Pivot burrowing of scarab beetle (Trypoxylus dichotomus) larva

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Brief Communication

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Pivot burrowing of scarab beetle (*Trypoxylus dichotomus*) larva

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Abstract

Scarab beetle larvae do not have appendages to shovel soil and their trunk is thick compared to their body length; hence, their movement through the soil is perplexing. Here, we found that the last larval instars of *Trypoxylus dichotomus* burrow in two different ways, depending on the hardness of the soil. If the soil is soft, the larvae keep their body in a straight line and use longitudinal expansion and contraction; if the soil is hard, they flex and rotate their body. It is thought that the larvae adapt to diverse soil conditions using two different excavation methods.

The soil is home to many organisms, and how they move through soil has been studied in several species (e.g., rats, lizards, snakes, nematodes, eels, spiders, ants, earthworms) by analyzing the shape of their burrows or/and using X-ray scanning technology.[1-11] Among them, earthworms have attracted much attention as a research model because they can burrow through soil without appendages, and their dynamics have recently been elucidated via an observation system using transparent superabsorbent polymers.[12] Furthermore, applications of these excavation techniques are being developed.[13-15] Earthworm morphology seems to be suitable for burrowing in terms of the drag force from the soil, as their trunk is narrow compared to

their body length. However, the shape of some organisms in the soil is suitable for burrowing. For example, the larva of the scarab beetle (*Trypoxylus dichotomus*), which spend their life in the soil until adult emergence. Their trunk is very thick compared to their body length; the transverse sectional diameter of a earthworm is approximately 2 mm[16], whereas that of the last instar larvae is ~20 mm. In other words, the transverse sectional area of beetle larvae contributing to resistance from the soil is almost 100 times larger than that of a earthworm. Therefore, there the burrowing mechanism must be different from that of earthworms under similar conditions. Although beetle ecology in the soil has been reported in several studies[17,18], little is known about how beetle larvae move.

Here, we analyzed the burrowing mechanisms of beetle larvae. Beetle larvae were placed on the soil surface to make sure they could burrow into the soil (Figure 1A). In order to observe the burrowing behavior, a two-dimensional (2D) observation tank (130 × 210 × ~20 mm) was constructed (Figure 1B); we succeeded in observing the dynamics of the larvae under a 2D soil condition (Figure 1C, Supplementary Movie 1). The larvae burrowed by rotating themselves (Figure 1D, Supplementary Movie 1). Rotation was observed regardless of sex. All observed individuals proceeded towards the bottom and stopped when rotating at the bottom layer (Figure 1C).

Next, we investigated whether the hardness of the medium being excavated could change the dynamics of burrowing. Different amounts of soil were used to compare compacted soil (relative volume of soil: 1) and non-compacted soil (relative volume of soil: 1/2). The speed of burrowing was faster (Figure 1D, Supplementary Movie 1) and the degree of rotation was smaller when burrowing in non-compacted soil (Figure 1D, Supplementary Movie 1). However, in these observation systems, there were several areas that could not be observed clearly to analyze the dynamics because the soil covered their body. Therefore, we

searched for a burrowing medium that would aid in observing the burrowing dynamics clearly. We constructed a complete 2D observation system by stacking cylindrical paper straws of two diameters cut into 2 cm segments (Figure 2A). By changing the diameter of the straw (using 6 mm and 10 mm diameter straws), the area of the lumen changes, which is thought to have the similar effect as soil comp action changes.

As a result, we could observe quite clearly how the burrowing larvae rotate. Through the 6 mm straws, the larvae proceeded towards the bottom by rotating and stopped at the bottom layer just as in the hard soil (Fig. 2C, Supplementary Movie 3). The larvae were seen to rise to the surface without moving to the bottom layer through the 10 mm straws, contrary to in the soft soil (Fig. 2D, Supplementary Movie 5). Although beetle larvae are known to be able to change their burrowing direction depending on CO_2 concentration[17], their movement may also be motivated by the size of the surrounding particles or by sensing other factors that vary with size.

