

1 **Short title:** Movements and castes in a termite

2

3 **Caste-biased movements by termites in isolation**

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19

20 **Abstract**

21 The caste system of termites is an example of phenotypic plasticity. The castes differ not only  
22 in morphology and physiology, but also in behavior. As most of their behaviors within  
23 colonies involve nestmates, it is difficult to extract innate differences among castes. In this  
24 study, we focused on movement patterns of isolated individuals of *Hodotermopsis sjostedti*.  
25 We observed distinct clusters in movement patterns over 30 min, which indicates that termites  
26 have multiple innate modes of movement. The use of these modes is biased among castes,  
27 among which neotenics had a caste-specific mode and soldiers moved more actively than  
28 workers or neotenics. These caste biases may reflect different adaptive responses to social  
29 isolation. Our study provides a basis for a deeper understanding of the roles of individual  
30 movements in social behaviors.

31

32 **Summary Statement**

33 Movement patterns of termites in isolation were described for different castes. We proposed  
34 movements as a novel caste-specific characteristics in social insects.

35

## 36 **Introduction**

37 Phenotypic plasticity refers to a flexible, epigenetic regulation of organismal phenotypes in  
38 response to environment (West-Eberhard, 2003). Because the regulation is usually adaptive to  
39 environment, phenotypic plasticity is usually considered as a product of evolutionary  
40 adaptation (Pigliucci, 2005; Pfennig et al., 2010). Phenotypes include not only morphology  
41 and physiology, but also behaviors (Hau and Goymann, 2015). While morphological  
42 plasticity is associated with ontogeny (such as molting), behavioral plasticity can emerge  
43 among individuals even with the same morphology or developmental stages. Therefore, the  
44 degree of behavioral plasticity can be characterized as crosstalk between internal (e.g.,  
45 morphological, physiological, neuronal) and external (i.e., environmental, social) factors of  
46 individuals (Nussey et al., 2007; Dingemanse et al., 2009).

47 Insects provide plenty of examples of phenotypic plasticity, many of which are realized  
48 in response to hormones (Gilbert and Epel, 2009). Some eusocial insects, such as ants and  
49 termites, have morphologically distinct castes (Bourke and Franks, 1995; Eggleton, 2010).  
50 Each caste is a specialist of a particular colonial task associated with its morphology, and this  
51 specialization can lead to sophisticated division of labor among nestmates (Wilson, 1971). For  
52 example, soldier castes in termites have specialized mandibles that play a key role in nest  
53 defense (Eggleton, 2010). Workers of the leaf-cutting ant *Atta cephalotes* can be divided into  
54 small- and large-sized castes called minim and media. Minim ants deposit pheromones on  
55 their foraging trails more frequently than do media ants (Evison et al., 2008). Accumulating  
56 evidence indicates that social interactions among nestmates alter individual hormone levels  
57 that regulate molecular mechanisms involved in caste differentiation, thus optimizing caste  
58 ratios in colonies (Fewell and Gadau, 2009; Bourke, 2011). The degree of behavioral  
59 plasticity can depend on differences in social context among workers (Tanner, 2008; Tanner  
60 and Adler, 2009) and among castes (Ishikawa and Miura, 2012; Sun et al., 2013). To  
61 understand the role of behavioral plasticity in the division of labor in social insects, it is  
62 important to reveal innate properties of individual behavior and between-caste differences,  
63 ideally in the absence of social interactions.

64 One of the most elemental behavioral components of animals is their movement pattern.  
65 Although simply defined as a change in the spatial location of individuals with time,

66 movement plays a central role in determining the fate of individuals (Nathan et al., 2008). In  
67 social insects, interactions among nestmates are essential to their lives. This need may result  
68 in the evolution of efficient movement characteristics of individual social insects. For  
69 example, workers of the termite *Cornitermes cumulans* show Lévy walk characteristics in  
70 their free walking patterns, which is known to be an efficient search movement (Miramontes  
71 et al., 2014). Since each morphological caste is assigned a role in the colony, we can  
72 hypothesize that these roles are correlated with caste-specific movement patterns. However,  
73 caste-specific patterns are largely unexplored.

