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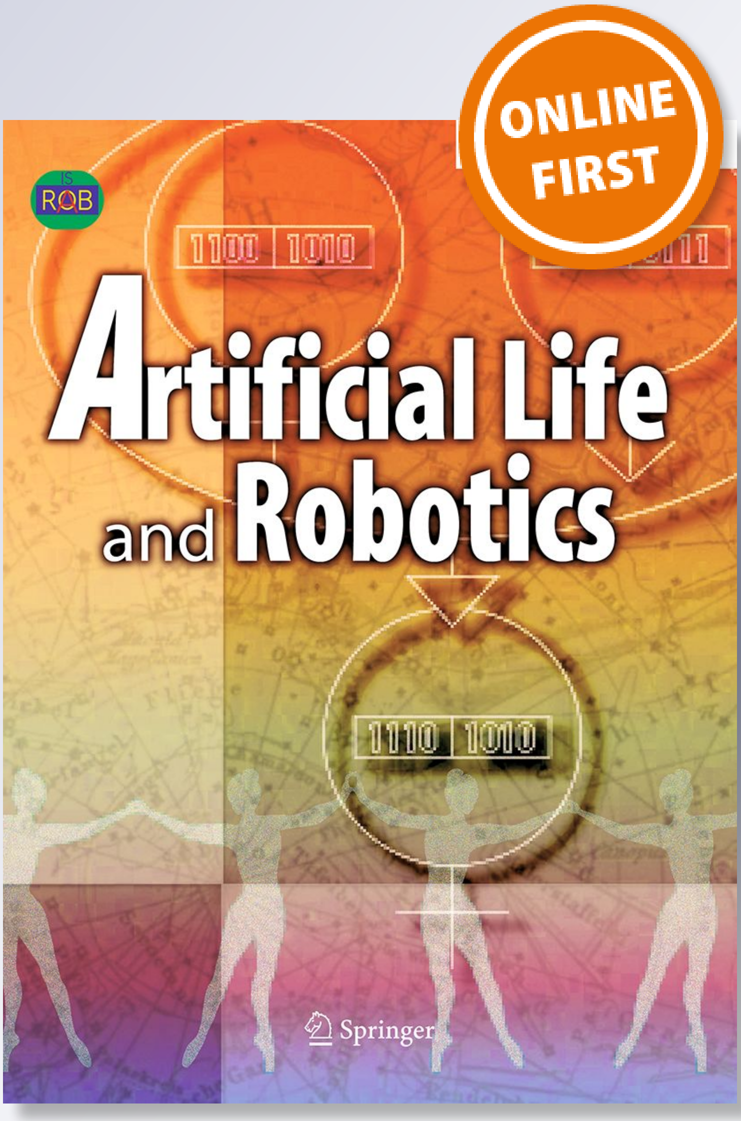

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ORIGINAL ARTICLE

Behavioral pattern of pill bugs revealed in virtually infinite multiple T-maze

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Abstract

A behavior called turn alternation has been studied extensively in terrestrial isopods. This behavior is seen when they alternate their path choice on successive trials of the T-maze test. We made the multiple T-maze device which consists of two turntables with a T-maze mounted on each and examined the behavior of 36 pill bugs (*Armadillidium vulgare*) that each completed 130 successive T-maze trials. As a result, in addition to turn alternation, turn repetition (turning in the same direction on two successive turns) appeared at a rate of 20%. In the turn sequences, we observed segments consisting of successive turn alternations and defined the number of turn alternations in a segment as the length of it. Cumulative frequency distribution of segment lengths obeyed power law with exponent of 1.76. This result suggests that pill bugs in the multiple T-maze device behaved as Lévy walkers which forage in an environment, where resources are unpredictably distributed.