We analyzed the movie of larval dynamics using Fiji's Plugin "manual tracking" (version1.0). Beetle larvae have nine spiracles, which were used as indicators in the tracking analysis (Fig. 2B, E, F, S1, Supplementary Movie 4 and 6) (some parts, where the spiracles were not visible, were skipped.). Larvae could twist and turn their bodies in the 2D environment (Fig. 2E, red arrow). We first investigated the velocity of the spiracles. Movement of anterior parts was faster than posterior parts and the velocity in the 10 mm cylinder was faster than in the 6 mm cylinder (Fig. 2G). This velocity tendency (6 mm vs 10 mm) corresponds to the tendency in the soil compaction experiment (with vs without compaction). In addition, the change in angle between the three spiracles (3,5,9) was measured (Fig. 2H). Although there were two peak angles under both straw medium conditions, the position of the peaks was different. In 6 mm straws, the first peak was ~80° and the second peak was ~130°. In 10 mm straws, the peaks were at ~90° and ~150°. The frequency of the peaks was also different. In 10 mm straws, the first peak

distribution was less than in 6 mm. The two peaks indicate two modes of larval shape; the first peak indicates C-shaped shape, and the second peak indicates straight shape. It is assumed that there exists a relationship between larval shape and the presence or absence of pivot movement. Hence, the larval pivot burrowing can be changed depending on the surrounding environment.

From the experiments with different soil contents and different straw diameters, a correlation between velocity and the degree of rotation was evident. If the environment was conducive to fast progress, the larva went straight ahead; if not, they rotated.

Next, we examined the relationship between rotation position and larval shape. To investigate the location of rotation, we plotted the time transition of the Y-position of spiracle 3. With rotation, the Y-position fluctuated up and down periodically. Time series data of the angle were plotted. Angle and Y-position were inversely related（Fig. 2I, S2). This suggests that rotational burrowing may contribute to the larvae pushing (kicking) the soil with their tails to move downward (Fig. 2J). Another possible reason is that the rotation and pivoting motion causes local fluidization of the soil, thereby reducing the resistance.[19] The reason for the lack of a clear inverse relationship before reaching a certain depth is thought to be that the straw was not heavy enough to provide a drag force when pushed with the buttocks. Movement by pushing of the tail has been reported in tiger beetle larvae.[20] Although the tiger beetle larvae also burrow in the soil,[21,22] the shape of the hole is straight, and it is assumed that they do not burrow with rotation like the scarab beetle larvae. The tiger beetle larvae are not C-shaped like the scarab beetle larvae.[21,22] There is a possibility that larval morphology may cause rotation. In the future, comparisons with the tiger beetle can facilitate ecological evolutionary developmental biology (Eco-Evo-Devo) research.

In this study, we established a 2D observation system for beetle larvae and analyzed how they move. As a result, we discovered that scarab beetle larvae burrow by rotating. Burrowing dynamics changed when the hardness and size of the soil particles were

changed. In addition, the relationship between larval shape and timing of rotation was clarified, suggesting that rotated movement may be conducive to displacing the soil to move downwards. A 2D observation system was used in this study; it is unclear whether the same behavior would be observed in a 3D environment. X-ray CT technology would facilitate the reconstruction of images in a short time, which would elucidate burrowing dynamics. The rotational movement may be an effective way of burrowing by repeating simple movements. The significance of this rotational motion needs to be confirmed by reproducing it in a robot. Few studies have investigated the development of excavation robots that apply the burrowing mechanisms of animals (e.g., inchworms, shellfish, and earthworms).[15,23-25] The application of the burrowing mechanism of beetle larvae may also contribute to the development of new excavation technologies.

The rotational movement is caused by the shape of the larvae. Comparison of the larvae with those of other soil insects, such as the tiger beetle larvae[21,22], which burrows linearly, would facilitate Eco-Evo-Devo research. Additionally, the results of studies on the frequency, position, and direction of the rotation can be applied in the field of neuroethology.