74 The Japanese damp-wood termite *Hodotermopsis sjostedti* is a relatively basal species  
75 within termite phylogeny (Legendre et al., 2013). The elder instar larvae, called pseudergates,  
76 have the potential to differentiate into alates, neotenics (supplementary reproductives), and  
77 soldiers (Miura, 2001). Because the fourth instar and older larvae behave as the worker caste  
78 (Shimoji et al., 2017), we use the term “worker” instead of “elder instar larva” in this paper.  
79 This species provides a good model system to study caste differentiation, as morphological  
80 caste differentiation is regulated by nestmate interactions that induce hormonal changes in  
81 individuals (Cornette et al., 2008; Shimoji et al., 2017).

82 In this study, we tracked free-walking behavior of termites, and quantitatively evaluated  
83 the innate properties of individual movement using a video tracking system. From video  
84 analysis, we extracted movement characteristics of workers, soldiers, and neotenics. We also  
85 analyzed 14 morphological traits. By comparing innate movement properties with  
86 morphological traits, we explored the potential of movement patterns as caste-specific  
87 identifiers. We discuss how variation in innate movement patterns among castes operates in  
88 termite society.

89

## 90 **Materials and methods**

### 91 **Termites**

92 We collected colonies of *Hodotermopsis sjostedti* on Amami Island, Kagoshima prefecture, in  
93 April 2017. All colonies were brought back to the laboratory within their substrates, and were  
94 maintained at room temperature in the laboratory until the experiments. Sex was determined  
95 from external morphology (Miura et al., 2000). We chose females and males of three castes:  
96 workers, neotenics, and soldiers. The soldiers were easily identified by their large pigmented  
97 heads, and the neotenics were identified later by distinctly developed gonads found in their

98 dissected abdomens (Oguchi et al., 2016).

99

## 100 **Experimental design**

101 To compare the movement patterns of individuals of each caste (Fig. 1A), we recorded  
102 individual walking trajectories in an experimental arena (Fig. 1B). Each individual was  
103 marked with a black spot on the abdomen to make it easy to see. A single termite was placed  
104 in the arena and its movement was recorded for 35 min. We made the arena which was  
105 consisted of a white circular polystyrene surface (290-mm inside diameter) bounded by a  
106 plastic wall (50-mm height). The surface was cleaned with 70% ethanol before each trial. The  
107 arena was placed in a cardboard box to exclude any natural light and air currents, and the  
108 experiment was conducted at constant  $25 \pm 1$  °C under infrared light from a LED. A web  
109 camera (DC-NCR300U, Hanwha Japan) was positioned perpendicularly above the arena so  
110 that the arena filled the image frame (Fig. 1C). Video was recorded to a Windows PC in CCI-  
111 Pro-MR software (<http://www.cosmosoft.org/CCI-Pro-MR/>) at a resolution of  $640 \times 480$   
112 pixels and a frequency of 30 FPS. The coordinates of termite movement with time were  
113 extracted from each video in UMA tracker software (<http://ymnk13.github.io/UMATracker/>).  
114 After each recording, each individual was stored in 70% ethanol to confirm sex and caste  
115 under a microscope. In total, we recorded the movements of 10 male workers (3 from colony  
116 A, 4 from B, 3 from C), 10 female workers (3, 4, 3), 8 male soldiers (3, 2, 3), 9 female  
117 soldiers (3, 3, 3), 3 male neotenics (2, 0, 1), and 9 female neotenics (3, 3, 3) (Table S1).

118

## 119 **Movement analysis**

120 Data were analyzed in R v. 3.4.1 software (R Core Team, 2017) with the packages  
121 “CircStats”, “rcompanion”, and “MASS”. We measured nine characteristics of movement  
122 patterns from the videos: mean, maximum, and mode of instantaneous speed ( $\text{mm s}^{-1}$ ); SD of  
123 acceleration; total pausing time (s), number of pauses ( $\text{s}^{-1}$ ), total wall-following time (s); and  
124 shape (scale factor  $\rho$ ) and peak position (the absolute value of peak position  $\mu$ ) of the  
125 distribution of turning angle when it was fitted to a wrapped Cauchy distribution. To decrease  
126 the noise arising from video analysis during data analysis, we reduced the frame frequency to  
127 2 FPS. We discarded data of the first 5 min to avoid effects of handling. Two trials had a  
128 tracking time of <35 min. As termites sometimes stumbled and overturned, we watched each  
129 video and omitted such events from analyses.