Keywords Lévy walk · Multiple T-maze · Pill bug · Turn alternation · Turn repetition

1 Introduction

When animals move forward, the tendency to turn in the opposite direction of a preceding turn has been observed in a wide range of species [1–4]. This alternating turn behavior is considered beneficial during exploration and foraging [5, 6]. In terrestrial isopods, this behavior has been assumed to be generated by internal responses to leg movements [7] and has been studied extensively [8–28]. The main hypothesis regarding the mechanism underlying the behavior is based primarily on proprioceptive information from the previous turn that arises from bilaterally asymmetrical leg movements that occur when turning [11–14]. The alternating turn behavior which is thought to be generated by response to such internal sates is called turn alternation (TA) [15].

However, TA in the pill bug *Armadillidium vulgare* does not always appear to be simply based on proprioceptive information [25]. When pill bugs meet successive T-maze junctions, they can maintain a straight course by alternating their path choices. However, they sometimes stop after turning, return to the T-junction, and then turn down the other arm of the maze (directional change). By changing their direction, individuals that have begun turning in the same direction as they had at the last junction (turn repetition, TR) can reverse their behavior, and ultimately alternate their turns. For example, after making a second successive left turn (L), the individual can back up and initiate a right turn (R) instead, thus completing an alternation from L to R.

The function of directional changes has not been investigated, because they occur rarely and are usually omitted from data analysis. Recently, Moriyama et al. [26] used a multiple T-maze device [25] that offered animals more than 100 successive T-junctions at which they could choose to go left or right, and which provided ample data for statistical analysis to determine whether directional changes occur randomly or whether they are used to correct for TR.

When the experimenter gave pill bugs successive T-maze choices, in the middle of the second turn of a TR sequence, they sometimes performed a directional change and ultimately completed a TA. For example, in the middle of the second R of an R–R TR sequence, a pill bug executes a directional change, thus completing a "corrected" R–L TA. Of 597 turns that began as R–R TRs, directional changes

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in 142 resulted in a corrected R-L TA, yielding a correction rate of 23.8% [26]. Sometimes, a directional change resulted in a TR. For example, in the middle of the second R of an L–R TA sequence, a pill bug executes a directional change, thus completing a "corrected" L–L TR. This pattern occurred in only 43 of the 1665 potential L–R sequences (2.6%) [26]. Similarly, the rate of corrected L–R sequences (31.6%) was significantly higher than the rate of corrected R–R sequences (3.8%) [26].

Combining these data, the percentage of corrected TAs (26.5%) was significantly greater than the percentage of corrected TRs (3.2%) [26]. While this result suggests that directional changes most frequently reversed TRs to complete TAs and pill bugs may have a mechanism for maintaining TA, it also suggests that pill bugs still complete TRs with a high possibility of 73.5% despite having ability to maintain TA using directional change.

In this paper, we reanalyzed the data of our previous experiment [26] to investigate whether TR has ecological benefit for pill bugs or not.

2 Materials and methods

2.1 Animals

Five hundred pill bugs (*A. vulgare*) were caught on the grounds of Shinshu University for use as the main stock and were kept in a plastic container with a paper towel in the laboratory for 3 months. They were fed slices of carrot once per week. A moist atmosphere was maintained by wetting the paper towel every day. The temperature of the laboratory was kept at 23–25 °C.

Seven days before conducting the experiments, 51 individuals, each of which was 10–11 mm in length, were selected from the main stock and singly isolated in petri dishes. They were fed on a small piece of sliced carrot for 4 days and subsequently starved for 3 days to equalize their metabolic conditions and prevent excretion in the experimental apparatus.

2.2 Apparatus

The multiple T-maze device consists of two turntables with a T-maze mounted on each (Fig. 1). By turning the tables, the experimenter gave the animals successive T-maze choices. White inkjet printing paper was pasted onto the paths to prevent slipping. Transparent Teflon sheets were pasted onto the walls to prevent climbing.

The device was placed on a desk in the laboratory and was surrounded by white cloth to prevent strong shadows from being visible on the path, which could affect behavior.

