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Haruhiko Adachi (✉ h.adachi@fbs.osaka-u.ac.jp)

Osaka University

Makoto Ozawa

Osaka University

Satoshi Yagi

Osaka University

Makoto Seita

Osaka University

Shigeru Kondo

Osaka University

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

2 Haruhiko Adachi^{1*}, Makoto Ozawa², Satoshi Yagi², Makoto Seita¹, Shigeru Kondo¹

3 ¹. Graduate School of Frontier Bioscience, Osaka University, Suita, Osaka, 565-0871, Japan

4 ². Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan

5 * Corresponding author and address. E-mail: h.adachi@fbs.osaka-u.ac.jp

6

7 **Abstract**

8 Many organisms live in the soil but only a little is known about their ecology especially movement style. Scarab beetle larvae do
9 not have appendages to shovel soil and their trunk is thick compared to their body length. Hence, their movement through the
10 soil is perplexing. Here, we established the observation and analysis system of larval movement and found that the last larval
11 instars of *Trypoxylus dichotomus* burrow in two different ways, depending on the hardness of the soil. If the soil is soft, the
12 larvae keep their body in a straight line and use longitudinal expansion and contraction; if the soil is hard, they flex and rotate
13 their body. It is thought that the larvae adapt to diverse soil conditions using two different excavation methods. These results are
14 important for understanding the soil ecology and pose a challenge to engineer of newer excavation technology.

15

16 **Introduction**

17 The soil is home to many organisms, and how they move through soil has been studied in several species (e.g., rats, lizards,
18 snakes, nematodes, eels, spiders, ants, earthworms) by analyzing the shape of their burrows or/and using X-ray scanning
19 technology.¹⁻¹¹ Among them, earthworms have attracted much attention as a research model because they can burrow through

20 soil without appendages, and their dynamics have recently been elucidated via an observation system using transparent
21 superabsorbent polymers.¹² Furthermore, applications of these excavation techniques are being developed.¹³⁻¹⁵ Earthworm
22 morphology seems to be suitable for burrowing in terms of the drag force from the soil, as their trunk is narrow compared to
23 their body length. However, the shape of some organisms in the soil is not suitable for burrowing. For example, the larva of the
24 scarab beetle (*Trypoxylus dichotomus*), which spend their life in the soil until adult emergence. Their trunk is very thick
25 compared to their body length; the transverse sectional diameter of a earthworm is approximately 2 mm¹⁶, whereas that of the
26 last instar larvae is ~20 mm. In other words, the transverse sectional area of beetle larvae contributing to resistance from the soil
27 is almost 100 times larger than that of a earthworm. Therefore, there the burrowing mechanism must be different from that of
28 earthworms under similar conditions. Although beetle ecology in the soil has been reported in several studies^{17,18}, little is known
29 about how beetle larvae move.

30 **Result and Discussion**

31 Here, we analyzed the burrowing mechanisms of beetle larvae. Beetle larvae were placed on the soil surface to make sure they
32 could burrow into the soil (Figure 1A). In order to observe the burrowing behavior, a two-dimensional (2D) observation tank
33 (130 × 210 × ~20 mm) was constructed (Figure 1B); we succeeded in observing the dynamics of the larvae under a 2D soil
34 condition (Figure 1C, Supplementary Movie 1). The larvae burrowed by rotating themselves (Figure 1D, Supplementary Movie
35 1). Rotation was observed regardless of sex. All observed individuals proceeded towards the bottom and stopped when rotating
36 at the bottom layer (Figure 1C).

37 Next, we investigated whether the hardness of the medium being excavated could change the dynamics of burrowing. Different
38 amounts of soil were used to compare compacted soil (relative volume of soil: 1) and non-compacted soil (relative volume of

39 soil: 1/2). The speed of burrowing was faster (Figure 1D, Supplementary Movie 1) and the degree of rotation was smaller when
40 burrowing in non-compacted soil (Figure 1D, Supplementary Movie 1). However, in these observation systems, there were
41 several areas that could not be observed clearly to analyze the dynamics because the soil covered their body. Therefore, we
42 searched for a burrowing medium that would aid in observing the burrowing dynamics clearly. We constructed a complete 2D
43 observation system by stacking cylindrical paper straws of two diameters cut into 2 cm segments (Figure 2A). By changing the
44 diameter of the straw (using 6 mm and 10 mm diameter straws), the area of the lumen changes, which is thought to have the
45 similar effect as soil compaction changes.

46 As a result, we could observe quite clearly how the burrowing larvae rotate. Through the 6 mm straws, the larvae proceeded
47 towards the bottom by rotating and stopped at the bottom layer just as in the hard soil (Fig. 2C, Supplementary Movie 3). The
48 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
49 (Fig. 2D, Supplementary Movie 5). Although beetle larvae are known to be able to change their burrowing direction depending
50 on CO₂ concentration¹⁷, their movement may also be motivated by the size of the surrounding particles or by sensing other
51 factors that vary with size.