Materials and Methods

Insects

The last instars of Japanese rhinoceros beetle (*Trypoxylus dichotomus*) were commercially purchased (Finebeetle, Ehime, Japan) and kept individually in 500–1000 mL bottles filled with rotted wood flakes at 10–15 °C until use.

Observation of burrowing

The larvae were moved to a room with a temperature of 23 °C and could acclimatize for at least 30 min before the burrowing experiment was conducted. Larval movements in the glass tank (6 × 21 × 2 cm) were captured by a time-lapse camera

(DMCGX7MK2WK, Panasonic, Osaka, Japan). The larvae were incubated in a humus mat (Kanzen, Tsukinoyakinokoen, Gunma, Japan) and paper straws (6 mm, Shimojima, Tokyo, Japan) (10 mm, Zone Plus, Shiga, Japan) were cut into ~2 cm pieces for visualization.

Analysis of tracking

From the data obtained by time-lapse photography, the coordinates of the spiracles present in each body segment of the larvae were obtained for each time series using Fiji's macro manual tracking (version1.0). The data were skipped when the spiracles were unclear. Based on the size of the tank, the distance per pixel was calculated and used for subsequent determination of velocity between the spiracles. In the time lapse, the images were taken every second, so the displacement of each image of each trachea was directly used as the velocity. The angle was calculated from the positional information of the three spiracles. All analysis after obtaining the positional information was done in Julia language (version1.5).

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Competing interests

The authors declare no competing interests.

Authour contributions

Conceptualization: H.A.; Methodology: H.A., M.O., S.Y. and S.K.; Validation: H.A., M.O., S.Y. and S.K.; Formal analysis: H.A.; Investigation: H.A.; Visualization: H.A.; Writing – original draft: H.A. and S.K.; Writing – review & editing: H.A., M.O., S.Y., M.S. and S.K.; Supervision: H.A and S.K.; Project administration: H.A and S.K.; Funding acquisition: H.A. and S.K.