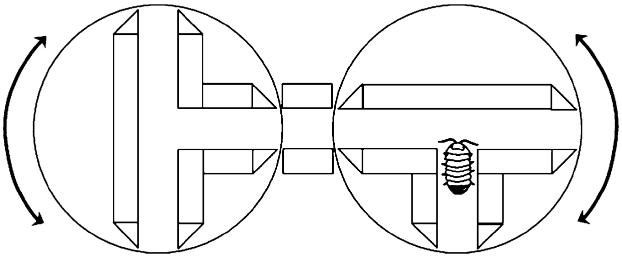

Fig. 1 Multiple T-maze device. Diameter of each turntable: 5 cm, length of the connection alley: 1 cm, distance between junctions: 6 cm, and width of paths: 0.8 cm

A video camera (Panasonic HDC HS-9) was placed above the device and was used to record behavior.

2.3 Procedure

We slowly inserted one end of a rectangular piece of paper under the legs of each subject to remove the animal from the petri dish. The subject on the paper was safely lowered onto the entrance at the base of a T-maze and allowed to enter the maze spontaneously. Subsequently, the device was covered with a white box that had a transparent ceiling that prevented the pill bugs from being disturbed by the experimenter's breath.

All pill bugs moved forward to the junction and turned left or right. Just after turning at the junction, the turntable was rotated, such that it led to the next T-maze through the connection alley. The preceding turntable was rotated to make that the stem of its T meets the connection alley. Thus, the pill bugs entered the stem of the next T when leaving the arm of a preceding T, and in this way, were forced to repeatedly make turning choices.

Molting individuals were not used. Trials were stopped when individuals stayed stationary for more than 1 min. Each individual was used once and returned to the university ground after the trial. The paper that covered the paths in the T-maze was changed between individuals.

2.4 Turns and turn pairs (TA and TR)

Turns were categorized into four types. Normal left turn (nL) was defined as traveling along the maze stem, turning left at the choice junction, and passing the connection alley into the stem of the next T-maze. Normal right turn (nR) was defined similarly, but for the right.

Directional change was defined as stopping before reaching the next junction and reversing direction. Corrected left turn (cL) was defined as turning right at a junction, initiating a directional change, and reaching the junction of the next T-maze. Corrected right turn (cR) was defined similarly for when the direction of the first turn was to the left and the corrected direction was to the right. Thus, left (L) turns included nL and cL turns, and right (R) turns included nR and cR turns.

TA was defined as a pair of consecutive turns of L–R or R–L. TR was defined as a pair of consecutive turns of L–L or R–R.

3 Results

3.1 TA and TR frequencies

36 pill bugs each completed 130 turns. Each experiment lasted for about 20 min. At the beginning of experiments, most individuals moved slowly and sometimes stopped for several seconds. After this relatively inactive and unstable period, the activity of the animals increased, and their level of locomotion remained stable throughout the rest of the experiment. Because of this initial instability, the first 20 turns for each individual were omitted, and only the subsequent 110 trials were used for analysis. As a result, types of turn pairs (TA or TR) were determined for 109 pairs of consecutive turns for each individual (109 turn pairs for 36 individuals = 3924 total turn pairs).

Among the 3924 turn pairs, the overall counts for TA and TR were 3242 (82.6%) and 682 (17.4%), respectively, and the TA count was significantly larger than 50% of the total (Chi-square test: $\chi^2 = 1670$, $P < 0.001$).

In the turn sequences, we can observe TA and TR segments, where only TAs or TRs continue for several trials. For example, in the turn sequence consisting of "LRLRLRLLRLRRR", we can find two TA segments, each of which consists of six (LRLRLRL) and three (LRLR) consecutive TAs, and two TR segments, each of which consists of one (LL) and two (RRR) consecutive TRs. In this paper, we call the number of consecutive TAs or TRs in the TA or TR segments as the "length" of the segment.

In the relative frequency distribution of the lengths of TA segments, we can see 31 kinds of lengths (Fig. 2). The total frequency was 567, the mode was at the length of 2 (27%), and the maximum length was 51 (Fig. 2).

