RESEARCH ARTICLE | OCTOBER 19 2020

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Takaharu Shokaku ✉ ⓘ ; Toru Moriyama ⓘ ; Hisashi Murakami ⓘ ; Shuji Shinohara ⓘ ; Nobuhito Manome ⓘ ; Kazuyuki Morioka ⓘ

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Takaharu Shokaku,[1,a)] **Toru Moriyama,**[2] **Hisashi Murakami,**[3] **Shuji Shinohara,**[4] **Nobuhito Manome,**[4] and **Kazuyuki Morioka**[1]

AFFILIATIONS

[1] Department of Network Design, Meiji University, Nakano, Tokyo 164-8525, Japan
[2] Faculty of Texitile Science and Technology, Shinshu University, Ueda, Nagano 386-8567, Japan
[3] Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Tokyo 153-8904, Japan
[4] Faculty of Engineering, The University of Tokyo, Bunkyo, Tokyo 113-8656, Japan

[a)] Author to whom correspondence should be addressed: tshokaku2@gmail.com

ABSTRACT

In recent years, various animal observation instruments have been developed to support long-term measurement and analysis of animal behaviors. This study proposes an automatic observation instrument that specializes for turning behaviors of pill bugs and aims to obtain new knowledge in the field of ethology. Pill bugs strongly tend to turn in the opposite direction of a preceding turn. This alternation of turning is called turn alternation reaction. However, a repetition of turns in the same direction is called turn repetition reaction and has been considered a malfunction of turn alternation. In this research, the authors developed an automatic turntable-type multiple T-maze device and observed the turning behavior of 34 pill bugs for 6 h to investigate whether turn repetition is a malfunction. As a result, most of the pill bug movements were categorized into three groups: sub-diffusion, Brownian motion, and Lévy walk. This result suggests that pill bugs do not continue turn alternation mechanically but elicit turn repetition moderately, which results in various movement patterns. In organisms with relatively simple nervous systems such as pill bugs, stereotypical behaviors such as turn alternation have been considered mechanical reactions and variant behaviors such as turn repetition have been considered malfunctions. However, our results suggest that a moderate generation of turn repetition is involved in the generation of various movement patterns. This study is expected to provide a new perspective on the conventional view of the behaviors of simple organisms.

Published under license by AIP Publishing. https://doi.org/10.1063/5.0009531

I. INTRODUCTION

Long-term measurement and analysis of animal movement can provide new insights into behavioral characteristics. For such tong-term analyses, it is desired to automatically observe animal movements without burdening the experimenters. In recent years, various automated tracking systems in ecology have been developed to obtain accurate and highly resolved behavioral data.[1] These systems also have the advantage of reducing human effort for tracking.[2,3] For example, a tracking system to observe the long-term behavior of small animals quantitatively has been developed.[4] A system that enables real-time tracking has also been developed.[5] It is more difficult to perform tracking that distinguishes multiple individuals than tracking single individuals, but some systems that track multiple individuals have also been developed.[2,3,6–9] Furthermore, in recent years, systems that enable real-time observation of multiple individuals have been developed.[10,11] Tracking systems have also been developed in natural environments, not only in the laboratory.[12–15] Animal observation instruments also have a purpose of reducing disturbance under experimental environments. Noises that affect the behaviors of animals must be removed in experiments. Then, a noiseless tracking system for multiple individuals has been developed.[16] Automated long-term observation of animals can acquire accurate movement trajectories that could not

be measured manually. As an example of a system to acquire trajectories, there is an instrument that observes a millimetric-sized walker such as ants.[17] There is a system for analyzing the relationship between visual and spatial memory in fruit flies.[18] The system can measure trajectories with movement distance over 1000 times or more the length of a subject. In addition, there is an instrument for tracking a subject at a wider angle than a conventional fixed camera[19,20] and a system for obtaining three-dimensional trajectories of aquatic microorganisms.[21] A behavior measurement system for small animals using a force plate is also proposed.[22] This system does not rely on image processing. Most of the above instruments can record a large amount of unrestricted behaviors of the target animals in wide spaces without directly interfering with the animal. On the other hand, behavior analyses in ethology sometimes require extracting a specific behavior of animals, not unrestricted behaviors. The conventional instruments will need longer observations to extract enough of the specific behavior from unrestricted behaviors. An experiment in which the environment is manipulated to increase chances of observing a specific behavior is called an experimental observation.[23] There are few studies to develop instruments for automatic and long-term observation in the viewpoint of experimental observations. As one of few examples for actively manipulating the environment, there is a system for examining the relationship between the phototacticity and movement of microorganisms.[24] This study aims to develop an automatic observation instrument that specializes for the turning behavior of pill bugs based on the experimental observation. Then, the instrument can measure enough amount of turn alternation (TA) behaviors for analyses to obtain new knowledge in the field of ethology. In particular, this study aims to discover movement generation of pill bugs by spontaneous manifestation based on some decision-making, not only by mechanical reaction. This requires observing many of the alternation behaviors of pill bugs by the proposed unique instrument. When pill bugs are forced to turn in an L-shaped passage and walk freely after exiting the passage, they tend to move forward while turning in the direction opposite to that of the passage. Such alternation of turns is found in a wide range of species (e.g., unicellular organisms,[25] insects,[26] rodents,[27] and humans[28]) and is thought to be beneficial in foraging and exploration.[29–32] In isopods, including pill bugs, this alternation of turns is called turn alternation (TA),[33,34] and its mechanism and adaptive significance have been actively studied.[32–54] However, pill bugs occasionally elicit turn repetition (TR).[53] This is a phenomenon in which the animal turns in the same direction as previously (such as when they are forced to turn in an L-shaped maze) and has been considered as a failure of TA due to exogenous disturbance. However, it has been suggested by behavioral experiments with pill bugs that the occurrence of TR is related to the generation of adaptive behavior.[52,55,56] For example, in an experiment by Moriyama, the subject was placed in a multiple T-maze apparatus and was continuously given a T-junction 200 times.[52] Then, in each subject, the rate of occurrence of TA every 10 trials was calculated, and the time sequential change of the TA rate was analyzed. As a result, the rate was stable at a high value in half of the subjects. However, in the other half, TR often appeared at a high frequency, and the TA rate varied greatly. Subsequently, the subjects were given a dead-end multiple T-maze task. Then, the population that had varied the TA rate greatly climbed the wall of the passage and escaped the maze. Because the escaped