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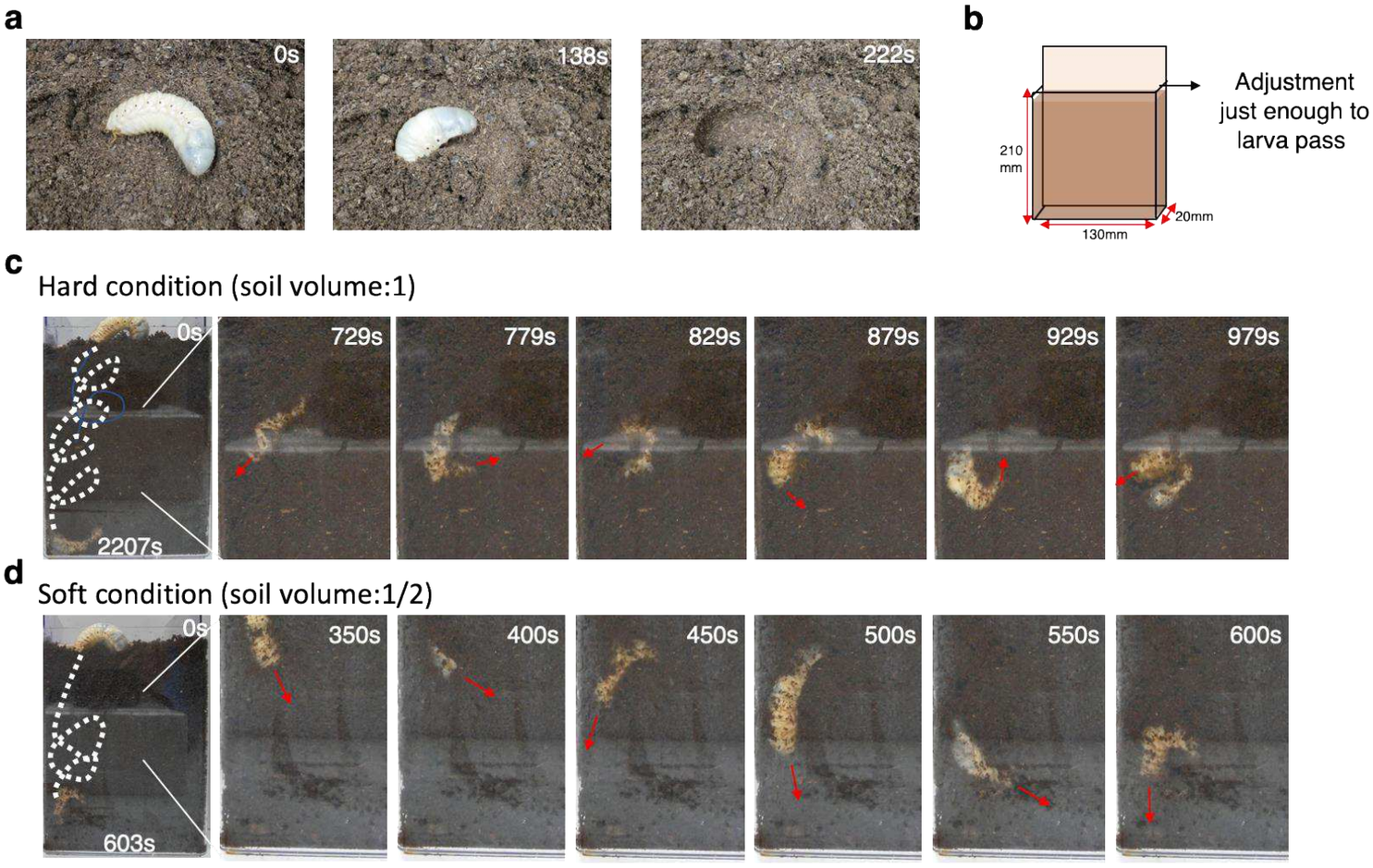
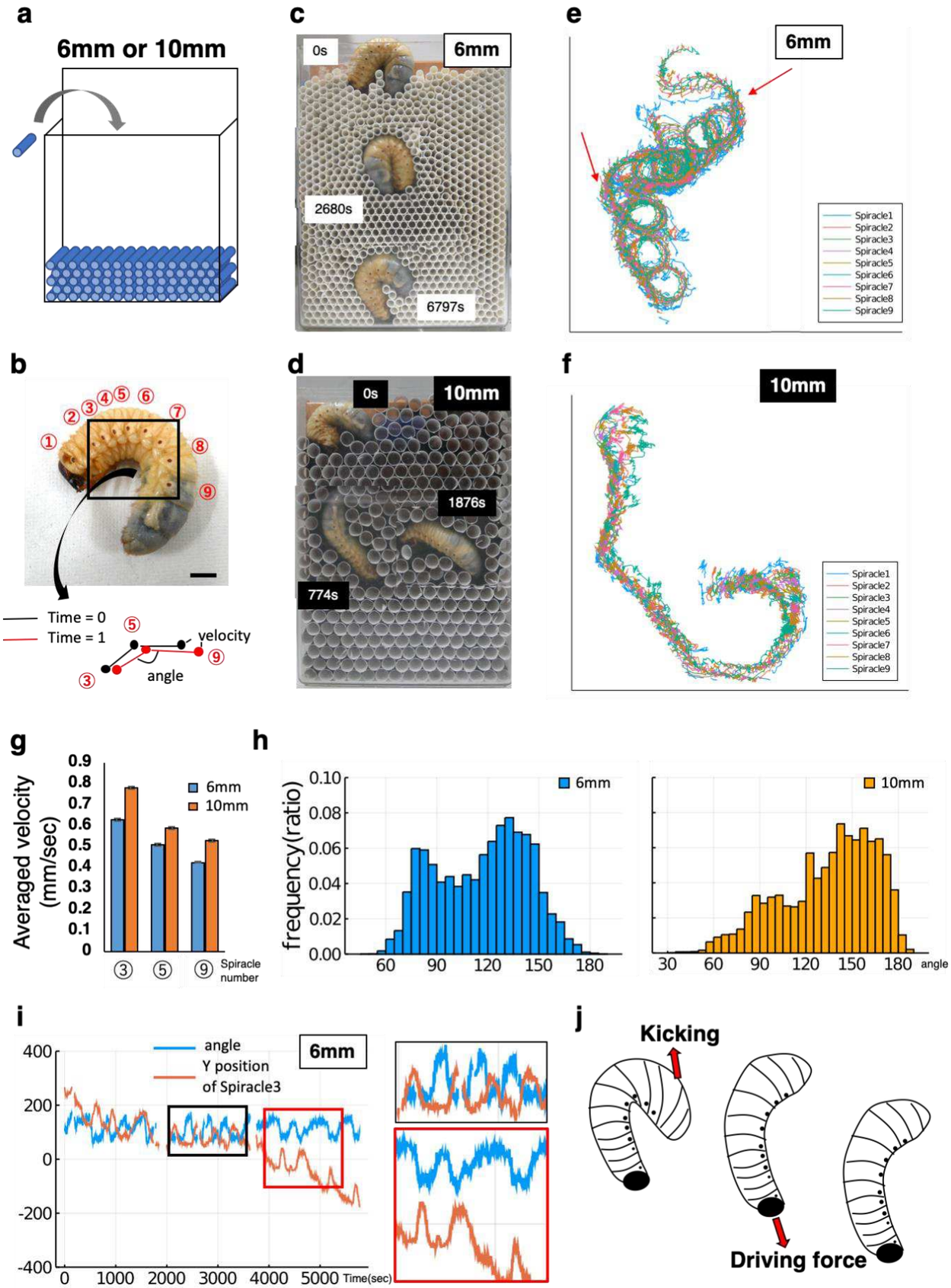