In the relative frequency distribution of the lengths of TR segments, we can see only six kinds of lengths. The total frequency was 583, the mode was at the length of 1 (88%), and the maximum length was 12.

3.2 Analysis of distribution of segment lengths

The cumulative relative frequencies of lengths of TA segments were plotted on a double logarithmic scale and the approximation function of the plots was estimated (Fig. 3). As a result, we observed that the plots followed power function with the exponent of 1.76 (coefficient of determination: $R^2 = 0.94$) (Fig. 3).

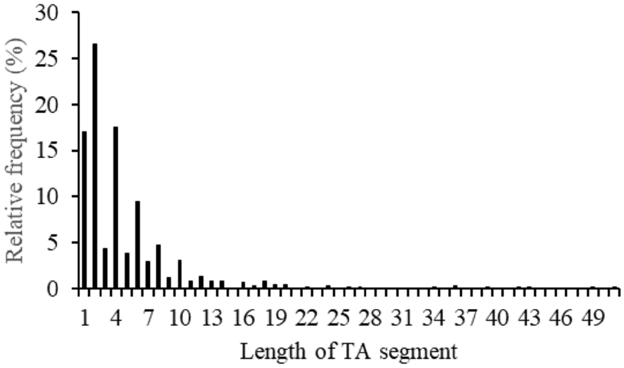

Fig. 2 Relative frequency distribution of the lengths of TA segments

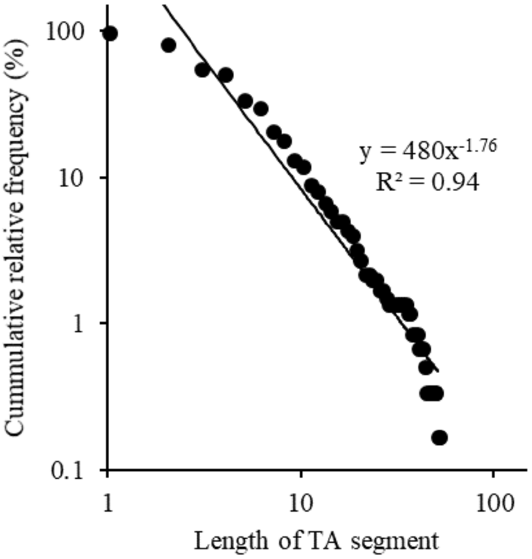

Fig. 3 Cumulative relative frequencies of lengths of TA segments on a double logarithmic scale. Black line is the approximation function for the plots

The cumulative relative frequencies of lengths of TR segments were plotted on a double logarithmic scale and the approximation function of the plots was estimated, too. As a result, we observed that the plots followed power function with the exponent of 3.43 ($y = 111x^{-3.43}$, coefficient of determination: $R^2 = 0.99$).

4 Discussion

In the turn sequences, while there are various lengths for TA segments (31 kinds), length of 1 is dominant in TR segments (88%). This result suggests that pill bugs have mechanisms not only to continue TAs but also to generate TR with length

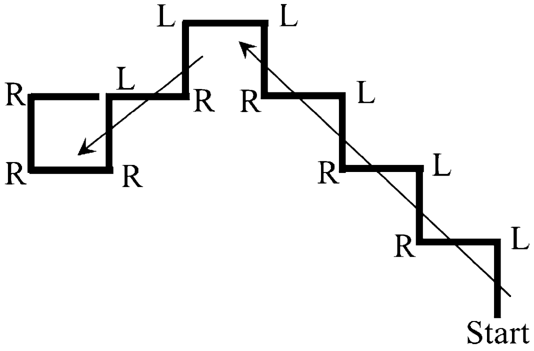

Fig. 4 Example of trajectory of a pill bug's movement. The turn sequence consists of LRLRLRLLRLRRR. Black arrows represent displacements of consistent directions for the TA segments (LRLRLRL and LRLR)

of 1 to cease succession of TAs, which result in TA segments with various lengths.