individuals eventually reached a dark, moist place suitable for survival, climbing the wall is considered to be an adaptive behavior for survival. These results suggest that some TRs are endogenous and are closely associated with the generation of adaptive behaviors. However, there had been no experimental result showing that generation of TR directly affects the behavior of pill bugs and provides benefits for their survival. To be the first case, Murano et al. devised a method to represent a sequence of turns obtained from the multiple T-maze task of a pill bug as a trajectory on a plane and analyzed the effect of TR on the formation of the trajectory.[57] In this method, a zigzag trajectory in which TAs emerged continuously was defined as a straight movement, and a TR was defined as directional change. As a result, the sequence of turns of each subject was expressed as a trajectory in which straight lines of various lengths were connected in various directions. For analysis, Murano et al. counted the number of straight movements of length l from the trajectories of all subjects and examined the probability distribution of l.[57] It was found that the shape of the distribution was a long-tail type formed by a large number of short straight movements and a very small number of long straight ones, and the distribution was well fitted to the distribution of the Lévy walk model.[58] Theoretically, a Lévy walk-like movement is expected to allow animals to make the most efficient search for ecological resources,[59,60] and the validity of this hypothesis has been investigated in experiments and observations of various species (insects,[61–63] jelly fish,[64] marine vertebrates,[65–67] seabirds,[68] and bacteria[69]). In pill bugs, a free-walking experiment using a servosphere device confirmed a Lévy walk-like behavior.[70] It is interesting to note that Murano et al. suggested that even in the limited condition of a multiple T-maze, pill bugs moderately elicited directional changes (i.e., TRs) to generate a variety of the lengths of straight movements (i.e., sequences of TAs), which resulted in the Lévy walk-like movement pattern.[57] However, in their experiment, because the data volume of each subject was not sufficient for analysis, data for all individuals were collected and analyzed. In such an analysis, even if the distribution of the lengths of the straight movements of each subject is not long-tail type, they may be synthesized to give a pseudo-long-tailed distribution.[71] Therefore, to more correctly determine whether the movement of pill bugs is the Lévy walk style, it is necessary to obtain a sufficient amount of data for each individual for analysis. However, Moriyama's apparatus was manual and limited to obtaining about 200 trials per subject.[52] To solve this problem, Shokaku and Morioka developed an automatic turntable-type multiple T-maze device that automatically records the turning of the pill bugs for a long time.[72] In this paper, at first, the outline of this device is explained. Next, we report the results of the behavioral analysis of 34 pill bugs that completed the multiple T-maze task for more than 6 h on this device.

II. AUTOMATIC TURNTABLE-TYPE MULTIPLE T-MAZE DEVICE

A. Related works on automatic animal behavior measurement instruments

As mentioned in Sec. I, there are many systems that automate animal behavior measurement. This study first aims to obtain patterns of movement of pill bugs by measurement using the

instrument we have developed. Similarly, several instruments have been proposed to measure movement of animals. Most of them acquire movement trajectories using vision-based systems. A movement measurement system using image-based simultaneous tracking of multiple subjects has been proposed.[73] There is also a system for obtaining three-dimensional trajectories of aquatic microorganisms using multiple cameras.[21] There are various image processing methods to measure animal behaviors, and their comparisons have been made.[74] In addition, advanced instruments that integrate actuators and other sensors with image processing have been developed. For example, there are measurement instruments including actuation by motors to capture the subject's movement trajectory.[19,20] These can measure wider movements than fixed cameras. A system that attaches AR markers to ants and measures behavior has been developed.[75] In particular, as an example of long-range observation of the behavior of millimetric-sized walkers such as ants, there is a robot platform that follows a subject in real time by image processing and measures the subject's trajectory.[17] A sphere type measurement system has also been developed. The system measures a movement trajectory of a subject by rotating the sphere in a direction opposite to the traveling direction of the subject.[70] In this way, many animal behavior measurement instruments integrate robot elements such as motor control and image processing. The proposed instrument in this study also has a similar system configuration using image processing and motors as described later. In this system, a single subject is placed in the device, and real-time measurement is performed on the subject by motor control and image processing with a camera. The measurement is automatically performed. As described previously, the proposed instrument contributes to more sophisticated animal behavior analyses than conventional movement trajectory measurements, based on automated and long-term measurement, the measurement specialized for the turning behavior of pill bugs.

B. Hardware configuration

The device consisted of the maze, drive, control unit, and the protective wall (Fig. 1). The maze unit consists of two turntables each with a T-maze made of resin printed by a 3D printer on the upper surface and a connection passage connecting the two mazes (Fig. 2). The lengths of the right, left, and center passages of each maze are 5 cm each, that of the connecting passage is 2 cm, and the distance between the intersections of both mazes is 12 cm. The width of each passage is 0.8 cm, and the height of the walls is 2 cm. Vinyl tape is attached to the inside of the wall to prevent the subjects climbing, and ink jet printer paper was attached to the floor to prevent their legs from slipping. The side of the maze was surrounded by resin plates. The drive unit consists of stepping motors (SMs) (Sustainable Robotics, SPU-01b) for rotating turntables and gears that connects the motors to the turntables (Fig. 1). Because the motor and the turntable are connected via gears, vibration transmitted from the motor to the turntable was greatly reduced.[76] Additionally, to prevent the vibration of other possible factors, anti-vibration sheets (AS ONE Co., Haplagel Sheet) are placed at various points in the device. The signal transfer type of the motor is serial, and the motor and computer are connected by a serial-to-USB conversion cable. The control unit is composed of a digital video camera (DVC) for subject imaging (SONY, HDR-CX370) and a personal
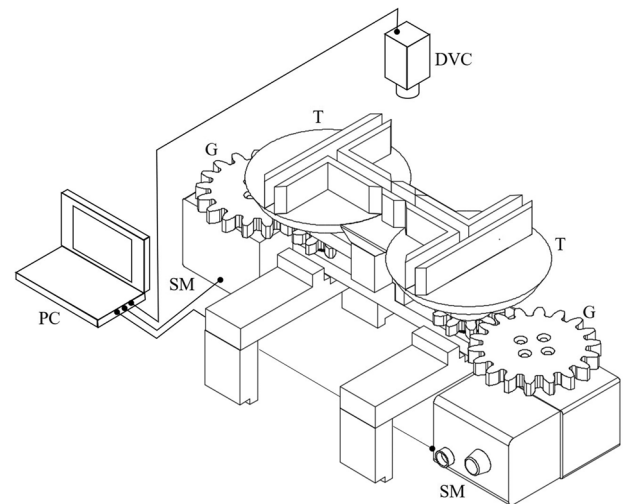