130 First, we computed the instantaneous speed as the distance covered by an individual from one  
131 frame to the next. As the distribution of instantaneous speed was bimodal, with peaks at  
132 around 0 and 20 mm s<sup>-1</sup>, we evaluated the mode of instantaneous speed during moving from  
133 the values of the second peak. Acceleration was computed as the change in the instantaneous  
134 speed. As the mean of acceleration must be 0, we calculated the SD of the acceleration as a  
135 descriptive parameter. We assumed that a termite remained motionless when the  
136 instantaneous speed was <2 mm s<sup>-1</sup>. As the duration of analysis differed among individuals,  
137 we standardized the sum of pausing time and the number of pauses by dividing by the  
138 duration of analyzed time. We considered that an individual showed wall-following behavior  
139 when it was <10 mm from the wall, and the sum of wall-following time was standardized as  
140 for pausing time. The direction of movement was computed as the angle between the  
141 corresponding displacement and the horizontal, and turning angle was identified as the  
142 magnitude of change in the direction of movement from one frame to the next. To identify the  
143 degree of angular correlation in termite movements, which naturally comes from local  
144 scanning behavior of animals (Bartumeus and Levin, 2008), we fitted the data of turning  
145 angle to a wrapped Cauchy distribution using the maximum likelihood estimation method.  
146 The estimates of scale factor  $\rho$  and the absolute value of peak position  $\mu$  of the distribution  
147 were obtained using the *wrpcauchy.ml* function.

148

### 149 **Morphometric analysis**

150 To evaluate morphological differences among castes and sexes, we measured body parts  
151 (Koshikawa et al., 2002, 2005) of all stored individuals in image analysis software (cellSens  
152 Standard; Olympus, Japan) (Fig. S1). We measured the following 14 distances under a  
153 stereomicroscope (SZX-16; Olympus, Japan): head length (from the base of the mandible to  
154 the posterior margin of the head), maximum head width, head width at base of mandibles,  
155 labrum width, post-mentum length, post-mentum width, left mandible length (straight cross  
156 length from the condyle to the tip), pronotum length, pronotum width, mesonotum width,  
157 metanotum width, femur width (hind femur), femur length (hind femur), and tibia length  
158 (hind tibia) (Fig. S1).

159

### 160 **Statistical analyses**

161 To distinguish the movement and morphological patterns among castes, we used cluster

162 analysis with Euclidean distance matrices for degree of similarity and Ward's method for  
163 clustering. We created separate dendrograms for movement patterns (using the nine  
164 characteristics) and morphological patterns (using the 14 body part measurements). All  
165 variables were standardized by Z-transformation before analysis.

166 To compare dominant movement components among castes, we performed a principal  
167 components analysis (PCA) using the function *prcomp*. PCA was performed for movement  
168 patterns and morphological patterns independently, and variables were prepared as in the  
169 cluster analysis. The Kaiser–Meyer–Olkin measure of sampling adequacy gave a value of  
170 0.6412 for the dataset of movement patterns (classified as “mediocre”) and 0.9431 for that of  
171 morphological patterns (classified as “meritorious”; Kaiser, 1974). We reduced the variables  
172 to two principal components (PC1 and PC2) in each analysis as representative characteristics.  
173 Then we compared these PC scores between castes and colonies using Scheirer–Ray–Hare  
174 (SRH) tests (Sokal and Rohlf, 1995) with the function *scheirerRayHare*. We also compared  
175 PC1 between sexes and colonies of each caste using SRH tests.

176 Finally, we examined the relationship between movement traits (PC1) and morphological  
177 traits (PC1). We tested the correlation for each caste and sex using Kendall's coefficient of  
178 concordance, pooling original colonies in each caste and sex. The significance level  $\alpha$  was  
179 adjusted to 0.00833 using Bonferroni's method.

