Supplemental materials for

Complex relationship between tunneling patterns and individual behaviors in termites

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SI text

Phylogenetical analysis

To reconstruct the evolutionary history of tunneling behaviors of lower termites, we first mapped the presence of tunneling behaviors on the phylogeny. We assumed that the multiple-piece or central nesting termites do tunneling through the soil, while single-piece nesting termites do not (Abe 1987). We counted *Hodotermopsis sjostedti* as a multiple-piece nester, based on the recent study (Kitade et al. 2012), although this species was previously considered as a one-piece nester. Note that there are some observations of tunneling behaviors even in single-piece nesting termites, especially in laboratory conditions (Castle 1934; Morgan 1959; Nkunika 1988; Waterhouse and Norris 1993; Scheffrahn et al. 2003); however we considered that they do not tunnel in natural condition as the frequency of their tunneling behaviors would be much lower than the actual multiple-piece nesters. Moreover, note that the wood roach *Cryptocercus*, which is the sister group of all modern termite species, does not dig in soil but excavates inside the gallery of well-rotten logs (Bell et al. 2007). Because of the lack of study, the information of many Rhinotermitidae (*Parrhinotermes*, *Dolichorhinotermes*, *Termitogeton*, *Serritermes*, and *Glossotermes*) is limited. In this study, we regarded them as one-piece nesters (Mizumoto and Bourguignon 2020). Recent molecular phylogeny can be found in Bucek et al. 2019 or Bourguignon et al. 2015, but these do not include genus *Paraneotermes*. Thus, based on the morphological phylogeny (Krishna 1961), we inserted *Paraneotermes* and *Kalotermes* into the molecular phylogeny. We conducted the ancestral state estimates using a maximum parsimony approach implemented in Mesquite v3.6 (Maddison and Maddison 2018).

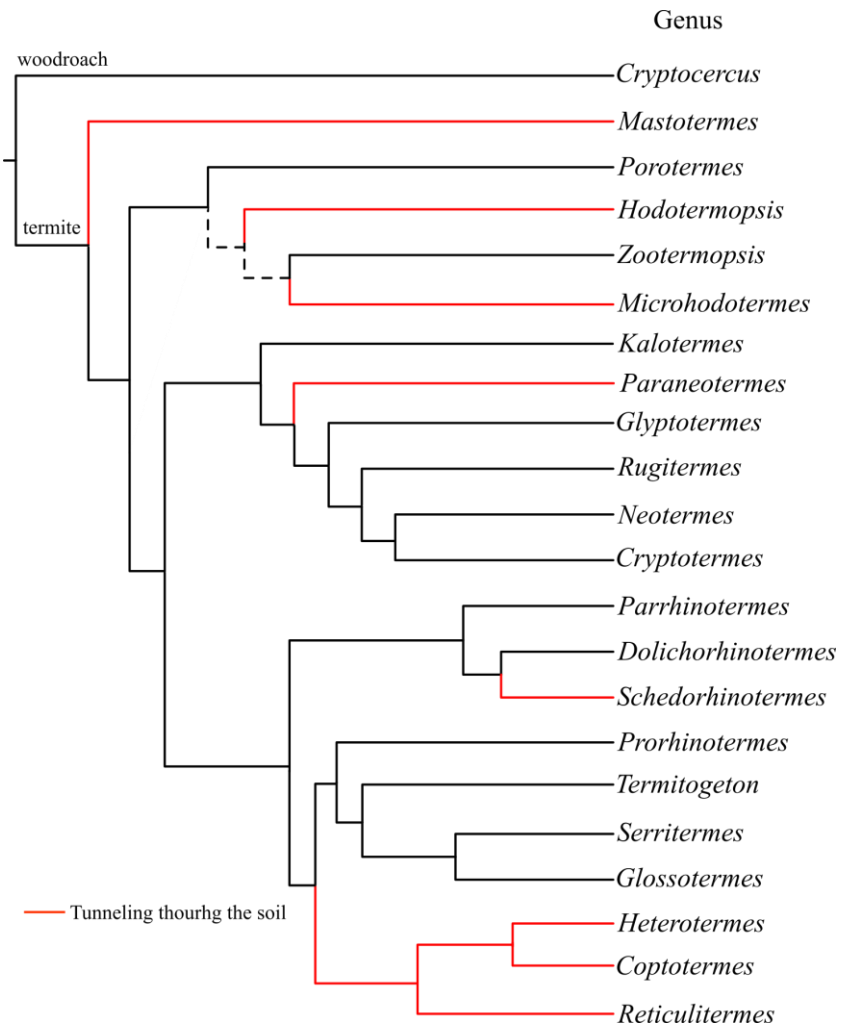

Figure S1. Detailed phylogeny of lower termites (modified from Krishna 1961; Bourguignon et al. 2015) with the information of tunneling behaviors. The ancestral states were reconstructed with maximum parsimony. Tunneling through the soil has evolved at least four times independently in Mastotermitidae, Hodotermitidae, *Paraneotermes*, and Rhinotermitidae. Dashed lines indicate ambiguous reconstruction.