FIG. 1. Hardware configuration of the automatic turntable-type multiple T-maze device. DVC: digital video camera, G: gear, PC: personal computer, SM: stepping motor, and T: turntable.
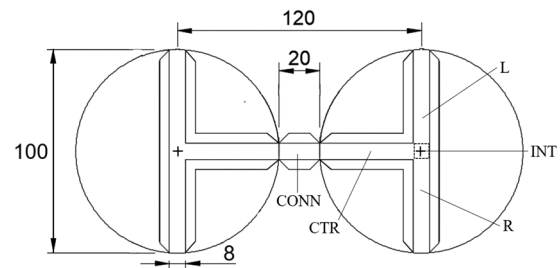

FIG. 2. Maze unit. CONN: connection passage, CTR: central passage, INT: intersection, L: left passage, and R: right passage. The unit of length is millimeters.

computer (PC) (Apple, MD388J/A). The camera is mounted 32 cm above the device. The standard of the video signal is High-Definition Multimedia Interface (HDMI), and the camera and computer are connected by an HDMI–universal serial bus (USB) conversion cable. The protective wall is made of styrene boards that cover the entire device. Inside the wall, six LED lights are placed above the maze and maintain the illuminance of the passage at 35 lx.

C. Software configuration

1. Pill bug positioning

This processes the images acquired by the digital video camera to specify the barycenter coordinates of the subject. The important thing in the image tracking is to employ an imaging method that provides a clear contrast between the subject and the background.[1] Therefore, the following processing was performed. The captured images (size: 1280×720 pixels) are sent from the camera to the computer at 60 fps, where grayscale and blur processing are executed to remove noise. Next, after an appropriate threshold is set for the
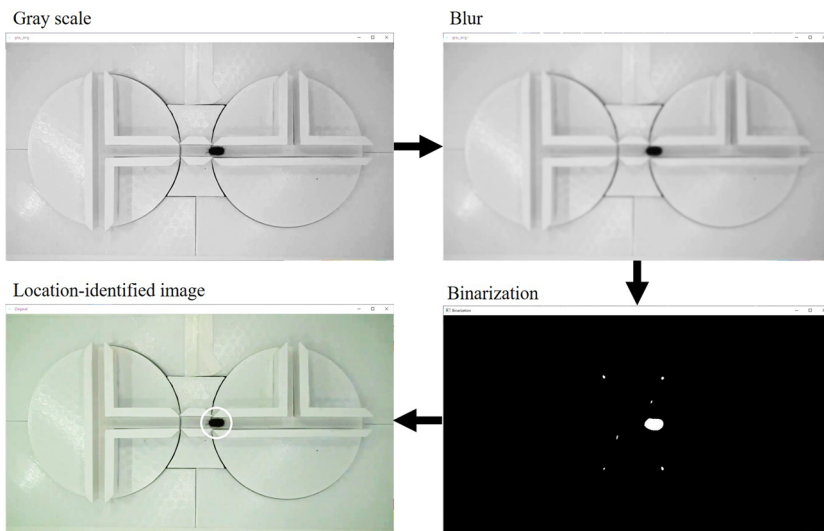
FIG. 3. Image processing procedure. Gray scale: Convert color images from PC to grayscale images Blur: Blur the grayscale image to remove noise. Binarization: A threshold value is set for the blurred image, and then the binarization process is performed to reverse the black and white. Location-identified image: The white area from the binarized image is defined as the subject.

brightness, a binarization process is performed, and finally, a reversal process of the white and black regions is performed. Using these processes, the subject and the background are displayed on the monitor as white and black areas, respectively. In order to extract only the region corresponding to the subject, the connected pixels are regarded as one region, and a labeling process of assigning a number to each connected component is performed. The number of pixels of each connected component is regarded as an area, the areas are compared, and the barycenter of the largest area is determined as the position of the subject. The barycentric coordinates of this white area ($1 \leq x \leq 1280$, $1 \leq y \leq 720$; the unit is pixels) are defined as the position of the subject (Fig. 3). One of the reasons why image tracking of organisms is difficult is that biological organisms are deformable objects.[77] The pill bug used in this study was hardly deformed when recorded from directly above. Therefore, image tracking can be done without manual correction. The library used for image processing is OpenCVSharp3-AnyCPU (v.4.0.0.20181129). The program is developed using Visual Studio C#.

