

1 Hidden role plasticity of the reproductive caste in a morphologically differentiated 2 termite society

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13 14 Abstract

15 Reproductive division of labor is the defining characteristic of eusocial insects, separating germline-
16 like reproductives from soma-like workers. While most studies have focused on worker sterility, it is
17 generally assumed that developing reproductives invest only in maturation, not in colony labor. Here
18 we show that nymphs (pre-alates) in a highly structured termite society can contribute to colony labor
19 under natural conditions. During a rare colony emigration event of a marching termite,
20 *Longipeditermes longipes*, we observed developed nymphs carrying brood items to the new nest. In ~8
21 hours of field observation, ~155,000 termites emigrated with ~35,000 brood and ~30,000 food items
22 transported, and nymphs participated exclusively in brood transportation. The traffic flow of nymphs
23 was positively correlated with brood-carrying workers but negatively correlated with soldiers.
24 Movement patterns were not different between brood-carrying and non-carrying nymphs, suggesting
25 that nymphs express nursing worker-like behavior at minimum costs, consistent with the theoretical
26 prediction of task allocations. These results reveal that caste flexibility exists even in highly canalized
27 societies and emerges under ecological stress.

28 29 Introduction

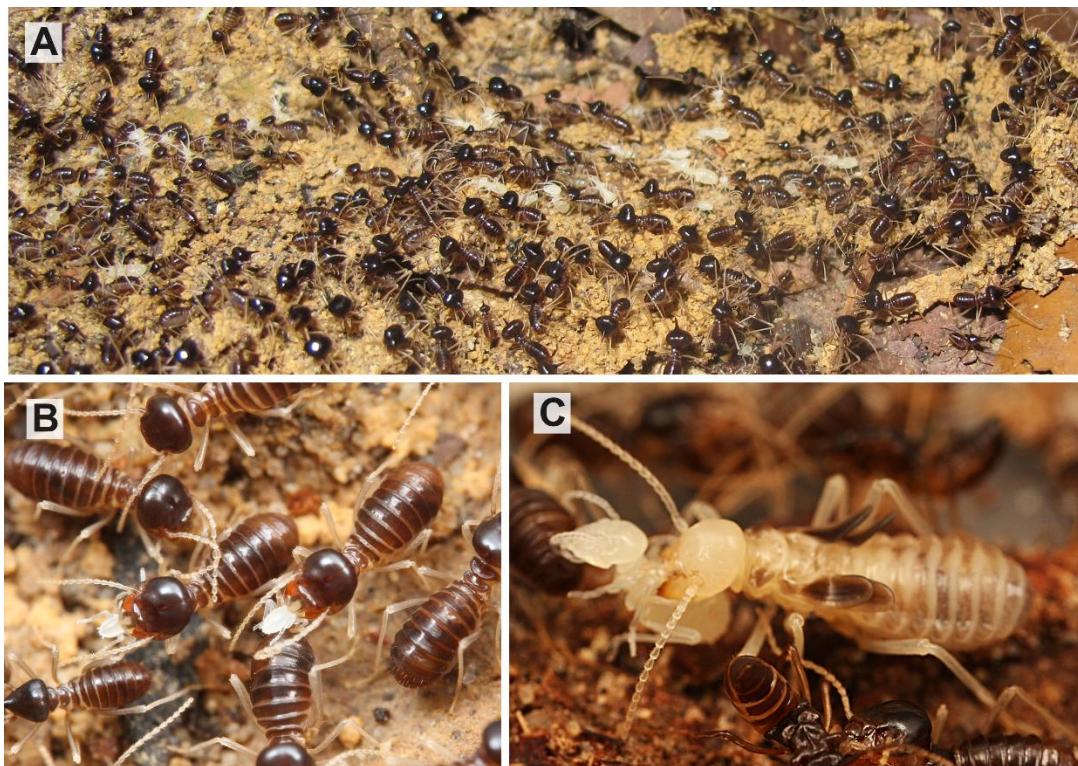
30 Reproductive division of labor is the defining characteristic of eusocial insects (Wilson 1971,
31 Crespi and Yanega 1995, Boomsma and Gawne 2018), where a few individuals (kings and queens)
32 monopolize reproduction, while non-reproductive workers specialize in colony tasks, such as foraging,
33 defense, and brood care. Role flexibility, or the level of division of labor, plays a central role in social
34 evolution, since specialization allows high efficiency and diverse functions by overcoming life history
35 trade-offs (Bernadou et al. 2021, Bell-Roberts et al. 2024, Field 2025). Much of the focus has centered
36 on the sterility and totipotency of worker castes: whether they can revert to reproduction or are
37 irreversibly canalized (Crespi and Yanega 1995, Boomsma and Gawne 2018, Revely et al. 2021, Turner
38 et al. 2025). On the other hand, the opposite of this problem has often been untested, and developing
39 reproductives are assumed to invest solely in their maturation and do not contribute to colony tasks.