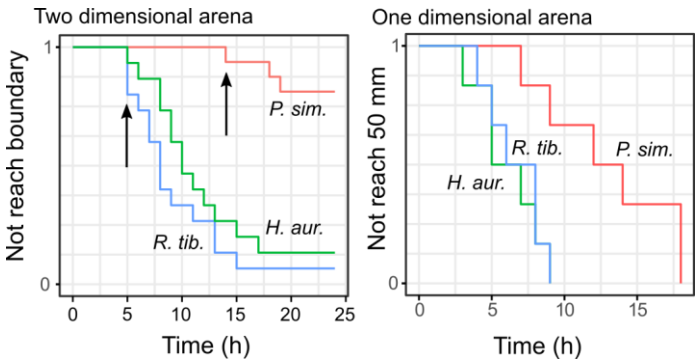

Figure S2. The time until a group of termites reach a wall (two dimensional) or create a 50mm tunnel (one-dimensional arena). Arrows indicate the time at which we analyzed the tunneling patterns for the two dimensional arena.

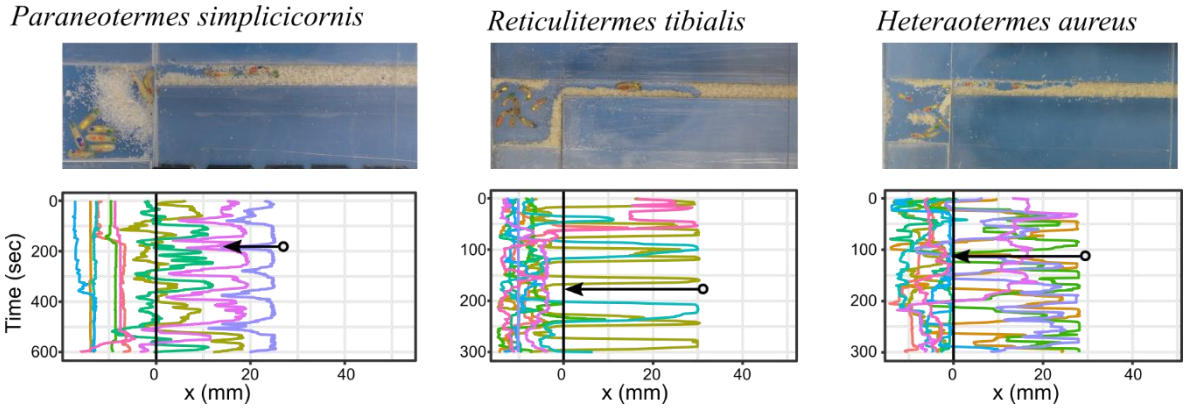

Figure S3. Experimental setup for behavioral observations with representative x-axis trajectories of each termite. Different individuals are shown in a distinct color. Black arrows show examples of backward movements, where *R. tibialis* and *H. aureus* often go back to the entrance of the tunnel, while *P. simplicicornis* mainly move only within a limited range within the tunnel.