2. T-maze control

This controls the motors of the drive unit to rotate the turntables appropriately in accordance with the movement of the subject moving in the maze. After startup, when the right, left, or central passage of each maze is connected to the connection passage and the subject is in a passage that is not connected to the connection passage, the program turns the maze to connect the passage that the subject is in to the connection passage (Fig. 4). At the same time, another maze is turned to connect the central passage to the connection passage. Based on the above, a method of rotating the motor based on the position information of the subject will be described. The position information of the subject is a position of the subject's barycenter based on the upper left from an image of 1280 × 720 resolution transmitted in real time, which is the position of the subject. In this case, in order to determine whether or not the subject has turned in a T-junction, the subject is rotated when the position of the subject proceeds beyond any dotted lines from intersection

of T-maze. The pill bug may turn back to the central passage and re-enter the turntable of the former side. At that time, when the subject on the turntable of the former side selected a passage again, the passage selected by the subject and the connection passage were reconnected. In some cases, the subject re-selected another passage after the passage selected by the subject was connected to the connection passage. In those cases, we connected the passage that the subject had re-selected to the connection passage. The above method was used to prevent the subject from escaping the device. By repeating these procedures, the apparatus continuously gives the subject a T-maze task. The rotation speed of the motor used in this device was not sufficient to connect the passage. As a result, the subject sometimes escaped from the passage. Therefore, the gear ratio of the motor to the turntable is 1:2, so that the subject can be led to the
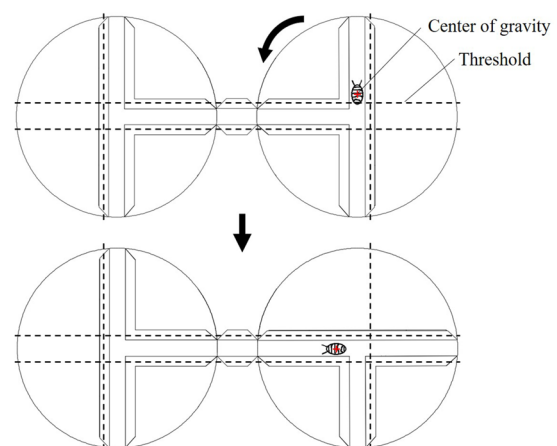

FIG. 4. Overview of turntable rotation. Red cross: pill bug's barycenter. Dotted line: the threshold for turning the maze.
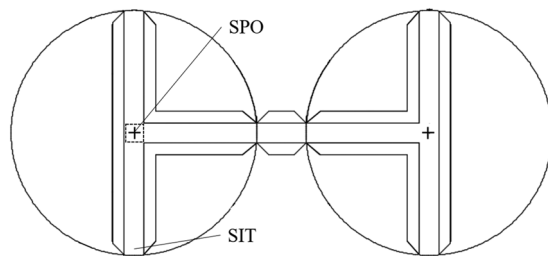
FIG. 5. Initial state of the T-maze and subject insertion point. SIT: Subject insertion point. SPO: Starting point of the motor control program.

next passage without escaping from the device. The target speed of the turntable is initially set at $6°/s$, and the speed is increased at a $225°/s^2$ acceleration until a maximum speed of $120°/s$. As a result, we were able to connect the passage before the subject reached the end of the passage.

3. Experiment flow

In the experiment, before the subject is put into the maze, the central passage of each maze is manually connected to the connection passage. (This is the initial state of the apparatus, and the shape of the entire passage becomes "H-shaped.") Next, a subject is inserted from the left passage of the left maze labeled as "SIT" in Fig. 5, and at the same time, the subject positioning program is started. After the subject moves in the passage, when its barycenter enters the intersection "SPO," the motor control program is started.

III. SUBJECTS

One hundred pill bugs, Armadillidium vulgare, were collected in Hachioji, Tokyo ($35°39'59.2''N$, $139°18'57.6''E$), and kept in a large plastic case with soil and dead leaves in the laboratory. The temperature was kept at $24°C–25°C$. A light with a timer was installed on top of the case, which was set to provide 35 lx illumination inside the case between 6 am and 6 pm. Water was sprayed into the case every day. For feeding, carrot pieces were placed on a piece of paper towel placed on the soil and replaced every other day. Dead individuals were removed as soon as they were found. Seven days before the experiment, individuals with a length of 9 mm–12 mm without deficit of antennae or legs were extracted from the plastic case and individually housed in lidded plastic petri dishes containing pieces of water-moistened paper towel. A piece of carrot was placed on the paper towel. and several drops of water were added on it once a day. To prevent the subject from excreting in the maze, the carrot was removed 3 days before the experiment. The paper towel was also replaced.

IV. EXPERIMENTAL METHOD

On the day of the experiment, the subject was taken out of the petri dish and left in another petri dish with a piece of dry paper towel for about 30 min. Thereafter, the subject was put on an elongated drawing paper inserted under the legs and moved to the passage of the maze. When the subject walked and arrived at the intersection, the door of the protection wall was closed, and

the experimental device started moving. Subjects that stood still for 5 min were removed, and their records were not used for analysis. At a random time after 6 h from entry, the device was stopped, and the subject was removed from the maze. Before each experiment, the wall of the passage was wiped with absorbent cotton containing ethanol, and the inkjet printer paper on the floor was replaced. During the experiment, the temperature was kept at $24°C–25°C$, the humidity was about 60%, and the floor illumination was kept at about 35 lx. Each subject was used only once for the experiment. The experiments were recorded at daytime for those conducted between 6 am and 6 pm and nighttime for those conducted between 6 pm and 6 am.

