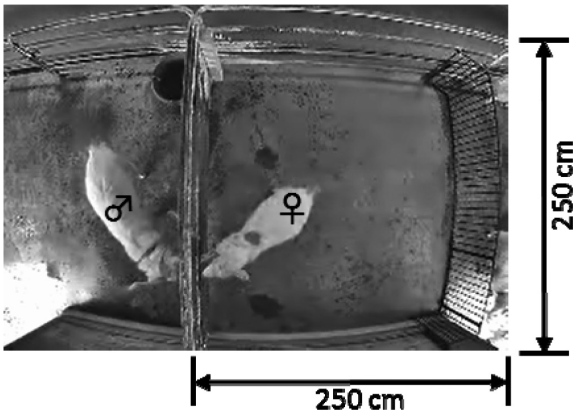

Fig. 1. Image captured from video recording data. One side of the observation pen was adjacent to the male paddock. A marker was attached to the back of the female goat to track goat movements.

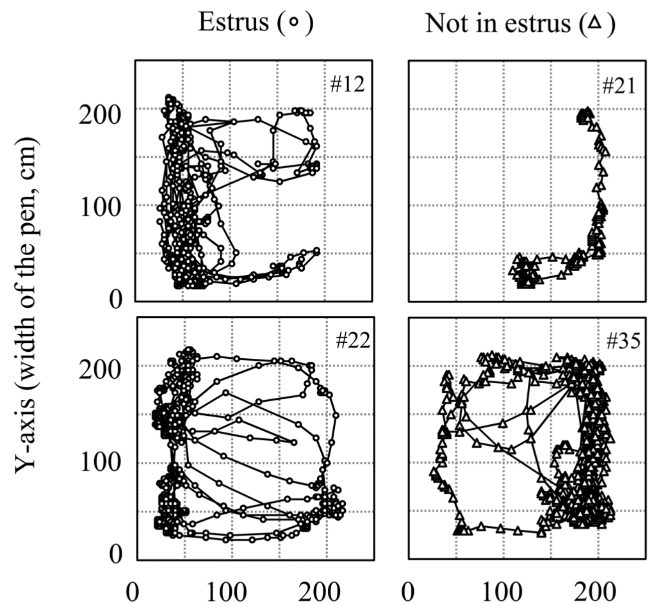

Fig. 2. Trajectory data obtained from individual goats in estrus (open circle) and not in estrus (open triangle). Data were plotted every 0.5 sec for 10 min.

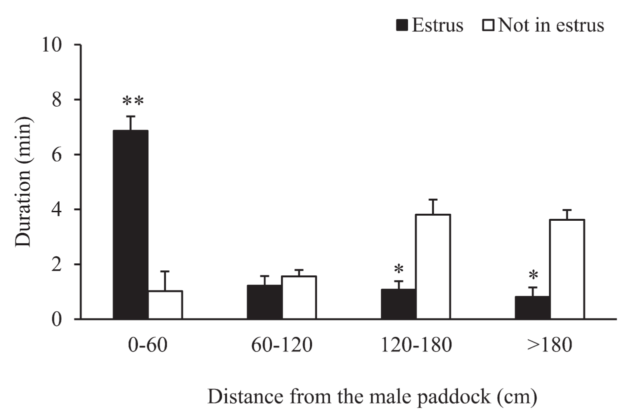

Fig. 3. Proximity to the male paddock during the 10-min observational period. Data represent the mean ± SE. * $P < 0.05$ and ** $P < 0.01$ compared with goats not in estrus.

the male," were assessed on a time-series graph, with the time scale along the horizontal axis and the distance from the male paddock along the vertical axis, as shown in Fig. 4. When the distance from the male paddock (corresponding to the x-coordinate of the marker) decreased from >120 cm to <60 cm within nine consecutive data points (corresponding to 4 sec), the status was considered to be "approaching the male". "Staying near the male" was defined when the x-coordinate of the marker was < 60 cm (corresponding to the area adjacent to the male paddock). The goats in estrus (left panels), frequently approached the male and stayed near the male (< 60 cm) more than half the observation time (particularly long in the case of #33). In contrast, the goats not in estrus (right panels) remained at the other side of the area adjacent to the male paddock. "Approaching the male" was detected in some goats not in estrus (#15 and #35), but the goats did not stay near the male. Thus, the vertical coordinate remained at a higher value (approximately > 120 cm) and did not drop below 60 cm.

The average concordance rates for "approaching the male" between video-tracking analysis and video-recorded observation by a human observer were 97.5% and 72.5% for goats in and not in estrus, respectively. For "staying near the male," the concordance rates of the two methods were 84.5% and 82.2% for goats in and not in estrus, respectively. The results obtained by video-tracking analysis and video-recorded observation by a human observer indicated that the number of approaches to the male and the duration of staying near the male paddock were significantly higher for goats in estrus than goats not in estrus (Table 1).

