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Observation of Self-Assemblage Behavior in African Nightcrawler (*Eudrilus eugeniae*)

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ABSTRACT

Self-assemblage is a form of social behavior observed in some earthworm species, where individuals spontaneously group with conspecifics. This behavior has been previously documented in *Eisenia fetida*, an epigeic species widely used in vermiculture. However, the presence of similar behavior in *E. eugeniae*, another commonly used vermicomposting species, remains untested. This study aimed to investigate the self-assemblage behavior of *E. eugeniae* through a Y-tube choice test, evaluating individual preferences for conspecific presence. The experiment was conducted under controlled laboratory conditions using 30 replicates for both main (with conspecifics) and control (without conspecifics) treatments. Results showed a significant preference for the conspecific group in the main test ($\chi^2 = 6.5$, $p = 0.01$), while no directional bias was observed in the control. However, decision-making time did not differ significantly between treatments ($p = 0.63$). These findings indicate that *E. eugeniae* displays active self-assemblage behavior. Further research is needed to identify the mechanisms underlying this behavior and its implications for applied soil ecology.

1. Introduction

Collective behavior refers to the emergence of coordinated group patterns through repeated local interactions between individuals without requiring complex cognition or centralized control (Carlesso & Reid 2023). Collective behavior often occurs in flocking birds, schooling fish, ant colonies, and other social animal groups (Couzin *et al.* 2005). Collective behavior often emerges in response to various ecological factors such as temperature, light, resource availability, or the presence of conspecifics (Maknun 2017). These local interactions can lead to group cohesion or synchronized movement, support the spread of information, and increase survival, reproductive success, and overall fitness (Giardina 2008).

One phenomenon of collective behavior in biological systems is self-assemblage, where individual components spontaneously organize into functional group structures through environmental cues (Whitesides & Grzybowski 2002). Self-assemblage can emerge from simple rules and stimuli, leading to complex structures and group cohesion without centralized control. Zirbes *et al.* (2012) demonstrated self-assemblage behavior in Eisenia fetida, where individuals tend to cluster and remain in conspecific groups. Volatile aggregation signals may mediate this behavior, though the specific compounds remain unidentified. Understanding self-assemblage behavior is also crucial in ecological contexts, where it may contribute to survival and ecosystem functioning.

In ecological contexts, self-assemblage in earthworms may improve their efficiency as decomposers by enhancing moisture retention, increasing mating opportunities, and supporting group-level survival in microhabitats (Dominguez *et al.* 2001; Blouin *et al.* 2013). Such clustering has been observed to accelerate vermicomposting, as seen in studies using *E. fetida* and related species (Tahir & Hamid 2012; Hazra *et al.* 2018).

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However, it is still unknown whether self-assemblage behavior also occurs in other earthworm species, thus creating an issue that needs to be studied. One species that has attracted attention is *Eudrilus eugeniae*, which shows similar ecological traits and behavioral patterns to *E. fetida*, especially in its epigeic lifestyle, high surface activity, and frequent use in vermiculture systems. These similarities suggest that *E. eugeniae* may also have the potential for self-assemblage behavior, although this has not been empirically tested.

2. Materials and Methods

2.1. Materials

This experimental study was conducted at the Animal House, Laboratory of Animal Behavioral Physiology, IPB University. Adult individuals of *E. eugeniae* were purchased from CV. Ilman Jaya Organik Nusantara, Bogor, Indonesia. The worms were maintained in plastic containers filled with moist soil (60-70% humidity) (Ahmed and Al-Mutairi 2022) and covered with pararanet to control light exposure. Moisture was monitored using a 4-in-1 soil tester, and the worms were fed daily with tofu dregs at room temperature (27°C), with environmental conditions recorded using a 4-in-1 environmental meter.

The experimental setup included a semi-transparent Y-tube with two circular target chambers (8 cm diameter), two connecting branches (9 cm), a 9 cm main stem, a filter cloth, and a camera for documentation. Additional materials included distilled water and 70% ethanol for cleaning**.**

2.2. Experimental Procedures

Six *E. eugeniae* test worms were used, each tested five times, resulting in 30 replications per condition. The worms were acclimated for seven days before testing. A Y-tube choice test was used to assess self-assemblage behavior (Zirbes *et al.* 2012). Each branch of the Y-tube led to a target chamber (Figure 1). In the main experimental condition, one of the chambers (Figure 1 zone C) contained ten adult *E. eugeniae*, while the other chamber was empty. A filter cloth was used to prevent worms in the stimulus chamber from escaping or physically interacting with the test worms.