V. RESULTS

A. Analysis of TA

Forty subjects were examined, and 34 (85%) continued walking for more than 6 h. The data, barycentric coordinates and turning direction, from the first record to that after 6 h of these 34 subjects were used for analysis. The body length was $11 ± 0.81$ mm (Mean ± SD, $n = 34$), and the sex ratio (female:male) was 1.3:1. The turning direction of the subject was identified from the record obtained from the turntable's motor control program. However, when the subject changed direction in the passage after turning, the turning direction was redefined to be the reverse of the previous direction following the definition of Moriyama et al.[53] When two successive turns were in different directions, the set of the turns was defined as a TA. When two successive turns were the same in direction, the set of the turns was defined as a TR. The total number of turns of a subject was $1800 ± 440$ (Mean ± SD, $n = 34$), and the TA rate was $47% ± 31%$ (Mean ± SD, $n = 34$). The number of subjects with TA rates significantly greater than 50% (one-sided binomial test) was 18 (53%). The TA or TR rate is 50% for completely random movement, namely, theoretical Brownian walks. In this TA dominant group, the TA rate of a subject was $74% ± 11%$ (Mean ± SD, $n = 18$), and that in the TA nondominant group was $17% ± 14%$ (Mean ± SD, $n = 16$). We used Mann-Whitney's U test to evaluate differences between the two groups in the median of body length, we used Fisher's exact probability test to evaluate differences between the two groups in sex ratio, and we used F-test and Welch's t-test to evaluate differences between the two groups in the total number of turns. There was no significant difference between the two groups in body length [$E(U) = 51$, $V(U) = 180$, $z = 0.93$, $P = 0.35$], sex ratio (Fisher's exact probability test, $P = 1$), or total number of turns [$F_{15,17} = 4.7$, $P = 0.0042$; $P = 0.21$, $t(welch) = −1.3$, $df = 32$]. The F-test and t-test were performed for the TA rate r between the day and night groups. There was no significant difference in the TA rate r between the day and night groups ($F_{8,24} = 0.80$, $P = 0.63$; $P = 0.85$, $t = −0.19$, $df = 32$).

B. Analysis of movements

1. Anomalous diffusion

From the time-series record of the turns of each subject, each turn was extracted as a polygonal line bent 90° to the left or right (⌐, ⌐), and the lines were connected in the order of time series on a coordinate plane. By this method, a TA was represented as a zigzag trajectory (⌐⌐), and a TR was represented as a directional change (⌐⌐).
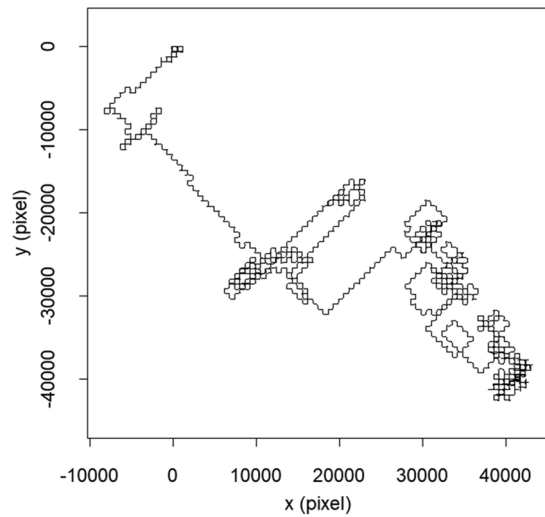
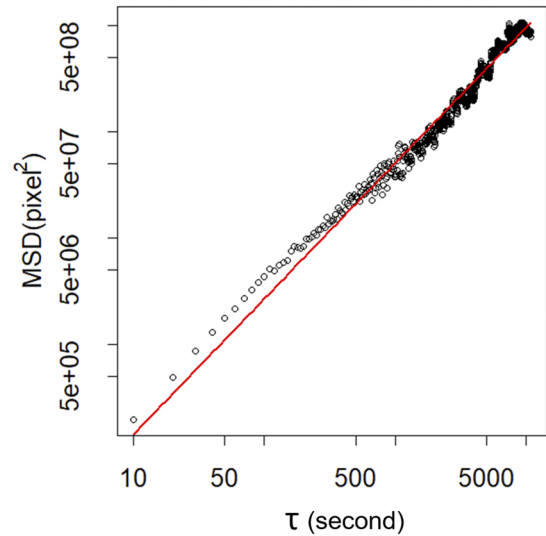**FIG. 6**. Trajectory of a subject (Subject No. 4 in Table I).

FIG. 7. Plots of the MSD of a subject in the anomalous diffusion group (Subject No. 4 in Table I, α = 1.3). The red line is an approximated power function.

As a result, the time-series record of the turns of each subject was represented as a serial trajectory in which zigzag trajectories of various lengths were connected in various directions (Fig. 6). In this paper, this virtual trajectory is simply called a trajectory. To evaluate how the trajectory of each subject spreads, the mean square displacement (MSD) of the trajectory was examined. The mean square displacement is the average value of the square value of the distance of the section [$(x_{t+\tau}, y_{t+\tau})$, (x_t, y_t)], where the particle having been at (x_t, y_t) has moved during the time width τ within the total movement time T.[78] When the total number of sections N is T/τ, the MSD is obtained by the following formula:

$$MSD(\tau) = \frac{1}{N}\sum_{i=1}^{N}\sqrt{(x_{t_i+\tau}-x_{t_i})^2 + (y_{t_i+\tau}-y_{t_i})^2}. \quad (1)$$

In this experiment, $t_{i+1} = t_i + \tau$ and MSD values were plotted when τ was changed from 10 s to $T/2$ (T = 6 h) at 10-s intervals. As a result, in 25 subjects (74%), the plot was well approximated by the power function expressed as the following formula:

$$MSD(\tau) = D\tau^{\alpha}. \quad (2)$$

The significance of the function was examined by linear regression analysis for the plots on the log–log graph (Fig. 7). When the coefficient of determination R^2 (R_7^2: in Kvalseth[79]) was 0.8 or more, the function was defined to be fitted significantly. When the diffusion exponent α is 1, the MSD linearly increases in proportion to τ. Diffusion of a particle for which the MSD has such characteristics is commonly referred to as normal diffusion and is observed in theory when a particle moves in a Brownian motion.[80] However, when α is other than 1, the MSD nonlinearly increases in proportion to τ. Diffusion of a particle with such characteristics is called anomalous diffusion. In anomalous diffusions, one where α is greater than one is called super-diffusion and one where α is less than one is called sub-diffusion.[80] In this study, the movements of 16 subjects (64%) were categorized as super-diffusion (α was 1.3, standard deviation 0.11), 2 (8%) as normal diffusion ($0.95 \leq \alpha < 1.04$), and 7 (28%) as

sub-diffusion (α was 0.81, standard deviation 0.11) (Table I). The proportions of the TA dominant group in the super-diffusion group and normal diffusion group (13 subjects, 72%) were significantly higher than that in the sub-diffusion group (no subject, 0%) (Fisher's exact probability test, P = 0.0016). There were no significant differences in body length and sex ratio between the two groups. There was no correlation between the diffusion exponent and the TA rate. The F-test and t-test were performed for both α-value and R^2-value between the day and night groups. There was no significant difference in the α-value between the day and night groups ($F_{7,16}$ = 1.4, P = 0.65; P = 0.44, t = −0.78, df = 23). There was also no significant difference in the R^2-value between the day and night groups ($F_{8,24}$ = 0.74, P = 0.54; P = 0.96, t = −0.057, df = 32).