The ultimate goal of the present study was to develop an automated quantitative method for analyzing sexual behaviors during estrus in Shiba goats using tracking data obtained from video recordings. The video tracking technique that we established appears to be satisfactory for acquiring precise measurements of behavioral changes in goats, and it can probably be applied to other animals of a comparable size, such as sheep. Previously, the behavioral patterns of Shiba goats in estrus and the associated endocrine mechanisms have been studied in detail based on intensive direct observations [3, 12, 13]. In the present study, our behavioral analysis based on video tracking data quantitatively detected goats in estrus, which approached the male more frequently and spent more time near the male goat. The two behaviors, i.e., "approaching the male" and "staying near the male," identified using video tracking data, agreed well with the video-recorded observations by a human observer. Our results suggest that using the experimental setting employed in this study, these parameters can be employed as reliable indicators of proceptivity in female goats. More long-term observational studies, such as continuous observations during one estrus cycle, may help to elucidate the time-course changes in estrous behaviors, including their intensity and duration.

When female goats are in estrus, they repeatedly approach the

Table 1. Behavioral patterns of goats in and not in estrus analyzed using video tracking data (*Tracking*) and video-recorded observations by a human observer (*Observation*)

Item	Analysis	Estrus (n = 8)	Not in estrus (n = 8)
Duration of walking (min/10 min)	Tracking	1.1 ± 0.2	0.7 ± 0.2
Total length of movement (m/10 min)	Tracking	59.6 ± 8.0	43.4 ± 10.8
Duration of standing (min/10 min)	Tracking	6.4 ± 0.5	7.1 ± 0.7
Number of approaches to the male (times/10 min)	Tracking	4.5 ± 0.6*	1.9 ± 0.8
	Observation	4.6 ± 0.6*	2.4 ± 0.8
Duration of staying near the male (min/10 min)	Tracking	6.9 ± 0.5**	1.0 ± 0.4
	Observation	6.8 ± 0.5**	1.0 ± 0.4

Data represent the mean ± SE. * $P < 0.05$ and ** $P < 0.01$ compared with goats not in estrus.

male and wag their tail, before moving away and then approaching the male again [4]. Tail wagging may visually attract males from a distance and/or help distribute pheromones from the anogenital region [18]. In the present study, we did not include this behavior for the analysis because it cannot be identified by the video-tracking method we employed. However, given the importance of this behavior in the process of successful mating in goats, it may be worth establishing a methodology that can automatically measure the tail wagging rate by video tracking or any other alternatives techniques. Alternatively, it has been reported that female goats in estrus prefer to be in close proximity to males rather than females [14], whereas female–female interactions, such as female–female mounting, are more frequent when a male is not present [15]. These findings suggest that the display of sexual behaviors is influenced by the presence of male goats. In the present study, the observation pen was set in the area adjacent to the male paddock, thereby allowing us to determine the proximity of female goats to males. When the x-coordinate of the marker was < 60 cm, it indicated that the target goat was located in an area adjacent to the male paddock, where they could choose to make contact with the male through the bars of the pen. This pattern was confirmed by human observations, which showed that the duration of remaining near the male paddock was longer for goats in estrus than goats not in estrus. Female goats exhibited the highest frequencies of proceptivity when the male goat was confined to a small pen, whereas the frequency of proceptivity was the lowest when the male goat was completely free to interact with the female goats [15]. Thus, the experimental conditions employed in our study might have helped to stimulate proceptivity in goats in estrus. Consequently, the analysis of the video tracking data clearly recognized the behavioral pattern of staying near the male paddock for a long time as a sign of estrus. In contrast, the goats not in estrus preferred to stay far from the male, which was reflected by the longer time spent >160 cm from the male paddock. As an index of proximity to male goats, a previous behavioral study recorded the positions of female subjects with respect to the male, with the female goats considered to be in close contact with the male if they were < 150 cm from the male [16]. Thus, staying > 160 cm from males may be a sign of sexual non-proceptivity.

It is generally recognized that animals in estrus are more active than when not in estrus. In cows, the physical activity levels recorded by pedometers were found to be 2–4 times higher during estrus compared with those when not in estrus [17]. Similar results were obtained in dairy goats [18] when their activities were monitored continuously throughout the estrus cycle. However, pedometers simply assess the activities of animals by measuring their acceleration rate, whereas video-tracking analysis can yield more detailed information, including the actual lengths of movements, velocity of walking and positioning information. We observed that the activity parameters did not differ between goats in and not in estrus, which might have been related to the experimental setting, in which the female subjects were allowed to make contact with the male through the bars and the goats in estrus spent more than half of the observation time staying near the male paddock. The close proximity to a neighboring male pen might have contributed to the decreased activity parameters, as observed in a previous study in which the estrus activities of goats were monitored using pedometers [18]. From a methodological perspective, our data were derived from the analysis of 10-min observational periods, and it might have been difficult to determine the possible differences in physical activity between goats in and not in estrus within this short period of time. Nevertheless, the positioning information and other variables that can be calculated by vector analysis based on video tracking data are useful for assessing more complex features of goat behavior (e.g., proximity to males).