Worms were rinsed with distilled water before each trial, and all apparatus components were cleaned with 70% ethanol between trials to avoid contamination all apparatus components were cleaned with 70% ethanol between trials to avoid contamination. A single *E. eugeniae* earthworm was placed at the end of the Y-tube (Figure 1 A zone). A choice was considered to have occurred when the worms chose one of the target chambers, while in the control trial, both chambers were left empty. The decision time (time taken to make a choice) was recorded using a stopwatch, with a maximum observation duration of 45 minutes.

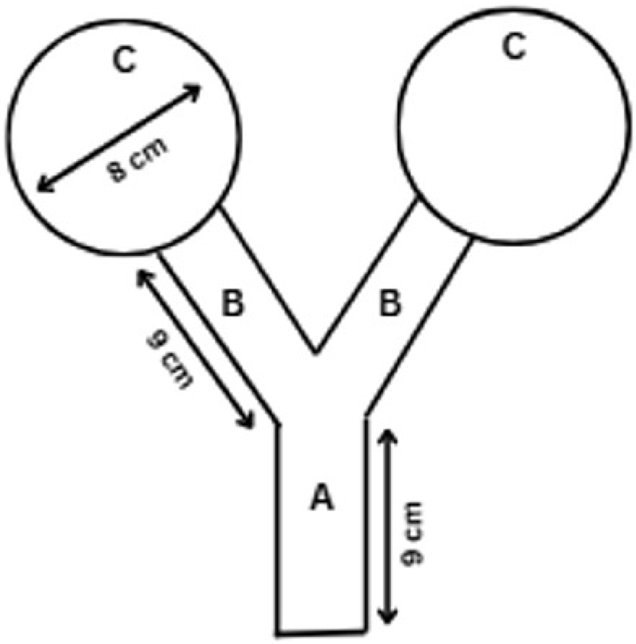

Figure 1. Y-tube used in the self-assemblage assay of *E. eugeniae.* (A) Neutral zone (9 cm) where a single individual was introduced at the start of the trial, (B) two lateral arms (9 cm each) through which the worm could move, (C) circular target chambers (8 cm in diameter) that were either filled with 10 conspecific individuals or left empty depending on the test condition

2.3. Statistical Analysis

Data analysis was performed using version 4.2.2 (R Core Team 2022) in RStudio (Posit, PBC). A chi-square goodness-of-fit test was used to determine whether *E. eugeniae* showed a significant preference for conspecifics in the Y-tube choice assay. Before comparing decision-making times between the experimental and control conditions, the Shapiro–Wilk test was applied to assess the normality of the data. The results showed that the data were not normally distributed in either condition (main: $p = 0.003$; control: $p = 0.03$). As the data did not meet the assumptions of normality, the non-parametric Mann–Whitney U test was employed for further analysis.

3. Results

3.1. Choice Preference in Y-tube Test

Behavioral observations using the Y-tube choice test showed that *E. eugeniae* had a significant tendency to move towards conspecifics, indicating the presence of self-assemblage behavior. In 22 out of 30 trials, individuals chose the arm leading to the conspecific group, a value significantly higher than random expectation (Chi-square test, χ^2 = 6.5, df = 1, p = 0.01) (Table 1). These results support the hypothesis that *E. eugeniae* actively seeks out its species. In contrast, the control experiment showed no significant difference between choices of the left or right branch (χ^2 = 0.5, df = 1, p = 0.46), indicating random movement and ruling out directional bias in the test experimental setup.

Figure 2 provides visual documentation of worm movement during the test to complement the statistical findings. The image shows an *E. eugeniae* individual (circled) approaching the chamber containing conspecifics, indicating a behavioral preference consistent with the quantitative data.

The comparison of decision-making time between treatments showed no significant difference. *E. eugeniae* took 22.64±1.91 seconds (Mean ± SE) in the main test and 21.20±1.83 seconds in the control (p = 0.63), suggesting that conspecific presence did not influence decision-making duration.

Table 1. Earthworm choices and number of experiments in Y-tube

Condition	Choice direction	Experiment number	p-value
Main experiment	Group way Other way	22 8	0.01*
Total		30	
Control experiment	Left Right	13 17	0.46
Total		30	

*A significant difference in the main test (Chi-Square test, p<0.05)

4. Discussion

Our study showed that a significant proportion of *E. eugeniae* individuals chose the conspecific chamber, indicating their ability to detect and respond to the presence of conspecifics, as confirmed by visual observation (Figure 2), where individuals were clearly observed aggregating in the conspecific chamber. This aggregation tendency resembles the behavior observed in *E. fetida*, as Zirbes *et al.* (2012) reported, where individuals were observed to join and remain in groups of their species. However, when comparing decision-

Table 2. Earthworm choice times for behavioral test in Y setup

Condition	Average time (s) ± SD	Average time (s) ± SE	p-value
Main experiment	22.64±10.45	22.64±1.91	0.01*
Control experiment	21.20±10.05	21.20±1.83	