180

## 181 **Results and Discussion**

182 The clustering of individual movement patterns gave a hierarchical structure: in some groups,  
183 individual trajectories were distinctly more similar to one another than to those in other  
184 groups (Figs. 2, S2). Clustering and heat map patterns revealed four clusters with the  
185 following characteristics: highly active movements with frequent wall-following (cluster 1);  
186 highly active movements with less frequent wall-following (cluster 2); mixed movement of  
187 cluster 1 and 2 (cluster 3); and little active movement with frequent pauses (cluster 4) (Figs. 2,  
188 S2). This suggests that *H. sjostedti* has different movement modes, corresponding to different  
189 clusters, which can be statistically distinguished (Fryxell et al., 2008).

190 Clusters 1 to 3 encompassed all castes, whereas cluster 4 was neotenic-specific (Fig. 2).  
191 The expression of different movement modes was significantly caste-biased (Fisher's exact  
192 test,  $P = 0.006$ ), as soldiers favored cluster 1 and workers favored cluster 2 (Fig. 2). The first  
193 component, PC1, explained 54.51% of the total variance. The highest loadings for this  
194 component were associated with how actively termites moved (high loadings for PC1 include



195 mean speed, pause time, and number of pauses) or moving speed (mode and maximum speed).  
196 PC2 explained 17.67% of the total variance and had the highest loadings for the total wall-  
197 following time and the SD of acceleration. PC1 was significantly different among castes and  
198 among colonies (SRH test: caste,  $H_2 = 11.990$ ,  $P = 0.002$ ; colony,  $H_2 = 10.074$ ,  $P = 0.006$ ;  
199 caste  $\times$  colony,  $H_4 = 3.350$ ,  $P = 0.501$ ; Fig. 3), whereas PC2 was not different among castes or  
200 colonies (SRH test: caste,  $H_2 = 4.169$ ,  $P = 0.124$ ; colony,  $H_2 = 0.098$ ,  $P = 0.952$ ; caste  $\times$   
201 colony:  $H_4 = 6.413$ ,  $P = 0.170$ ; Fig. 3). A sex difference was not detected in any caste (SRH  
202 test: worker,  $H_2 = 0.571$ ,  $P = 0.450$ ; soldier,  $H_2 = 3.343$ ,  $P = 0.068$ ; neotenics,  $H_2 = 0.692$ ,  $P =$   
203 0.405).

204 In stark contrast, cluster analysis using individual morphology gave two distinct clusters,  
205 one of which contained all soldiers and the other was a mixture of workers and neotenics (Fig.  
206 S3). As evident from the heat map, the soldier cluster was characterized by larger size in all  
207 body parts (Figs. S3, S4B). Similarly, PCA reduced the 14 morphological characteristics to  
208 two major PCs, of which PC1 explained 82.78% of the total variance and PC2 explained  
209 4.65%. As PC1 was positively correlated with all measurements, it was considered to indicate  
210 general body size. PC1 was significantly different among castes (SRH test: caste,  $H_2 = 32.664$ ,  
211  $P < 0.001$ ; colony,  $H_2 = 2.558$ ,  $P = 0.278$ ; caste  $\times$  colony:  $H_4 = 0.821$ ,  $P = 0.936$ ; Fig. 3),  
212 whereas PC2 was not significantly different among castes or colonies (SRH test: caste,  $H_2 =$   
213  $5.725$ ,  $P = 0.057$ ; colony,  $H_2 = 2.931$ ,  $P = 0.231$ ; caste  $\times$  colony:  $H_4 = 4.185$ ,  $P = 0.382$ ; Fig.  
214 3). We did not find any correlation between morphological patterns (PC1) and movement  
215 patterns (PC1) in any caste or sex (female worker:  $T = 26$ ,  $P = 0.601$ ; male worker:  $T = 20$ ,  $P$   
216  $= 0.728$ ; female soldier:  $T = 12$ ,  $P = 0.260$ ; male soldier:  $T = 16$ ,  $P = 0.720$ ; female neotenic:  
217  $T = 22$ ,  $P = 0.477$ ; male neotenic:  $T = 1$ ,  $P = 1$ ; Fig. S5). Overall, we concluded that the caste-  
218 biased movement patterns reflect specific characteristics of the castes themselves rather than  
219 morphological differences associated with castes.