52 We analyzed the movie of larval dynamics using Fiji's Plugin "manual tracking" (version1.0). Beetle larvae have nine
53 spiracles, which were used as indicators in the tracking analysis (Fig. 2B, E, F, S1, Supplementary Movie 4 and 6) (some parts,
54 where the spiracles were not visible, were skipped.). Larvae could twist and turn their bodies in the 2D environment (Fig. 2E,
55 red arrow). We first investigated the velocity of the spiracles. Movement of anterior parts was faster than posterior parts and the
56 velocity in the 10 mm cylinder was faster than in the 6 mm cylinder (Fig. 2G). This velocity tendency (6 mm vs 10 mm)
57 corresponds to the tendency in the soil compaction experiment (with vs without compaction). In addition, the change in angle

58 between the three spiracles (3,5,9) was measured (Fig. 2H). Although there were two peak angles under both straw medium
59 conditions, the position of the peaks was different. In 6 mm straws, the first peak was $\sim 80^\circ$ and the second peak was $\sim 130^\circ$. In
60 10 mm straws, the peaks were at $\sim 90^\circ$ and $\sim 150^\circ$. The frequency of the peaks was also different. In 10 mm straws, the first peak
61 distribution was less than in 6 mm. The two peaks indicate two modes of larval shape; the first peak indicates C-shaped shape,
62 and the second peak indicates straight shape. The two peaks show indicate two modes of larval morphology shape; the , first
63 peak indicates C-shaped shape, and the second peak indicates straight shape. It is assumed that there exists a relationship
64 between larval shape and the presence or absence of pivot movement. Hence, the larval pivot burrowing can be changed
65 depending on the surrounding environment.

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

68 Next, we examined the relationship between rotation position and larval shape. To investigate the location of rotation, we
69 plotted the time transition of the Y-position of spiracle 3. With rotation, the Y-position fluctuated up and down periodically.

70 Time series data of the angle were plotted. Angle and Y-position were inversely related (Fig. 2I, S2). This suggests that
71 rotational burrowing may contribute to the larvae pushing (kicking) the soil with their tails to move downward (Fig. 2J).

72 Another possible reason is that the rotation and pivoting motion causes local fluidization of the soil, thereby reducing the
73 resistance.¹⁹ The reason for the lack of a clear inverse relationship before reaching a certain depth is thought to be that the straw

74 was not heavy enough to provide a drag force when pushed with the buttocks. Although the tiger beetle larvae also burrow in
75 the soil,^{20,21} the shape of the hole is straight, and it is assumed that they do not burrow with rotation like the scarab beetle larvae.

76 The tiger beetle larvae are not C-shaped like the scarab beetle larvae.^{20,21} There is a possibility that larval morphology may

77 cause rotation. In the future, comparisons with the tiger beetle can facilitate ecological evolutionary developmental biology
78 (Eco-Evo-Devo) research.

79

80 In this study, we established a 2D observation system for beetle larvae and analyzed how they move. As a result, we discovered
81 that scarab beetle larvae burrow by rotating. Burrowing dynamics changed when the hardness and size of the soil particles were
82 changed. In addition, the relationship between larval shape and timing of rotation was clarified, suggesting that rotated
83 movement may be conducive to displacing the soil to move downwards. A 2D observation system was used in this study; it is
84 unclear whether the same behavior would be observed in a 3D environment. X-ray CT technology would facilitate the
85 reconstruction of images in a short time, which would elucidate burrowing dynamics. The rotational movement may be an
86 effective way of burrowing by repeating simple movements. The significance of this rotational motion needs to be confirmed by
87 reproducing it in a robot. Few studies have investigated the development of excavation robots that apply the burrowing
88 mechanisms of animals (e.g., inchworms, shellfish, and earthworms).^{15,22-24} The application of the burrowing mechanism of
89 beetle larvae may also contribute to the development of new excavation technologies.

90 The rotational movement is caused by the shape of the larvae. Comparison of the larvae with those of other soil insects, such as
91 the tiger beetle larvae^{20,21}, which burrows linearly, would facilitate Eco-Evo-Devo research. Additionally, the results of studies
92 on the frequency, position, and direction of the rotation can be applied in the field of neuroethology.

93

94 **Materials and Methods**

95 **Insects**

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

98 **Observation of burrowing**

99 The larvae were moved to a room with a temperature of 23 °C and could acclimatize for at least 30 min before the burrowing
100 experiment was conducted. Larval movements in the glass tank (6 × 21 × 2 cm) were captured by a time-lapse camera
101 (DMCGX7MK2WK, Panasonic, Osaka, Japan). The larvae were incubated in a humus mat (Kanzen, Tsukinoyakinokoen,
102 Gunma, Japan) and paper straws (6 mm, Shimojima, Tokyo, Japan) (10 mm, Zone Plus, Shiga, Japan) were cut into ~2 cm
103 pieces for visualization. Each experiment was conducted three times.

104 **Analysis of tracking**

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

111

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117 discussion.

118

119 **Competing interests**

120 The authors declare no competing interests.

121

122 **Authour contributions**

123 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:
124 H.A.; Investigation: H.A.; Visualization: H.A.; Writing – original draft: H.A. and S.K.; Writing – review & editing: H.A., M.O.,
125 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.

126

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130

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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)).

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

195 **(a)** A schematic of the pseudo two-dimensional (2D) observation system for analysis. Cut paper straws were stacked up. **(b)**

196 Tracking analysis scheme. Nine spiracles were tracked by the ImageJ plugin “manual tracking” (Version 1.0) from three

197 animals in each experiment. The angle was calculated from three spiracles (3,5,9).

198 **(c and d)** Pseudo 2D observation image burrowing in 6 mm and 10 mm cylinders. **(e and f)** The trajectory of nine spiracles

199 burrowing in 6 mm and 10 mm cylinders. Red arrows show the twisting and turning points. **(g)** The average velocity of the

200 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

201 mm cylinders from three beetle movement. **(i)** Relationship between rotation and the velocity of spiracle 3 in 6 mm cylinders.

202 Blue line shows the angle calculated from three spiracles (3,5,9), and the orange line shows the vertical position of spiracle 3

203 each time. (Other data in the 6 mm cylinders and 10 mm cylinders are in Fig. S2).

204

Figures

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)).

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 spiracle 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).

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