40 Termites exhibit diverse social systems, including several evolutionary events of true workers
41 (Thompson et al. 2000, Roisin and Korb 2010). As in other eusocial Hymenoptera (e.g., ants, honeybees,
42 and stingless bees), termite species with true workers exhibit morphologically distinct worker and
43 reproductive lines, which are irreversibly differentiated at an early stage of development. Worker lines
44 perform all the colony tasks and lack the totipotency to become dispersing alates. On the other hand,
45 individuals in the nymphal line nutritionally depend on workers and do not contribute to the colony

46 tasks. A laboratory study suggests that nymphs may be able to feed independently without workers
47 present (Crosland and Su 2006). However, the nymphal line is widely assumed to be free of work
48 responsibilities in natural conditions, due to the observation difficulties arising from their cryptic life
49 and seasonality.

50 The marching termite *Longipeditermes longipes* (Termitidae: Nasutitermitinae) develops a highly
51 structured society with large colony size and multiple morphological worker and soldier castes (Miura
52 and Matsumoto 1998). This species does not have a permanent nest; instead, all members occasionally
53 emigrate from their nests to new locations (Figure 1). These rare events expose the hidden natural
54 behavior of nymphs in the field setting. Here we report the field observation that nymphs contributed
55 to brood transport during a natural emigration (Figure 1). This provides direct evidence that even in
56 highly canalized societies, developing reproductives can take worker-like roles under ecological stress.

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58

59 **Figure 1.** Nest emigration of *Longipeditermes longipes*. (A) The nest emigration trail.
60 Unlike a foraging trail, it includes broods (younger instar termites, identifiable by their
61 white coloration), is paved with structures, and is defended by soldiers. (B) Workers
62 carrying broods. (C) A nymph carrying a brood item.

63

64 **Materials and Methods**

65 *Estimation of traffic flow*

66 All observations were performed at Lambir Hill's National Park, Miri, Sarawak, Malaysia. On June
67 7th, 2023, we encountered the trail for nest emigration at 1:30 pm local time (N4.203589987,
68 E114.033425). From May 31 to June 12, we conducted daily field surveys of *L. longipes*, walking along
69 a ~4 km trail (Inoue-Pantu-Main) 1-2 times a day to search for trail formation. We typically
70 encountered 0-3 foraging trails per day. The trail for nest emigration is unique and distinct from
71 foraging trails: it occurs along a well-paved trail and contains workers transporting nestmates

72 (including eggs, larvae, and recently molted individuals) and nymphs, as well as a larger number of
73 termitophilous arthropods (Figure 1A). Such emigration trails are extremely rare: across 128 days of
74 fieldwork spanning 7 years of this species, the author encountered emigrating colonies only three
75 times (including this case) despite the large abundance of this species at the study site. The nest
76 emigration ended at 9:15 pm and lasted 7.63 hours.