When we draw turn sequence of a pill bug on a piece of graph paper, we can observe trajectory of its movement (Fig. 4). The trajectory consists of combination of TA segments with various lengths, which were connected by TRs. We can see TA segments as displacements of consistent directions and TR segments as directional changes (Fig. 4).

According to studies on trajectories of animal movements, animals exhibit scale-free movements or anomalous diffusion, which leads to efficient searches [29]. For example, the Lévy walks are described as a combination of many short movement steps and rare long-distance displacements and the length of these displacements follows power-law probability density function $P(x_i) \sim x_i^{-\mu}$, where x_i is the move length (displacement of consistent direction) and μ represents the power-law exponent ranging from $1 < \mu \leq 3$ [30].

The move length can be defined in different ways, such as the distance between consecutive landings on the sea surface for albatross [31–33] and the saccade interval length for fruit flies [34]. In our study, we defined length of TA segment as move length and investigated whether the pill bugs' log-scale movement lengths followed power-law distributions or not.

In the cumulative relative frequency distribution of the lengths of TA segments plotted on a double logarithmic scale, the plots followed power-law function with the exponent μ of 1.76, which is $1 < \mu \leq 3$ (Fig. 3). On the other hand, in the cumulative relative frequency distribution of the lengths of TR segments, the plots followed power-law function with the exponent μ of 3.43, which is $\mu > 3$. These results suggest that pill bugs in the multiple T-maze behaved as Lévy walkers performing many short movement steps and rare long-distance displacements (various lengths of TA segments) while changing directions of the movements (TRs).

In a study on foraging strategy, a Lévy walk with the power-law exponent μ ranging from $1 < \mu \leq 3$ was considered to be important in a natural environment in which resources are unpredictably distributed [35]. A Lévy walk (a) with $\mu = 2$ indicates optimal searching behavior in such an environment; (b) for the exponent $\mu \approx 1$, movement patterns are close to ballistic motion and this movement is useful to a foraging animal that is exploration foraging if resources are homogeneously distributed far from an animal's location; (c) for $\mu > 3$, the walk is approximated as Brownian motion and this motion is applicable for exploitation foraging if resources are abundantly distributed near an animal's location [35]. A Lévy walk with the exponent μ ranging from $1 < \mu \leq 3$, therefore, indicates a foraging pattern that balances exploitation and exploration foraging [36].

There are several hypotheses about the underlying mechanism of the Lévy walk. According to one hypothesis, Lévy walk arises from interactions of animals with external factors [37]. The other hypothesis, which has been supported by increasing evidence, states that Lévy walk is shaped by intrinsic factors of the animals' neuronal and/or physiological mechanisms [34, 38, 39]. For collective animals, it was reported that inherent noise in the group appearing as Lévy walk is well-balanced movement to facilitate dynamic collective motion and information transfer over the whole group rather than merely erroneous random motions derived from velocity matching among individuals [40]. Nagaya et al. [41] confirmed that the Lévy-like behavior in pill bugs on the servosphere, where movements of the animal are continuously recorded and compensated in such a way that the animal always locates on the top of the sphere and experiences a virtual unbounded two-dimensional field, was not triggered by any external factors. They suggested that the multi-scale walking behavior displayed by pill bugs is elicited by their intrinsic factors [41].

The main hypothesis regarding the mechanism underlying TA is based primarily on proprioceptive information from the previous turn that arises from bilaterally asymmetrical leg movements that occur when turning [11–14]. TA is thought to be generated by response to such internal sates [15].

On the other hand, TR has been considered to be generated by erroneous performance of the TA mechanism. Actually, the emergent rate of TR was 17%, while that of TA reached 83% in our experiment. However, our experiment also showed that TRs generated various kinds of lengths of TA segments and resulted in Lévy-like searching behavior for pill bugs. These results suggest that pill bugs have an intrinsic mechanism to elicit erroneous behavior for searching environment. Further experiments are needed to clarify the mechanism.

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