In summary, the present study reports the first analysis of behavioral changes associated with estrus in goats based on video tracking data. The trajectory data showed that the total movement lengths during the 10-min observation period did not differ between goats in and not in estrus. However, the quantitative behavioral analysis based on the video tracking data indicated that goats in estrus spent more time near the male paddock compared with goats that were not in estrus. The application of this technique to behavioral studies of goats may help to clarify novel aspects of estrus behaviors in goats, which might be difficult to detect or quantify based on direct observations.

Methods

Animals and housing

Adult female Shiba goats maintained at the Tokyo University of Agriculture and Technology were used in this study. Six to eight goats were housed in a paddock with an outside area of 25 m^2, a sheltered area of 15 m^2 and a natural photoperiod. Shiba goats are annual breeders in natural daylight conditions in Japan and have been used as an experimental model of ruminants. The goats were fed maintenance diets of alfalfa hay cubes twice a day (0900 h and

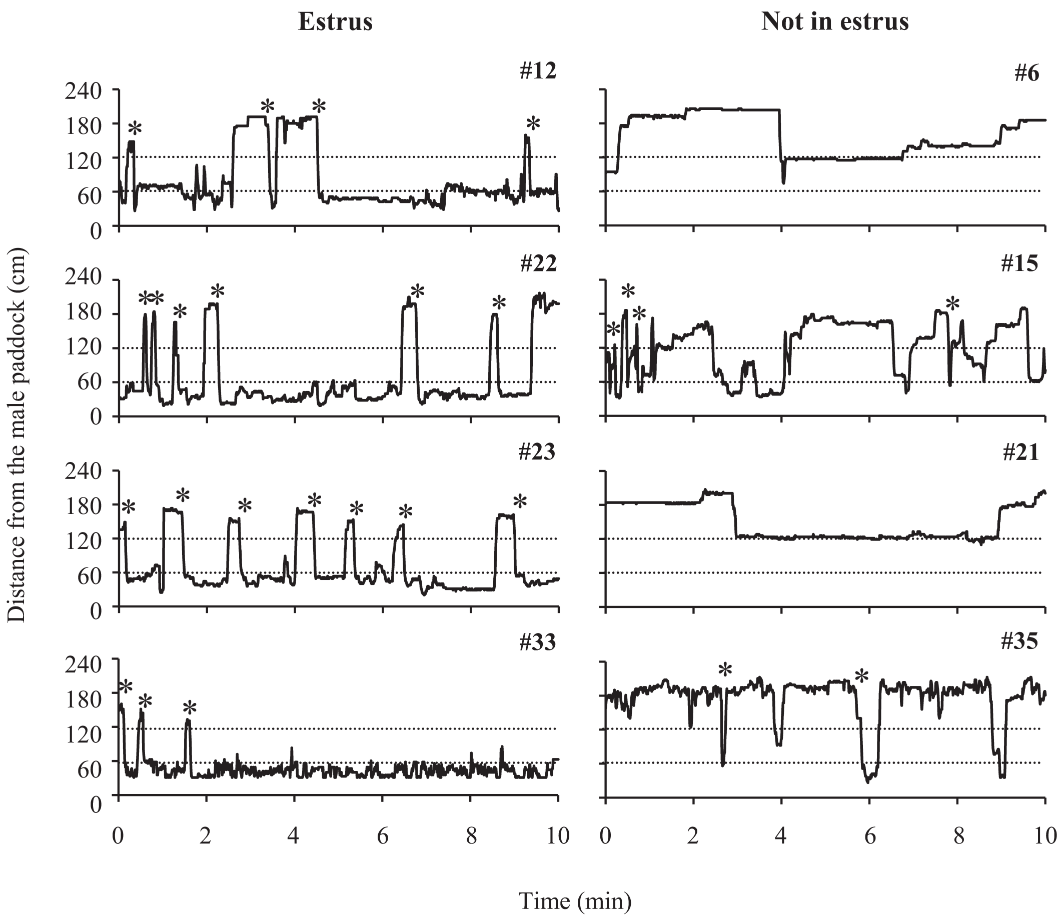

Fig. 4. Time-series graph showing goat movements, with the time scale along the horizontal axis and the distance from the male paddock along the vertical axis. Data were obtained every 0.5 sec for 10 min. When the distance from the male paddock decreased from > 120 cm to < 60 cm (horizontal dotted lines) within nine consecutive data points (corresponding to 4 sec), the status was considered to be "approaching the male." Asterisks indicate the occurrence of "approaching the male" according to video-tracking analysis.