*A significant difference in the main test (p<0.05)

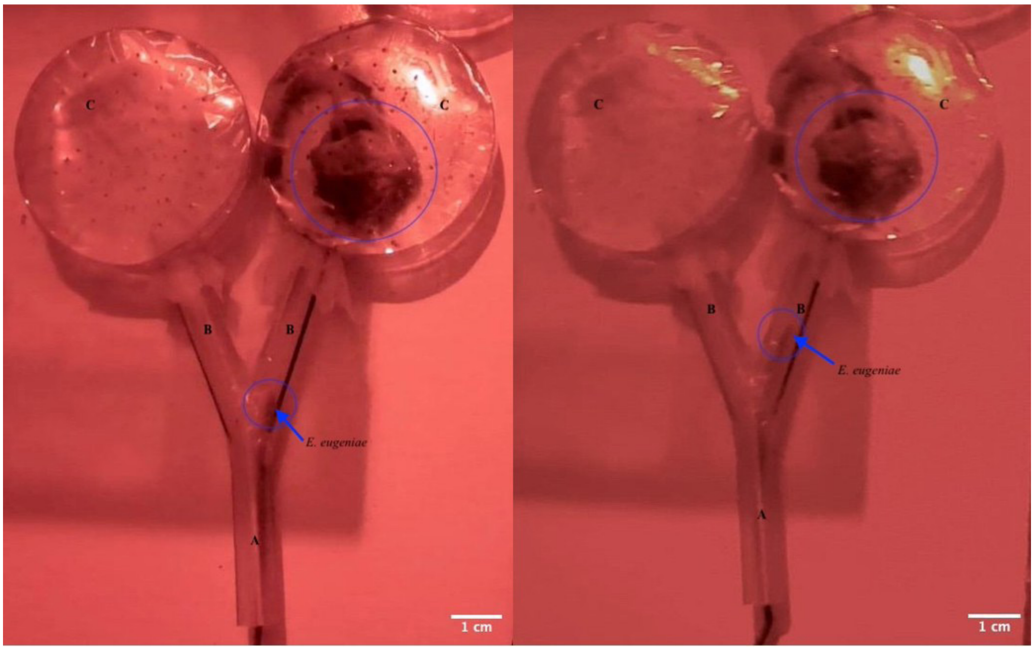

Figure 2. A representative image from the Y-tube choice test shows an *E. eugeniae* individual (circled) moving toward the chamber containing conspecifics. The chamber on the right contains 10 conspecifics; the left chamber is empty

making time, *E. eugeniae* showed no significant difference between the main and control conditions, suggesting that conspecifics did not affect path selection speed. Since this study aimed to assess immediate directional preference, the recorded behavior was limited to the moment individuals chose between the two arms of the Y-tube. Each trial was completed in under 30 seconds, and as such, long-term observations (e.g., at 5-10 minute intervals) were not part of the experimental design. Therefore, the interpretation of decision speed in this context reflects short-term response rather than prolonged exploratory behavior .

In contrast, *E. fetida* exhibited a significantly longer decision time in the presence of conspecifics ($p = 0.0001$) (Zirbes *et al.* 2012). Although the underlying mechanism remains unclear, Zirbes *et al.* (2011) identified volatile organic compounds (VOCs), such as ethyl pentanoate and ethyl hexanoate, as possible aggregation cues in *E. fetida*. These chemical signals may require exploratory behavior to be evaluated before movement decisions are made. In contrast, *E. eugeniae* did not show prolonged decision-making in the presence of conspecifics, which may suggest that its response to chemical cues is faster. However, the present study did not examine the specific role of chemical signals in *E. eugeniae* aggregation behavior. Further research is needed to identify the organic compounds or other factors involved in the movement decision-making process of *E. eugeniae*.

Earthworms respond to environmental cues, such as moisture and substrate texture, and social cues like pheromones. In the present study, environmental cues were minimized during testing by not placing soil or substrate inside the Y-tube apparatus. Additionally, humidity (60-70%) and temperature (27°C) were kept constant throughout all trials to reduce external influences on earthworm movement. Environmental cues guide individual orientation, whereas social signals, particularly aggregation pheromones, influence group-oriented behaviors (Satchell 1983; Blouin *et al.* 2013). Exploratory behavior, including head swaying, intermittent pausing, and substrate probing, plays a role in evaluating such cues before directional movement (Butt & Nuutinen 2005; Zirbes *et al.* 2011). Similar exploration has been documented in *Metaphire* sp., which was observed to navigate existing and newly formed tunnels by changing direction, moving back and forth, and revisiting paths (Karmila *et al.* 2023). While these behaviors are well-characterized in other species, similar detailed descriptions are still lacking for *E. eugeniae*. Future studies are needed to determine whether *E. eugeniae* engages in comparable exploratory movements during conspecific detection.