2. Lévy walk

Some particles that move in the manner of super-diffusion show Lévy walk.[81] Lévy walk is a random walk in which a particle repeats movement in random directions, and as its characteristic, the power law is observed in the probability distribution $P(l)$ of the distance l between directional changes[82] as below,

$$P(l) \sim l^{-\mu}. \quad (3)$$

In theory, the range of the Lévy index μ is $1 < \mu \leq 3$, and when μ approaches 1, diffusion exponent α approaches 2, when μ exceeds 3, α becomes almost 1.[83] Recently, the validity of the Lévy walk model has been actively investigated as a theory to explain the complex movement of organisms.[84] In this study, we investigated whether the movement of the super-diffusion group fits the Lévy walk mode or not. In their trajectories, the zigzag trajectory consisting of a series of TAs was defined as a straight movement, and a TR was defined as a directional change. The distance l was defined as the number of turns forming a straight movement between two directional changes as a start and an end point. According to this definition, the distance l in which TAs continued m times ($m \geq 1$) is $m + 1$ (Fig. 8).

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TABLE I. Characteristics of the movements of pill bugs.

Characteristics	Subject no.	R^{2a}	α^b	AIC weight Power	AIC weight Expt.[c]	μ	%TA
Lévy walk ($1 < \mu \le 3$)	1	0.98	1.2	0.87	0.13	1.5	90
	2	0.96	1.4	1.0	0.0	1.6	84
	3	0.97	1.2	1.0	0.0	2.0	72
	4	0.98	1.3	1.0	0.0	2.2	67
	5	0.95	1.4	1.0	0.0	2.4	72
	6	0.97	1.3	1.0	0.0	3.0	60
	7	0.90	1.1	1.0	0.0	3.0	55
Unidentified ($5 \le \mu$)	8	0.99	1.5	1.0	0.0	6.0	12
	9	0.97	1.3	1.0	0.0	6.0	13
	10	0.97	1.3	1.0	0.0	6.0	20
	11	0.97	1.2	1.0	0.0	5.6	43
	12	0.94	1.0	1.0	0.0	6.0	16
Brownian walk ($3 < \mu < 5$) (AIC weight: Expt.)	13	0.84	1.4	1.0	0.0	3.4	55
	14	0.87	1.2	0.0	1.0		87
	15	0.96	1.1	0.0	1.0		86
	16	0.98	1.3	0.0	1.0		83
	17	0.97	1.2	0.0	1.0		76
	18	0.87	1.0	0.0	1.0		70
Sub-diffusion ($\alpha < 1$)	19	0.96	0.9				7.7
	20	0.83	0.9				4.9
	21	0.89	0.9				18
	22	0.81	0.8				22
	23	0.88	0.7				11
	24	0.82	0.7				11
	25	0.82	0.7				34
Unidentified ($R^2 < 0.8$)	26	0.77					1.0
	27	0.75					81
	28	0.74					16
	29	0.73					0.54
	30	0.72					72
	31	0.69					74
	32	0.67					84
	32	0.39					65
	34	0.30					48

[a]R^2: Coefficient of determination for the power function fitted to the MSD plots.
[b]α: Diffusion exponent, AIC: Akaike information criterion, Power: power-law model.
[c]Expt.: exponential function model, μ: Lévy exponent, %TA: rate of turn alternation.

However, in a loop-shaped movement in which TRs continued o times ($o > 1$), l is only 1 and its frequency is $o - 1$ (Fig. 8). To investigate whether a power law is observed in the probability distribution of l, the rank of l (serial number assigned from the maximum value of l to the minimum one) is determined from the frequency distribution of l for each subject, and the rank distribution is analyzed.[70] A log–log plot of the rank distribution is considered to be useful for distinguishing between a power function (linear) and an exponential function (curve).[85] To quantitatively determine whether a rank distribution supports the power-law or exponential model, the Akaike information criterion (AIC)[86] for each model was calculated and the model with the higher AIC weight[87] was adopted. In this study,

truncated power-law, which is biologically more likely than pure power-law, was adopted as a power-law model.[67] When a distribution supported a power-law model, following recent theoretical and behavioral research trends,[88] in the case that the Lévy index μ was $1 < \mu \le 3$, the movement of the subject was determined to be a Lévy walk type (Fig. 9). When μ was ~1, the movement was determined to be a ballistic type, and when μ exceeded 3, the movement was determined to be a Brownian motion type (cf., Raposo *et al.*[89]). In the case of a distribution having a steep change in rank because its log–log plot becomes linear, the distribution may be determined to support the power-law regardless of whether the actual movement type is Lévy walk or Brownian motion. Therefore, in this study, when μ was
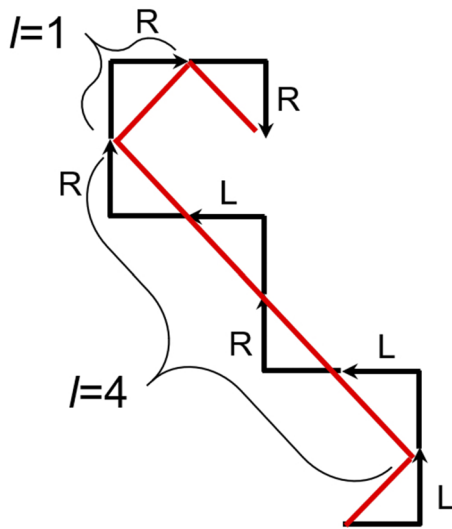
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FIG. 8. Definition of *l*. Black polygonal line with arrow: turn, L: left turn, R: right turn, red line: approximated straight movement. In this figure, a series of three TAs (L-R-L-R) constitutes a straight movement with the length *l* = 4. Of three TRs (one L-L and two R-R), a series of two TRs (R-R-R) constitutes a loop-shaped movement including a straight movement with the length *l* = 1.