220 Our results show that the emergence of different movement modes was caste biased. This  
221 indicates that movement patterns of individuals can indicate castes in termites. First,  
222 morphologically distinct soldiers showed greater activity than workers and neotenics (Figs. 3,  
223 S4A). Second, some neotenics, which cannot be distinguished from workers based on external  
224 morphological characteristics, showed a specific movement mode that was characterized by  
225 extremely low mobility with frequent pauses (Figs. 2, 3, S2). Because neotenics are the only  
226 reproductives among the castes analyzed in this study, the neotenic-specific movement mode  
227 should be related to physiological conditions involved in reproductive activity. Further  
228 investigation of individual movement patterns might enable us to find cryptic castes that

229 cannot be distinguished in their morphology and physiology across social insects (Robinson,  
230 2009).

231 As termites are social insects, the movements of isolated individuals can be regarded as  
232 searching for other nestmates (Miramontes et al., 2014). Our results show that many  
233 individuals spent a lot of time near the wall, although there was no difference among the  
234 castes (Fig. S4A). Such wall-following behavior can be seen in various social animals. For  
235 example, in ants social isolation results in increased locomotor activity and wall-following  
236 behavior (Koto et al., 2015). The wall-following behavior of woodlice results in aggregation  
237 close to the edge of the arena (Devigne et al., 2011). The same is true of schooling behavior  
238 by fish in a tank (Suzuki et al., 2003). Because physical heterogeneities affect the spatial  
239 distribution of organisms, wall-following behavior can increase the probability of  
240 encountering conspecifics. Thus, wall-following behavior might be an adaptive strategy to  
241 search for other individuals when social living animals are isolated.

242 In general, self-organized systems of social insects can achieve complex patterns such as  
243 the division of labor via social interactions (Camazine et al., 2001). One primary goal of  
244 research is to reveal how such complex collective behavior can emerge from individual  
245 behaviors. We found that different castes of termites in social isolation move in different  
246 ways (Figs. 2, 3), which should lead to caste-specific encounter patterns within a nest.  
247 Consequently, the rate of interactions between individuals could be determined by their  
248 movement patterns (James et al., 2008; Viswanathan et al., 2011; Mizumoto et al., 2017). The  
249 next step would be to reveal the feedback between movement patterns of individuals and  
250 encounters with other individuals in natural settings. By focusing on individual-based  
251 movement patterns as caste-specific characteristics, our study provides a novel direction for  
252 studying the division of labor in social insects.

253

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258

## 259 **Competing interests**

260 The authors declare no competing interests.



261

## 262 **Author contributions**

263 HS and NM designed the study and collected behavioral data. NM analyzed data. KO carried  
264 out morphometric analyses. HS, NM, KO, and SD wrote the manuscript. All authors gave  
265 final approval for publication.