Figure 1. Burrowing dynamics of scarab beetle (*Trypoxylus dichotomus*) larva

(**a**) Burrowing images. After beetle is put on the soil, they can burrow in a short time (< 4 min). (**b**) A schematic of the pseudo-two dimensional (2D) observation tank. The depth was adjusted just enough for larva to pass. (**c**) Pseudo 2D observation image, before and after burrowing, and during burrowing. (**d**) The dynamics of burrowing in soft sediment (half volume soil compared to (**c**)).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10mm
Spiracle number
h
frequency(ratio)
6mm
10mm
angle
i
angle
Y position of Spiracle3
6mm
Time(sec)
j
Kicking
Driving force

Figure 2. Observation system to analyze burrowing movement of scarab beetle (*Trypoxylus dichotomus*) larvae

(**a**) A schematic of the pseudo two-dimensional (2D) observation system for analysis. Cut paper straws were stacked up. (**b**) Tracking analysis scheme. Nine spiracles were tracked by the ImageJ plugin "manual tracking" (Version 1.0) from three animals in each experiment. The angle was calculated from three spiracles (3,5,9).

(**c** and **d**) Pseudo 2D observation image burrowing in 6 mm and 10 mm cylinders. (**e** and **f**) The trajectory of nine spiracles burrowing in 6 mm and 10 mm cylinders. Red arrows show the twisting and turning points. (**g**) The average velocity of the spiracle in 6 mm and 10 mm cylinders. (**h**) The histogram of the angle calculated from three spiracles (3,5,9) in 6 mm and 10 mm cylinders from three beetle movement. (**i**) Relationship between rotation and the velocity of spiracle 3 in 6 mm cylinders. Blue line shows the angle calculated from three spiracles (3,5,9), and the orange line shows the vertical position of spiracle 3 each time. (Other data in the 6 mm cylinders and 10 mm cylinders are in Fig. S2).