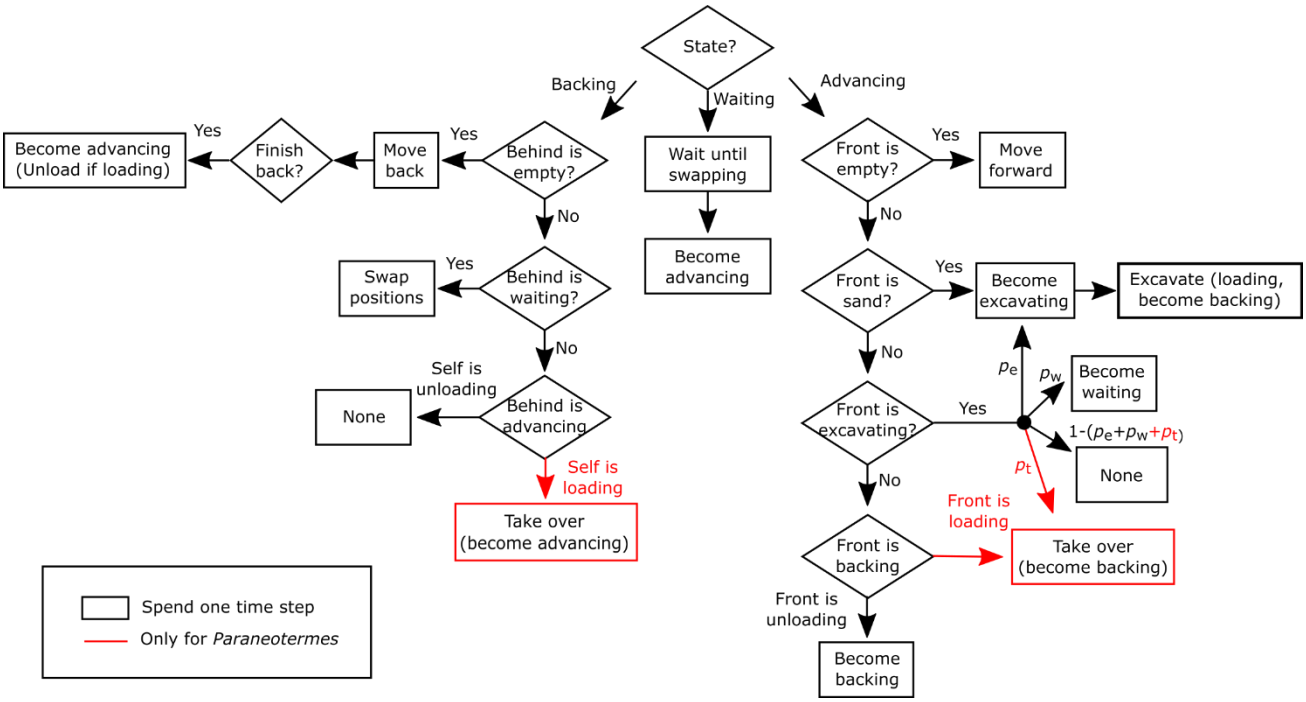

Figure S4 Model of termite collective excavation. The flowchart begins at the top with "State?". Once it reaches the endpoint, it goes back to the beginning, "State?". In case an individual enters the "None," it did nothing on that time step and then goes back to the beginning, "State?" at the next time step. Parameter values of p_e, p_w, and p_t are in Table S1.

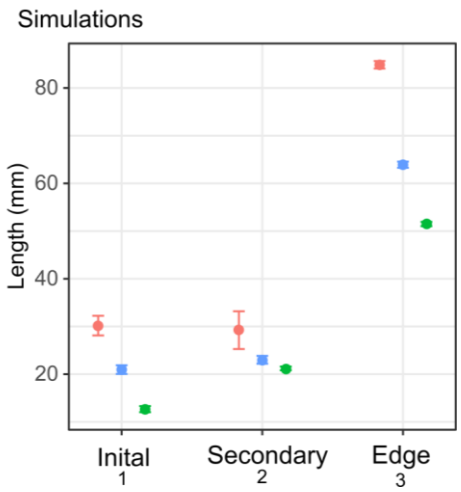

Figure S5. Comparison of the tunnel length for the simulations when divided into segments. Initial tunnel is the segment from the entrance to the first branch. Secondary tunnel is a segment between two branches. Edge tunnel is a segment reaching the face of a tunnel. When a tunnel has no branch, it only contains an edge tunnel. See also Fig. 2C.

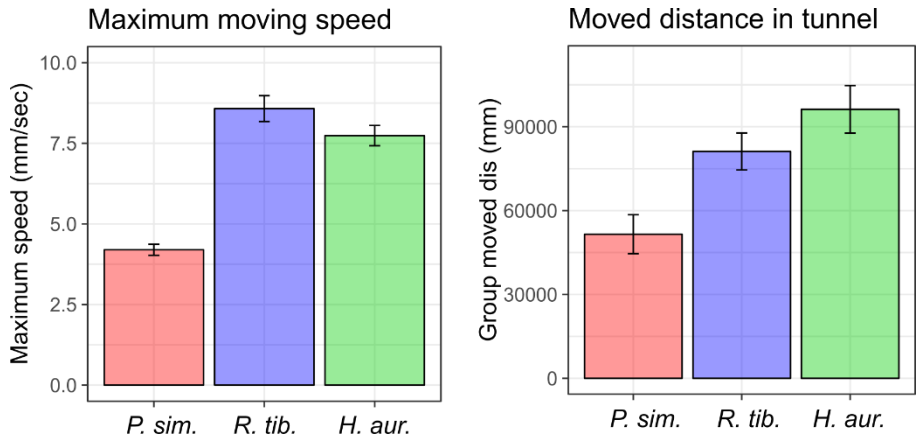

Figure S6. Comparison of the maximum instantaneous moving speed and total moved distance during observations. Maximum moving speed was measured for each individual separately, while moved distance was measured for each group by summing up the distance traveled by all members in a group inside a tunnel. As the observation was performed for 10 minutes every hour, we multiplied the distance by 6 to get the estimated values. Bars indicate mean ± S.E.