Beyond behavioral differences, aggregation in *E. eugeniae* may offer ecological benefits. Group formation may enhance survival in subterranean environments by stabilizing microhabitat conditions, reducing desiccation through moisture retention, and increasing reproductive success via encounter rates (Giardina 2008; Sivasubramaniam 2021). *E. eugeniae* preference for conspecific groups aligns with that of *E. fetida*, reinforcing the role of social cues in shaping collective behavior. In conclusion, in the absence of conspecifics or external cues, individuals exhibited random movement, reaffirming that aggregation in *E. eugeniae* is an active and cue-driven process. These results confirm that *E. eugeniae* exhibits aggregation tendencies similar to those of *E. fetida*.

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References

Ahmed, N., Al-Mutairi, K.A., 2022. Earthworms affect microbial population and soil fertility as well as their interaction with agriculture practices. *Sustainability* 14, 7803. https://doi.org/10.3390/su14137803

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Butt, K.R., Dai, J., Dendooven, L., Pérès, G., Tondoh, J.E., Cluzeau, D., *et al*. 2013. A review of earthworm impact on soil function and ecosystem services. *Eur. J. Soil Sci.* 64, 161–182. https://doi.org/10.1111/ejss.12025

Butt, K.R., Nuutinen, V., 2005. The behaviour of Lumbricus terrestris in relation to spatial distribution of food. *Biol. Fertil. Soils* 41, 198–203. https://doi.org/10.1007/s00374-005-0823-6

Carlesso, D., Reid, R.C., 2023. How to become one: the proximate mechanisms of self-assembly behaviour in social insects (Hymenoptera: Formicidae, Apidae). *Myrmecol. News.* 33, 149–168. https://doi.org/10.25849/myrmecol.news_033:149

Couzin, I.D., Krause, J., Franks, N.R., Levin, S., 2005. Effective leadership and decision-making in animal groups on the move. *Nature.* 433, 513–516. https://doi.org/10.1038/nature03236

Domínguez, J., Edwards, C.A., Domínguez, A., 2001. The biology and population dynamics of *Eudrilus eugeniae* (Kinberg) (Oligochaeta) in cattle waste solids. *Pedobiologia.* 45, 341–353. https://doi.org/10.1078/0031-4056-00090

Giardina, I., 2008. Collective behavior in animal groups: theoretical and empirical studies. *HFSP J.* 2, 205–219. https://doi.org/10.2976/1.2961038

Hazra, D., Sinha, R.K., Singh, K., Soni, B.K., 2018. Earthworms as natural bioreactors: their role in the conversion of rrganic wastes into vermicompost. *International Journal of Recycling of Organic Waste in Agriculture*. 7, 145–155. https://doi.org/10.1007/s40093-018-0211-0

Karmila, D., Widarto, H.T., 2023. Behavioural observation of earthworm *Metaphire* sp. as an initial domestication effort. *J. Sumberdaya Hayati.* 9, 70–80.
Maknun, D., 2017. Ekologi: *Populasi, Komunitas, Ekosistem Mewujudkan Kampus Hijau Asri, Islami dan Ilmiah.* Nurjati Press, Cirebon.
R Core Team, 2022. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.
Satchell, J.E., 1983. *Earthworm Ecology: From Darwin to Vermiculture*. Chapman and Hall, London.
Sivasubramaniam, S., 2021. The earthworm *Eudrilus eugeniae*: a model organism from regenerative biology. *J. Genet. Genomic Sci.* 6, 1–4. https://doi.org/10.24966/GGS-2485/100023
Tahir, T.A., Hamid, F.S., 2012. Vermicomposting of two types of coconut wastes employing *Eudrilus eugeniae*: a comparative study. *Int. J. Recycl. Org. Waste Agricult.* 1, 7. https://doi.org/10.1186/2251-7715-1-7
Whitesides, G.M., Grzybowski, B., 2002. Self-assembly at all scales. *Science.* 295, 2418–2421. https://doi.org/10.1126/science.1070821
Zirbes, L., Brostaux, Y., Mescher, M., Jason, E., Deneubourg, J., 2012. Self-assemblage and quorum in the earthworm *Eisenia fetida* (Oligochaete, Lumbricidae). *PLoS One.* 7, e32564. https://doi.org/10.1371/journal.pone.0032564
Zirbes, L., Mescher, M., Vrancken, V., Wathelet, J.P., Verheggen, F.J., Thonart, P., Haubruge, E., 2011. Earthworms use odor cues to locate and aggregate in soil. *PLoS One.* 6, e17752. https://doi.org/10.1371/journal.pone.0017752