1500 h). Clean water and mineralized salt were provided *ad libitum*. All the procedures were approved by the University Committee for the Use and Care of Animals at Tokyo University of Agriculture and Technology (#27-18).

Video recording

The study was conducted between November 2014 and January 2015. All of the female goats were checked for estrus once or twice daily and were considered to be in estrus when they allowed mounting by a male goat. We tested 16 goats (age = 2–9 years, body weight = 21–37 kg) that we confirmed as being in estrus (n = 8) or not in estrus (n = 8) between December 2014 and January 2015. An observation pen (2.5 m × 2.5 m) was set up in the corner of the female paddock, with one side adjacent to the male paddock. A network camera (DG-SF334, Panasonic Corporation, Kadoma, Japan) was fixed to the ceiling. A captured image from the video recording data is shown in Fig. 1. Before beginning the observations, one male goat was tied loosely with a rope to the adjacent side of the observation pen. A bright-colored circle marker (red or blue, 12 cm in diameter) was attached to the back of female goats to enable identification and tracking in video-recordings. The female goats were then moved gently into the observation pen by one observer (the first author of this paper: NE). The female and male goats were allowed to partially contact each other by poking their snouts through the bars of the adjacent side of the observation pen. After an adaptation period of approximately 10 min, the behaviors of the goats were recorded for 10 min using a network camera recorder (BB-HNP17, Panasonic) connected to a personal computer. The computer was located in a building at a distance from the goat paddocks. During video recordings, the observer remained in the building to prevent disturbing the behaviors of the goats.

Behavioral analyses by human observation

After finishing video recording, behavioral observations were performed by a well-trained observer (NE) using the video recordings. Based on previous descriptions of sexual behaviors during estrus in goats [1], we investigated the occurrence of two behaviors as indicators of proceptivity: "approaching the male" and "staying near the male.” The following criteria were used to define each behavior:

Approaching the male: Walking or running from the other side of the observation pen toward the area adjacent to the male paddock.

Staying near the male: Staying or behaving restlessly in the area adjacent to the male paddock and occasionally contacting with the male through the bars of the pen.

The data frames were scored as 0 (no behavior) or 1 (occurrence of a specific behavior) to analyze each behavior in terms of its frequency or duration. The cumulative period of time was expressed as the duration of the behavior.

Behavioral analysis based on video tracking data

The video recordings were processed using the ABDigitizer software (Chinou Jouhou Shisutemu, Kyoto, Japan). The positions and movements of the markers attached to the backs of the goats were tracked automatically, and the x- and y-coordinates of the central point of the marker were outputted every 0.5 sec. Thus, a 10-min video tracking dataset comprised 1200 frames.

Based on the results of the human observations, the following criteria were employed to identify each behavior from the video tracking data. The movement distance between two consecutive data points was determined by calculating the Euclidean distance, which represented the speed of movement (cm/0.5 sec).

Standing: Staying in one place and exhibiting no particular behavior. When the distance moved between two consecutive data points (corresponding to 0.5 sec) was < 3 cm, the status was considered to be “standing.”

Walking: When the distance moved between two consecutive data points was > 13 cm, the status was considered to be “walking.” The walking speed threshold (13 cm/0.5 sec) was determined as the mean minus one SD based on six control measurements from representative datasets. The range between 3 and 13 cm was used as the buffer zone.

Approaching the male: When the distance from the male paddock (corresponding to x-coordinate of the marker) decreased from >120 cm to < 60 cm within nine consecutive data points (corresponding to 4 sec), the status was considered to be “approaching the male.” These criteria were based on the analysis of human observation, in which a goat walked from the other side of the observation pen to the area adjacent to the male paddock and the x-coordinate of the marker decreased from 120 cm to < 60 cm within nine consecutive data points (the mean plus SD based on six control measurements).

Staying near the male: When the x-coordinate of the marker was < 60 cm (corresponding to the area adjacent to the male paddock), the status was considered to be “staying near the male.”

Statistical analyses

All of the data were expressed as the mean ± standard error (SE). Parameters related to the behavioral characteristics were analyzed using the Student’s *t*-test to compare the means between two groups (in estrus or not in estrus). Significant differences were accepted when $P < 0.05$. The concordance of the results obtained by video-tracking analysis and video-recorded observation by a human observer was examined according to a previously described method [9]. Briefly, an event was considered to be concordant if the behavior identified by video tracking analysis occurred within the range of three time points before or after the time point recorded in human observations. The concordance rate was calculated by dividing the number of concordant events (true positive) by the sum of the true positives, false positives and false negatives.

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