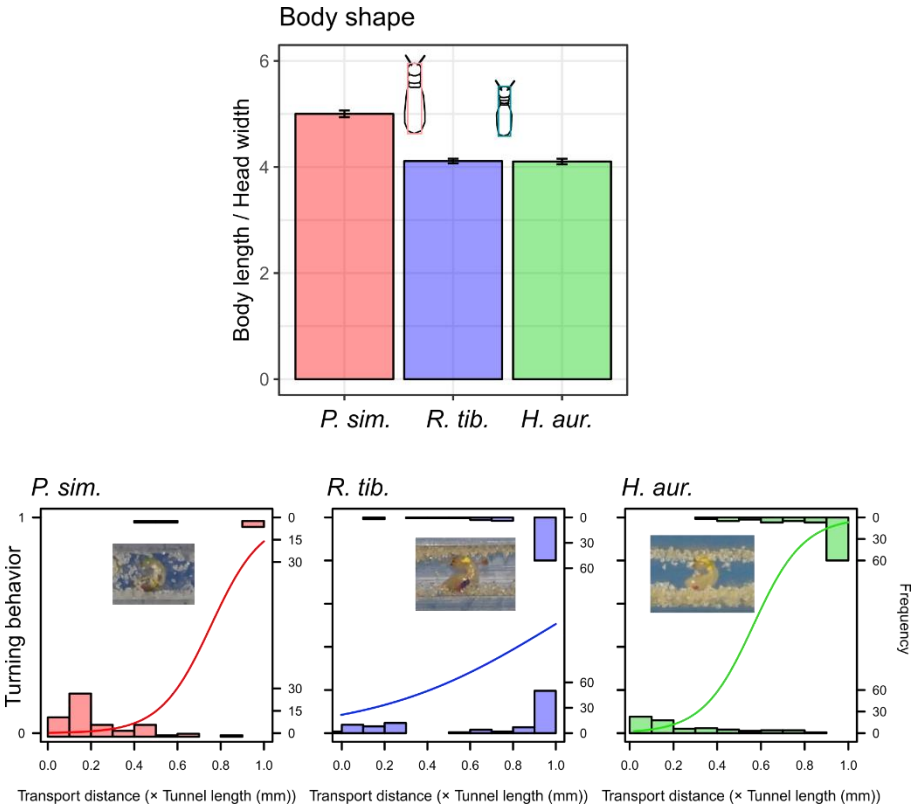

Figure S7. Comparison of body shape and turning behavior inside a tunnel. Body length and head width were measured for 30 individuals for each species. Bars indicate mean ± S.E. The mean values and s.d. for head width are 1.23 ± 0.09, 1.07 ± 0.07, and 0.95 ± 0.08 for *P. simplicicornis*, *R. tibialis*, and *H. aureus*, respectively Turning behaviors were measured for 1st row individuals which leave the tunnel face after excavating and when the length of the tunnel is longer than 40mm. Logistic regression curve is shown for each species, where we tested the relationship between transport distance and presence of turning behavior.

Table S1. Parameter values used in our simulations.

Species	p_e	p_w	p_t	α	β
Paraneotermes simplicicornis	0.06	0.30	0.59	0.541	0.765
Reticulitermes tibialis	0.26	0.56	NA	0.567	0.155
Heterotermes aureus	0.5	0.4	NA	0.483	0.232

Legends for supplementary videos.

Movie S1. Representative bucket brigade like tunneling behavior by *P. simplicicornis*. The video is played at 3.0x speed.

Movie S2. Representative individual transportation in tunneling behavior by *R. tibialis*. The video is played at 2.0x speed.

Movie S3. An example of the time development of the tunnel in our simulations. Left: visualization of the matrix which is under calculation, where the x axis indicates the length of each tunnel branch, while the y

axis indicates different branches with different tunnel faces. Grey cells contain sand, while white cells are empty. Colored cells are occupied by termites (legend is at right). First 20 mm is an entry area for introducing termites, and thus the maximum tunnel length is 100 mm. Right: visualization of tunnel development given a fixed branching angle (45°).

Reference for SI

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