77 We recorded the trail using a 4K video camera (HC-X1500-K, Panasonic) at 30 FPS with an LED
78 video light (Pixel G1s). Preliminary observations of this species' foraging trails showed that trails form
79 throughout the day; thus, using LED lights at night does not disturb their behavior. The trail was
80 diverged about 4.8 hours after starting the observation, so after that, the recording was on only one
81 trail. Workers carry food materials or nestmates. This can be easily distinguished by color. We
82 investigated the traffic dynamics by manually counting the number of individuals that passed in the
83 middle of the frame using BORIS (Friard and Gamba 2016). We counted them by passing direction
84 (outbound: going to the new nest; inbound: coming back to the old nest), caste (workers, minor
85 soldiers, major soldiers, or nymphs), and whether they were carrying items (none, food, or brood).
86 Traffic was sampled for 1 min every 10 min over the full 7.63-hour period, yielding 10% coverage. The
87 temporal development of traffic flow was compared between castes and carrying items using
88 Spearman's correlation analysis.

89

90 *Movement patterns of nymphs*

91 We watched all the videos to count the total number of nymphs transporting nestmates. We
92 trimmed all these events to create sub-clips. These clips included 9 nymphs transporting nestmates
93 and 21 without transporting anything. Then, we obtained the movement patterns of nymphs, using
94 Social LEAP Estimates Animal Poses (SLEAP) v 1.4.0 (Pereira et al. 2022), a deep-learning-based
95 animal pose estimation software on an Nvidia GeForce RTX 4090. We used a 4-node skeleton: head
96 (centered at the mouthparts); head-pronotum boundary, the middle of wing buds, and abdomen tip.
97 We labeled 88 frames from 9 videos for training, where 10% of the labeled frames were used for
98 validation (validation fraction = 0.1). We trained a U-Net-based model with a multi-animal top-down
99 approach, where SLEAP first detects the location of termites (centroids), crops centered images
100 around each detected location, and then predicts poses for each cropped instance (centered instance)
101 (Pereira et al. 2022). For this process, the receptive field size was set as 316 pixels for the centroid and
102 the centered instance. The augmentation was done by rotating images from -60 to 60 degrees. These
103 augmentations were applied dynamically during training, meaning that the number of unique labeled
104 frames used for training remained unchanged (Pereira et al. 2022). While tracking after inference, we
105 used the instance similarity method with greedy matching. All pose estimation data were converted
106 to HDF5 files for further analysis.

107 We used Python to format all HDF5 files for further analysis and converted them into FEATHER
108 files for analysis in R (R Core Team 2024). We employed a linear interpolation method to address
109 missing values in the dataset and a median filter with a kernel size of 5 to reduce noise. The data was
110 then downsampled to 5 FPS. After data formatting, we obtained each individual's location as the
111 center of the head and the tip of the abdomen. Moving speed was obtained by calculating the distance
112 displacements of the center. Also, turning angles were obtained by computing the changes in vector
113 from the tip of the abdomen to the head. The mean values of moving speed and turning angle were
114 compared between nymphs with and without nestmate items, using t-tests.