Figures

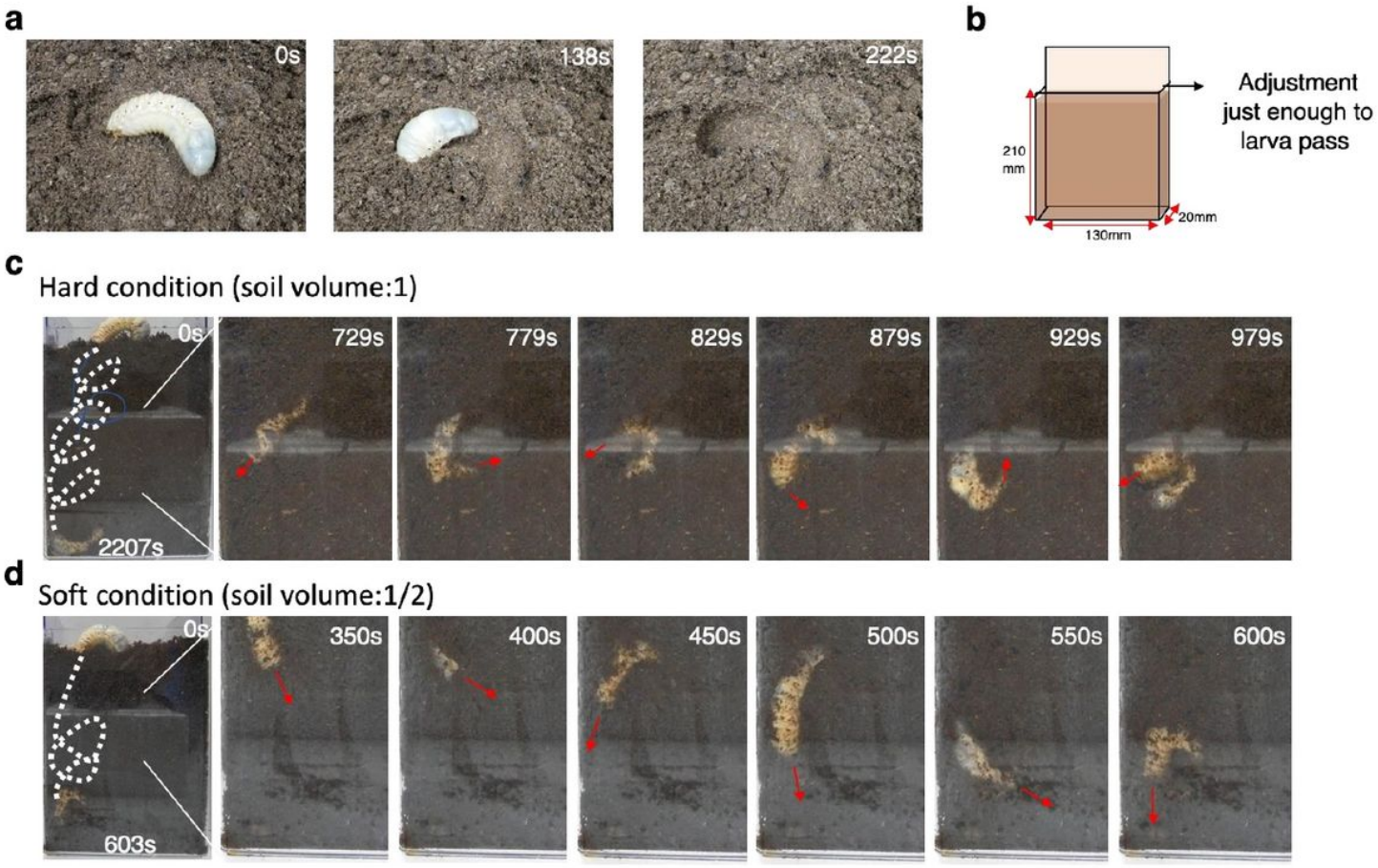

Figure 1

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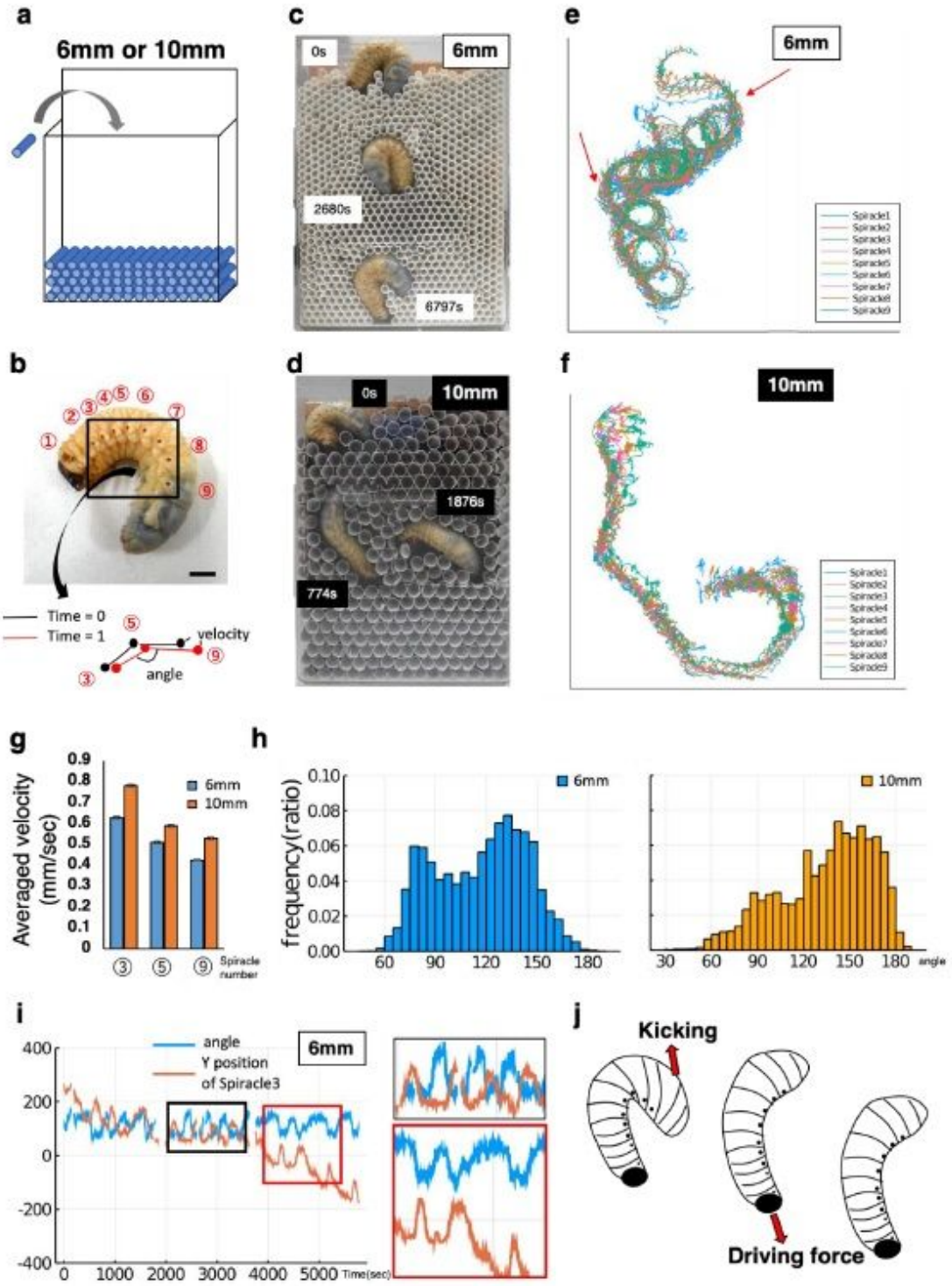

Figure 2

Observation system to analyze burrowing movement of scarab beetle (Trypoxylus dichotomus) larvae (a) A schematic of the pseudo two-dimensional (2D) observation system for analysis. Cut paper straws were stacked up. (b) Tracking analysis scheme. Nine spiracles were tracked by the ImageJ plugin “manual tracking” (Version 1.0) from three animals in each experiment. The angle was calculated from three spiracles (3,5,9). (c and d) Pseudo 2D observation image burrowing in 6 mm and 10 mm cylinders. (e and

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