266

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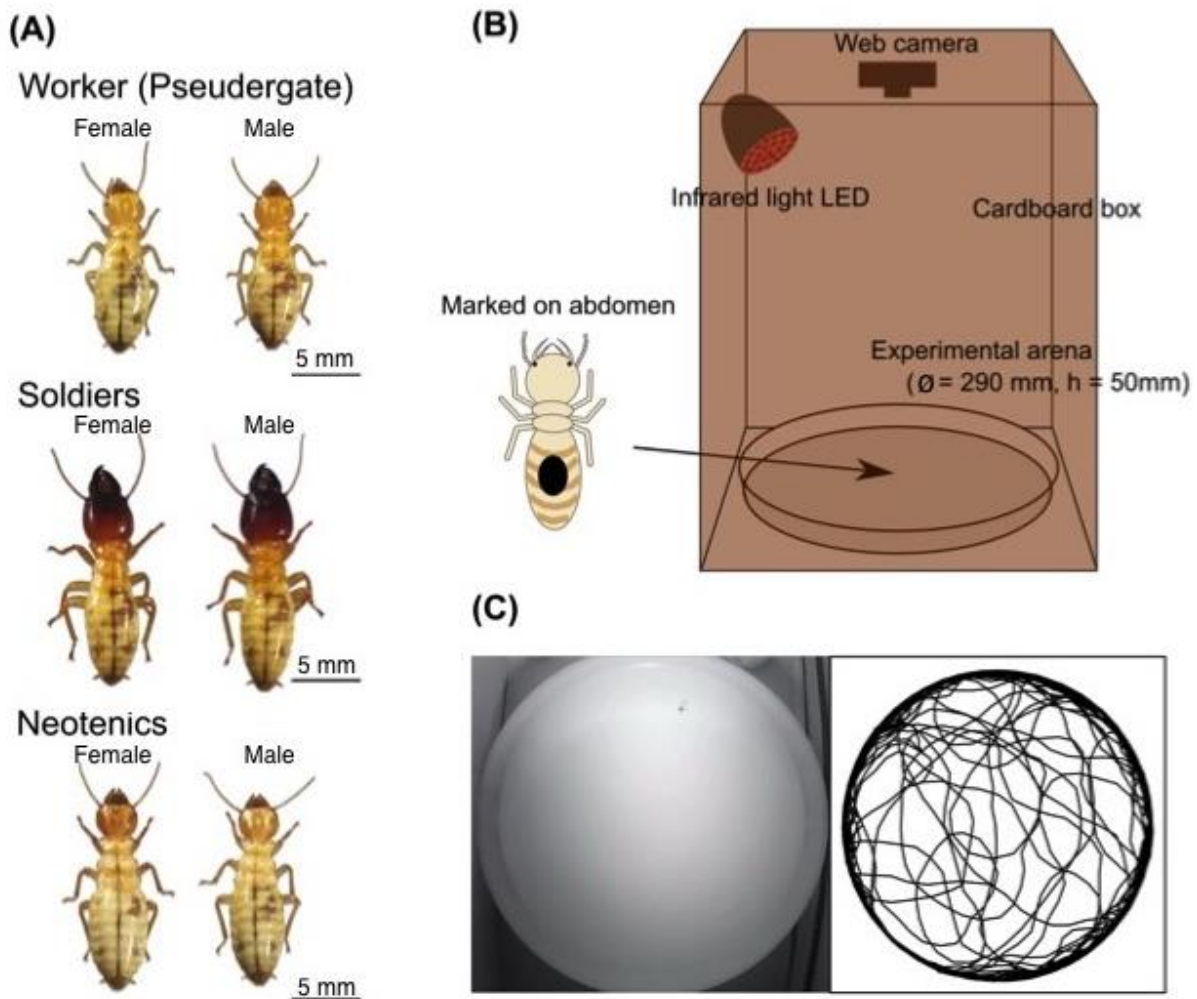
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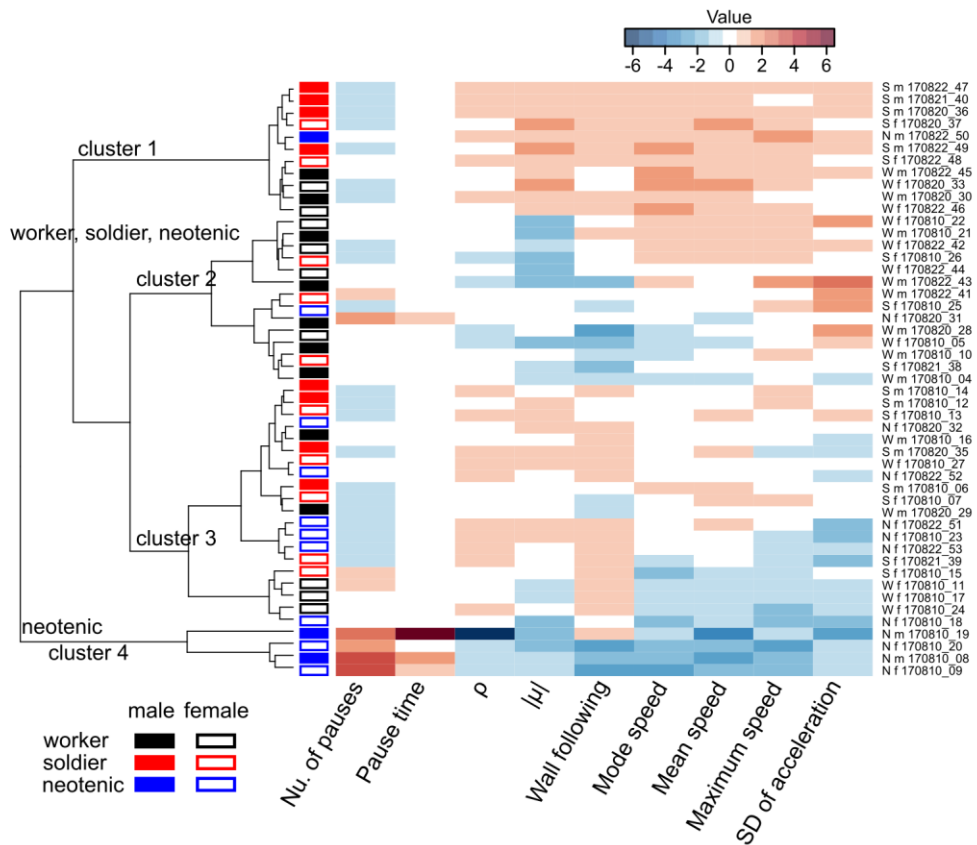
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368 **Figure 1.** Experimental procedure. (A) Castes and sexes used. Soldiers are easily identified  
369 by their large pigmented heads. Externally, neotenics are similar to workers, so they were  
370 confirmed by dissecting the abdomen after the experiment. (B) Observation equipment: an  
371 experimental arena in a cardboard box, recorded under infrared light. (C) A video capture and  
372 an example of behavioral trajectory.