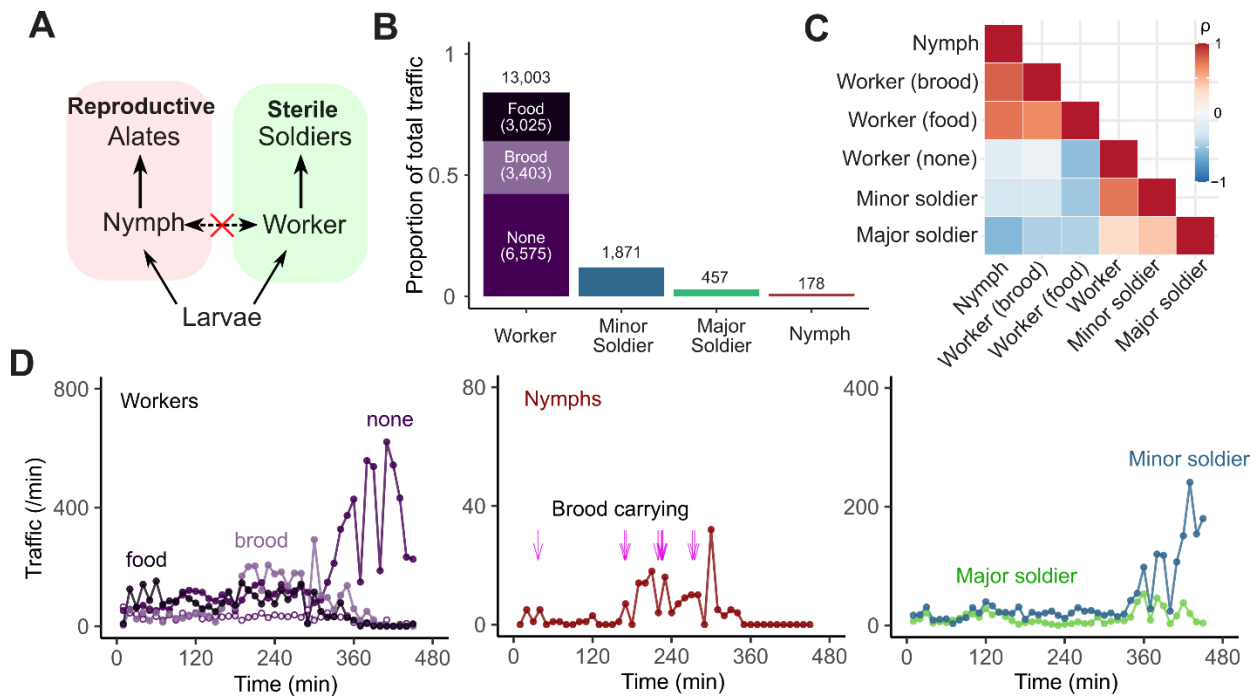
115 The data and codes are available on GitHub: [https://github.com/nobuaki-](https://github.com/nobuaki-mzmt/working_nymph_longi)
116 [mzmt/working_nymph_longi](https://github.com/nobuaki-mzmt/working_nymph_longi).

117

118 **Results**

119 During 8 hours of observations, we estimated ~155,090 individuals passed along the emigration
 120 trail, an order of magnitude greater than the estimated foraging population of this species (Gray and
 121 Dhanarajan 1974). Traffic was strongly biased outbound, with 91.6% (17,083) moving to the new nest,
 122 and only 8.4% (1,574) returning. Workers made up most of the outbound traffic (84%): 55% moved
 123 without items, 24% transported nestmates, and 21% transported food (Figure 2B, D). Minor and major
 124 soldiers accounted for 11.6% and 3.4% of outbound individuals, and soldiers never transport items
 125 (Figure 2B, D). Nymphs represented about 1% of the traffic (Figure 2B). Inbound traffic was dominated
 126 by workers without items (83.2%), followed by soldiers (14.8%), suggesting that ~10% of workers
 127 moved back and forth during emigration. As in the foraging trails (Gray and Dhanarajan 1974, Miura
 128 and Matsumoto 1998, Hoare and Jones 1998), outbound traffic was concentrated in the center of the
 129 trail, while inbound traffic was at both sides.

130 We observed 9 events of nymphs carrying broods (1.6% of nymphs, Figure 2D arrows). There were
 131 two distinct temporal patterns of traffic flow (Figure 2D). Workers carrying items and nymphs peaked
 132 3–5 hours after the start of observations, whereas workers without items and soldiers peaked toward
 133 the end. The traffic of nymphs was positively correlated with that of item-carrying workers but
 134 negatively correlated with that of soldiers (Spearman test, $P < 0.001$; Figure 2C). Movement analyses
 135 revealed no behavioral differences between nestmate-carrying nymphs and other nymphs. There were
 136 no significant differences in movement speed (t-test, $t_{17.4} = 0.45$, $P = 0.66$) and turning angles (t-test,
 137 $t_{24.9} = 0.24$, $P = 0.81$).
 138