greater than 5, the model of the distribution could not be determined. If a distribution supported the exponential model, it was determined to be a Brownian motion type (Fig. 10) (cf., Murakami *et al.*[90]). Of the 16 animals in the super-diffusion group, 7 (44%) moved in Lévy walk style. And five animals in the super-diffusion group and one animal in the normal diffusion group (33%) moved in Brownian motion style (Table I). The Lévy index μ of the Lévy
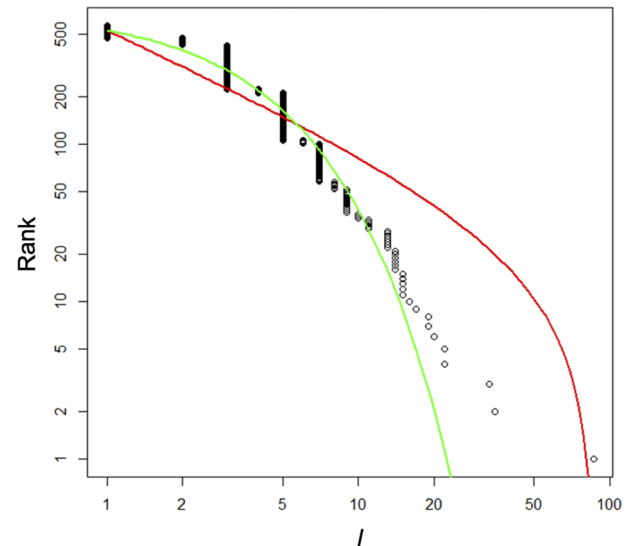

FIG. 10. Plots of the rank distribution of a subject in the Brownian motion group (Subject No. 17 in Table I). Green line: exponential model, red line: truncated power-law model.

walk group was 2.3 (standard deviation 0.63). In addition, a negative correlation was observed between μ and the TA rate r (Spearman's rank correlation coefficient $r_s = -0.79$, $P = 0.036$) and the regression line was expressed as $\mu = -0.0346r + 4.52$ (Simple regression analysis $F_5^1 = 8.9, p = 0.031$). Seven animals (100%) in the Lévy walk group and six animals (100%) in the Brownian motion group were members of the TA dominant group. There was no significant difference in the TA rate between the Lévy walk group (71%, standard deviation 12) and the Brownian motion group (76%, standard deviation 12).

Fisher's exact probability test was used to determine whether there was a significant difference in the day/night population ratio between the power and exp groups for the AIC values. No significant differences in day/night population ratios were observed between the power and exp groups (the Fisher's exact probability test $P = 1$). The F-test and t-test were performed for μ-value between the day and night groups. There was also no significant difference in the μ-value between the day and night groups ($F_{3,7} = 1.63$, $P = 0.75$; $P = 0.86$, $t = -0.18$, $df = 10$).

VI. DISCUSSION

A. Analysis of TA

Forty pill bugs were examined in the device, and 34 subjects (85%) continued walking for more than 6 h. This result indicates that this device is suitable for observing the walking of pill bugs for a long time. The TA rate of 47% is considerably lower than that of about 75% in the previous studies using a similar device.[52,53] One reason is considered to be that the distance between the intersections of the mazes (12 cm) is longer than that of the device of the previous studies (5 cm). For example, in the experiment of Watanabe and Iwata,[34] when the distance between intersections increased from 4 cm to
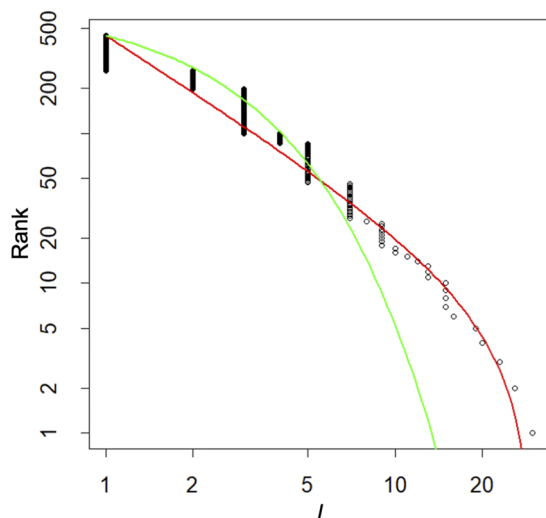

FIG. 9. Plots of the rank distribution of a subject in the Lévy walk group (Subject No. 4 in Table I, μ = 2.2). Green line: exponential model, red line: truncated power-law model.

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16 cm, the TA rate decreased from 82.2% to 55%. However, in this study, the TA rate in the TA dominant group, which is half of all analyzed subjects, was as high as 74%, which is close to the value of the previous studies. In an experiment using pill bugs and their closely related species, sow bugs, it was hypothesized that TA occurs when the difference in the momentum of the left and right legs that occurs during turning is switched immediately after turning, that is called the BALM (bilaterally asymmetrical leg movement) hypothesis.[41] Experiments have confirmed that the difference in the momentum after switching becomes smaller as the distance between the intersections increases and TA disappears when the difference is eventually canceled.[46] Based on these findings, it is assumed that the TA dominant group in this study has an inherent mechanism to maintain the difference in the momentum of the left and right legs for long distances, or acquired such a mechanism during the experiments. In contrast, the TA rate of the TA non-dominant group was 17%, which is well below 50%. This result reflects many of the group frequently eliciting TRs. It is assumed that TR is generated by a mechanism that does not switch the momentum difference between the left and right legs after turning, and maintains the momentum for a long distance. From these considerations, in the TA non-dominant group, it is presumed that there is an innate mutation in the BALM mechanism, or such a mutation was acquired during the experiments. The present device is not only capable of long-term automatic observation but also has the capacity to reveal unknown functions that may be latent in subjects. Verification of an unknown mechanism that maintains TA or TR for long distance is an issue for future research.

