# BEHAVIORAL EVIDENCE FOR INTERNAL FACTORS AFFECTING DURATION OF CONGLOBATION IN PILL BUGS (*ARMADILLIDIUM VULGARE*, ISOPODA, CRUSTACEA)\*

## SHORT COMMUNICATION

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Pill bugs individually walked an experimental pathway, then were induced to conglobate with a puff of air. After recovering, they were stimulated again. Sixty of 80 pill bugs conglobated both times, first moving either antennae (A) or legs (L) during recovery. Both AA and LL groups showed a significant positive correlation between first  $(t_1)$  and second  $(t_2)$  conglobation times. In the AL group, pathway locomotion time  $(t_0)$  was significantly positively correlated to both  $t_1$  and  $t_2$ . We conclude that pill bugs determine conglobation time based partly on their previous states.

Keywords: Conglobation - internal factor - isopod - pill bug - self-reference

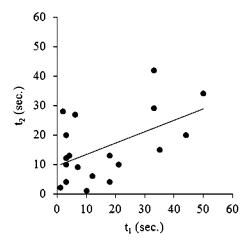
Pill bugs, *Armadillidium vulgare*, can conglobate, drawing their antennae inside to form uninterrupted spheres in response to strong vibrations or pressure. This behavior offers protection against predators such as spiders and ants [1, 5]. It also helps to conserve water [4]. Observations suggest that pill bugs recover from conglobation without external stimuli. In this study, we performed behavioral trials to investigate possible internal factors affecting duration of conglobation.

About 200 individuals were collected and kept in a plastic container with soil to a depth of 2 cm. They were fed carrot slices once a week. Humidity was maintained by wetting the soil daily. After two months, 80 individuals, each 9–10 mm in length, were selected and placed individually into petri dishes. Each was fed a small piece of carrot for three days then fasted for three days to equalize their digestive conditions.

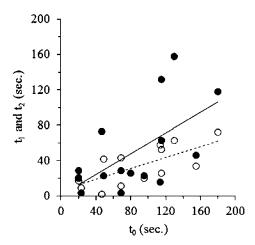
Subjects were individually placed in an arena (2 cm diameter) connected to an identical arena by a linear pathway (15 cm long). Each individual walked the path to reach the other arena then was placed on its back in a Petri dish (6 cm diameter).

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*Fig. 1.* Relationship between duration of conglobation for the first trial  $(t_1)$  and that for the second one  $(t_2)$  for the LL group. Solid line is the regression line  $(t_2 = 0.39 t_1 + 9.5)$ 



*Fig. 2.* Relationships of locomotion time along the straight pathway ( $t_0$ ) to duration of conglobation for the first trial ( $t_1$ ; solid circles) and to that for the second ( $t_2$ ; open circles) in the AL group. Solid line is the  $t_0 - t_1$  regression line ( $t_1 = 0.59 t_0 + 0.77$ ). Dashed line is the  $t_0 - t_2$  regression line ( $t_2 = 0.31 t_0 + 6.7$ )

In the first trial, the ventral surface of individuals was stimulated by a puff of air, and 69 of 80 conglobated. Each initially moved either its antennae or its legs when recovering. Pill bugs were stimulated a second time with air immediately after stretching their bodies in recovery, and 60 of 69 individuals conglobated.

Locomotion time along the straight pathway  $(t_0)$  and duration of conglobation  $(t_1 \text{ and } t_2, \text{ respectively})$  were measured in each trial. Of the 60 individuals that con-

globated both times, 20 first moved their antennae both times (Antenna-Antenna group; AA) and another 20 first moved their legs both times when recovering (Leg-Leg group; LL). Of the remaining 20 pill bugs, 15 moved their antennae in the first trial and legs in the second (AL), while five moved their legs in the first trial and antennae in the second (LA). The LA group was significantly smaller than the others (McNemar test;  $\chi^2 = 9.06$ , df = 6, p < 0.05).

The mean  $t_0 \pm S.E.$  (sec.) were 77.9 ± 10.9 (AA), 40.9 ± 3.4 (LL), 85.4 ± 12.8 (AL), and 83.4 ± 24.6 (LA). The value for the LL group was significantly smaller than the others (Kruskal–Wallis test; H=9.6, df=3, p<0.023). The mean  $t_1\pm S.E.$  (sec.) were 27.8 ± 6.4 (AA), 20.4 ± 5.8 (LL), 50.9 ± 12.5 (AL), and 20.6 ± 7.1 (LA), with no significant differences among them. The mean  $t_2\pm S.E.$  (sec.) were 25.9 ± 5.1 (AA), 16.8 ± 2.7 (LL), 33.1 ± 5.5 (AL), and 28.4 ± 9.1 (LA), and there were no significant differences among them.

In the AA and LL groups, there were significant positive correlations between  $t_1$  and  $t_2$  (Spearman's rank correlation; AA: rs = 0.657, p < 0.01; LL: rs = 0.476, p < 0.05) (Fig. 1). In the AL group, there were significant positive correlations between  $t_0$  and  $t_1$  (rs = 0.563, p < 0.05) and between  $t_0$  and  $t_2$  (rs = 0.725, p < 0.01) (Fig. 2), while there was no correlation between  $t_1$  and  $t_2$ .

Our correlation analyses imply that AA and LL individuals determined  $t_2$  based on  $t_1$  while AL individuals determined  $t_1$  and  $t_2$  based on  $t_0$ . This implication suggests that one factor affecting duration of conglobation is the previous internal state. Similar self-referential properties of prior states have been demonstrated in decision-making [2] and problem solving [3]. Further behavioral experiments are needed to reveal the neurological mechanism underlying pill bug conglobation and recovery.

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