139 **Figure 2.** Caste composition of nest emigration trail. (A) Simplified developmental
 140 pathway of *L. longipes*. Individuals irreversibly differentiate into either reproductive
 141 or sterile lines at an early stage in development. (B) Proportion of individuals passed
 142 during the observation. (C) Spearman correlation matrix showing correlations among
 143 the temporal traffic profiles of each caste during emigration. (D) Temporal dynamics
 144 of traffic flow for each caste. Open circles indicate backflow (only workers without
 145 items are shown), and arrows indicate instances of nymphs transporting nestmates.
 146

147

148 **Discussion**

149 In Nasutitermitinae termites, the caste system is highly canalized: workers, soldiers, and nymphs
150 commit to distinct roles without reversibility (Revely et al. 2021). Thus, as workers and soldiers lose
151 reproductive potential, nymphs are assumed to focus solely on reproductive development. Yet,
152 nymphs must have the capacity to perform basic colony tasks because, once they disperse as alates,
153 they must build a nest and care for their first brood (Nutting 1969). Social Hymenoptera, such as ants,
154 are also in the same condition, where newly emerged queens can perform worker-like tasks during
155 colony foundation, but hardly do so in their natal nests (Hölldobler and Wilson 1990). Our results
156 show that this potential for working can be expressed even before dispersal flights and colony
157 foundation; under strong ecological pressure, such as colony emigration, nymphs in *L. longipes* exhibit
158 working ability. Although the proportion of nymphs was small in the observed colony, such flexibility
159 could be ecologically significant depending on the colony status, where the proportion of nymphs can
160 be highly variable across seasons and ages (Chouvenc et al. 2022).

161 Furthermore, nest emigration provides a unique opportunity to measure the total colony size of
162 social insect colonies. We estimated that the colony size reaches much more than 150,000, as our
163 observations did not cover the entire colony, which is larger than most other termite species
164 (Mizumoto et al. 2022, Pequeno 2024). Size-complexity rule predicts that the larger cooperative
165 groups should have more division of labor, so that colonies can handle more tasks effectively in larger
166 group sizes (Bourke 1999). However, even in the larger colonies with multiple morphological castes of
167 *L. longipes*, our results highlight the importance of flexibility in meeting short-term labor needs.

168 Nest emigration is essential for many social insects to maintain the quality of their living place at
169 the expense of high costs and risks (McGlynn 2012). The entire colony members are exposed outside
170 the nest, and they need to rapidly transport all brood, nestmates, and food (Visscher 2007), with a
171 strong selective pressure for efficient coordination (Valentini et al. 2020). Such a sudden surge of
172 workload might have triggered nymphs to participate in transportation. Importantly, nymphs only
173 carried brood and not food, and their traffic flow strongly correlated with brood-transporting workers
174 (Figure 2C), where both peaked 3–5 hours after the start of observations, different from workers
175 without items and soldiers (Figure 2D). Thus, contributions of nymphs align with a safer “nursing”
176 role rather than risky foragers, although the difference between food and brood transportation could
177 be minimal during nest emigration. In addition, brood transport imposed little locomotor cost as long
178 as they were naturally walking on the trail, with no behavioral differences between brood-carrying
179 and non-brood-carrying nymphs. As nymphs are highly valued individuals within a colony, nymphs
180 engage in tasks but in a way that minimizes risk and investment, consistent with the predictions by
181 theories on task allocation (e.g., (Giraldo and Traniello 2014)).

182 In many social insects, task allocation within workers is often associated with their reproductive
183 potential, where the nurse role is often played by workers with higher reproductive potential, while
184 the foraging role is played by those with lower potential (e.g., (Tanaka et al. 2024)). Our observations
185 extend this principle beyond workers, showing that even developing reproductives can temporarily
186 take on low-cost and low-risk tasks under ecological stress. Although termite social behavior is
187 usually cryptic in their complex nests, rare migration events of marching termites provide a unique
188 window into the flexible behaviors of reproductive castes in a natural setting. These findings highlight
189 that the germ-soma boundary can be crossed, even strongly canalized, reminding the importance of
190 natural history observations to uncover the evolutionary plasticity of division of labor.

191

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