B. Analysis of movements

1. Anomalous diffusion

MSD was well fitted to the power function of the time width τ in the trajectories of 25 subjects, which are 74% of the 34 animals that continued walking for 6 h. From this result, it is considered appropriate to evaluate the degree of diffusion of the pill bugs in this study by the diffusion exponent α. Despite the restricted condition of the maze, all types of diffusion, super-, normal, and sub-diffusion, were observed. This result suggests that pill bugs have various types of movements. Of these types, only two subjects (8%) showed normal diffusion, which is characteristic of a Brownian motion-like random walk, and many subjects showed anomalous diffusion. This result suggests that most pill bugs do not determine the moving distance l for each time width τ at random, but rather by some internal mechanisms. Among the anomalous diffusion groups, the super-diffusion group (16 subjects, 64%) is presumed to elicit TA at a high rate and increase straight movements, while the sub-diffusion group (7 subjects, 28%) is presumed to elicit TR at a high rate and increase stagnant loop-like movements. In fact, the TA dominant group is the majority of the super-diffusion group (69%), while the non-super-diffusion (normal and sub-diffusion) group had only one of the TA dominant groups (11%).

2. Lévy walk

Of the 16 subjects in the super-diffusion group, approximately half (7%, 44%) showed Lévy walk-like movement. This ratio is almost the same as the result of Nagaya *et al.* (14 of 28 subjects) in which the pill bugs were allowed to walk freely on a servosphere.[70]

These results suggest that a certain number of Lévy walk-like movements appear in pill bugs showing super-diffusion, with or without restrictions (maze or free). However, the Lévy index μ obtained in our experiment was 2.3, while that obtained by Nagaya *et al.* was 1.5.[70] Lévy walk-like movement is considered to be a useful search strategy for animals that do not know the distribution of the resources scattered sparsely in the environment, and the movement with $\mu = 2$ is said to be the most efficient.[91] In addition, the efficiency of movement with μ of about 2 has been confirmed in various species (spider monkey,[92] fallow deer,[85] human being,[93] seabird,[68] and lymphocytes[94]). Wild pill bugs inhabiting the ground are expected to search for ecological resources such as scattered bait and evacuation sites as efficiently as possible while encountering numerous obstacles such as stones, fallen leaves, and branches. It is possible that the long multiple T-maze task performed in this experiment was a better model of the field conditions where pill bugs live than the free walking conditions of Nagaya *et al.*[70] In the Lévy walk group, a negative correlation was found between the TA rate r and the Lévy index μ. From the regression equation, when μ is 2, r is estimated to be 73%. In addition, when r becomes 100%, μ is estimated to be 0.91, and when r is 55%, μ is assumed to be 3. The r of 100% means that the zigzag movement continues during the entire time of the experiment, and the trajectory becomes one straight line, i.e., ballistic. An r of 44% approaches one when the random walker moves ballistically, and μ exceeds 3 when the walker moves in Brownian motion. In addition, when $\mu = 2$, which is an index of a highly efficient search, is given and the assumed r is 73%, it is implied that the subject elicits TRs moderately and results in a complex serial trajectory in which zigzag trajectories of various lengths are connected in various directions. In this experiment, Brownian motion-like movement was also observed. This movement is considered the most efficient search strategy for animals in resource-rich environments that do not require extensive search.[68] It is possible that subjects in the Brownian motion group had a lower desire to search than the Lévy walk group did and may have searched in a narrow range. Most of the Lévy walk and Brownian motion groups were members of the TA dominant group, and there is not a significant difference in the TA rate between the two groups. This result suggests that the Lévy walk-like and the Brownian motion-like movements of the pill bugs are not the result of the difference in the TA rate but the distribution of the successive number of TAs (distance l). This implies that the decision-making for the onset of a TR (directional change) is different between the two groups.

VII. CONCLUSION

In this study, an automatic observation instrument that specializes for turning behaviors of pill bugs was developed in order to obtain new knowledge in the field of ethology. This instrument integrated image processing and motor control and enabled long-term measurement of the turning behaviors of pill bugs. In addition, we succeeded in giving pill bugs a multiple T-maze task for 6 h using an automatic turntable-type multiple T-maze device. As a result of analyzing the trajectories of the subjects from the viewpoint of the random walk model, most were classified into normal/sub-diffusion, Brownian motion, and Lévy walk types. This result suggests that when the multiple T-maze task continues for a long time,

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pill bugs do not continue TAs mechanically but spontaneously elicit TRs to decide the timings of directional changes and make various lengths of straight movements, which result in complex trajectories. Research to elucidate the mechanism of this spontaneous expression of TR will be performed in the future. Moreover, how Lévy walks observed in organisms can emerge from interactions between the innate rule and the environment (e.g., T-maze) is still an open question in the context of Lévy walk studies.[95,96] Therefore, the proposed device and obtained data in the present study have the potential to reveal the generative mechanisms of Lévy walks. The distance between the intersections of the mazes of this device was set longer than that in the previous studies to control the motor rotation speed. If this distance is made shorter, TA will appear with a higher possibility, and trajectories different from those obtained in this study may appear. At present, this research group is improving the apparatus. In organisms with a relatively simple nervous system, such as pill bugs, it has been thought that innate behaviors such as TA result from innate mechanisms and variant behaviors such as TR are caused by malfunctions. This study, which suggests that spontaneous elicitation of TR is involved in the generation of a variety of movement patterns, is expected to provide a new perspective on the conventional explanation of behavioral mechanisms in simple organisms.

ACKNOWLEDGMENTS

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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