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377 **Figure 2.** Clustering of movement patterns. Ward's distance was calculated to make a  
 378 dendrogram and to order individuals. The movement patterns were divided into four different  
 379 clusters, only one of which was neotenic specific: cluster 1, highly active movement with  
 380 frequent wall-following behavior; cluster 2, highly active movement with less frequent wall-  
 381 following behavior; cluster 3, relatively low activity with frequent wall-following behavior;  
 382 cluster 4, very low activity with frequent pauses. Values in the heat map were Z-transformed.  
 383 Labels to the right indicate caste, sex, and individual: e.g., W\_m\_170810\_4 indicates a  
 384 worker male observed on 2017 August 10 with serial number 4 (see also Table S1A).

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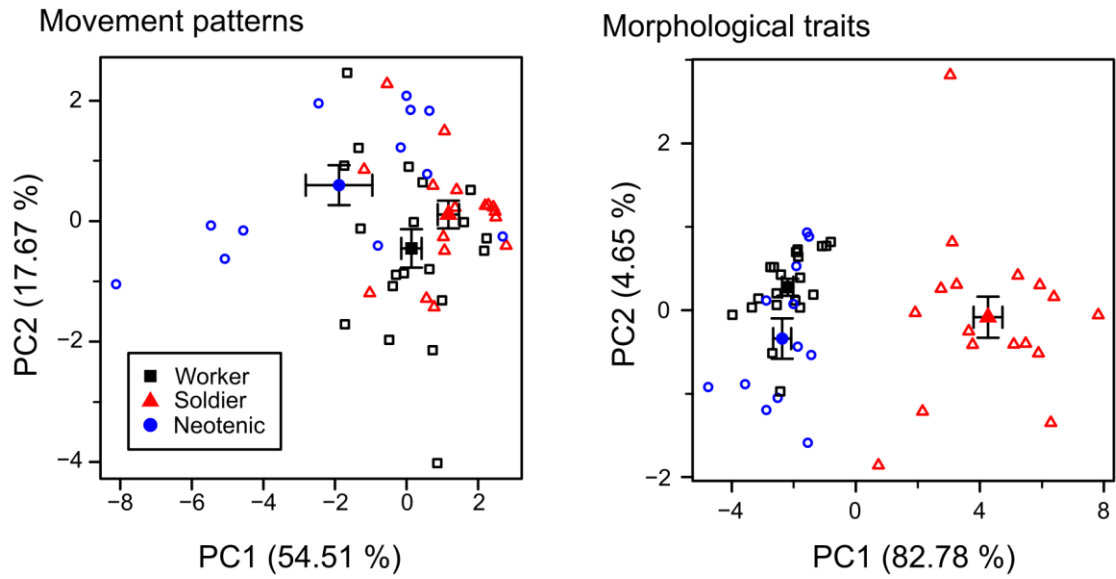
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394 **Figure 3.** Results of principal component analysis that reduced the variables to two principal  
395 components (PC1 and PC2) for each of movement patterns and morphological patterns. □△○  
396 Individuals; ■▲● mean of each caste. Bars: means  $\pm$  SEM.