Complex Relationship between Tunneling Patterns and Individual Behaviors in Termites

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ABSTRACT: The nests built by social insects are complex group-level structures that emerge from interactions among individuals following simple behavioral rules. Nest patterns vary among species, and the theory of complex systems predicts that there is no simple one-to-one relationship between variation in collective patterns and variation in individual behaviors. Therefore, a species-by-species comparison of the actual building process is essential to understand the mechanism producing diverse nest patterns. Here, we compare tunnel formation of three termite species and reveal two mechanisms producing interspecific variation: in one, a common behavioral rule yields distinct patterns via parameter tuning, and in the other, distinct rules produce similar patterns. We found that two related species transport sand in the same way using mandibles but build tunnels with different degrees of branching. The variation arises from different probabilities of choosing between two behavioral options at crowded tunnel faces: excavating the sidewall to make a new branch or waiting for clearance to extend the current tunnel. We further discovered that a third species independently evolved low-branched patterns using different building rules, namely, a bucket brigade that can excavate a crowded tunnel. Our findings emphasize the importance of direct comparative study of collective behaviors at both individual and group levels.

Keywords: collective behaviors, construction, self-organization, social insects, termites, tunneling.

Introduction

The coordinated behavior of group-living animals often creates complex group-level patterns (Camazine et al. 2001). Among these, nest structures and foraging tunnels built by social insects play an important role in their eco-

logical success by providing shelter and favorable micro-environments (Hansell 2005; Hughes et al. 2008). These structures differ widely among species, and the differences can result from a combination of variation in building behavior and variation in species-typical environments, including soil properties (Bollazzi et al. 2008; Tschinkel 2015; Perna and Theraulaz 2017). This leads to the fundamental question of what is the behavioral mechanism producing diverse structures. The prevailing theory is that group-level structures emerge from local interactions among individuals following simple behavioral rules (Theraulaz and Bonabeau 1995*a*; Camazine et al. 2001). Thus, different collective outcomes may be obtained either by differentiated behavioral rules or by regulation of a common set of rules to modify the interactions (Pratt and Sumpter 2006). Theoretical studies have supported the latter model; they predict that diverse structures can be explained by parameter tuning—the quantitative modification of a single set of behavioral rules shared among species—in ants (Franks et al. 1991; Khuong et al. 2011), termites (Bonabeau et al. 1998; Mizumoto et al. 2015; Ocko et al. 2019), and paper wasps (Theraulaz and Bonabeau 1995*b*; Karsai and Penzes 1998). However, because of the lack of comparative studies, there is no empirical evidence for the sharing of behavioral rules across species, and thus the key factor creating interspecific variation in patterns remains unknown.

In this study, we analyze the relationship between individual behavior and collective pattern in the tunneling behavior of termites. Both termites and ants dig underground tunnels to house colony members (Tschinkel 2003) and foraging tunnels to protect workers from desiccation and predators as they travel between the nest and foraging sites (Su and Bardunias 2005; Tschinkel 2011). Subterranean nest construction is often studied in ants, where branching pattern is a regulator of colony task organization (Buhl et al. 2006; Pinter-Wollman 2015). On the other hand, foraging tunnels are mainly studied in termites, where branching patterns can determine the efficiency of food search

(Lee et al. 2008). Although many studies have focused on the development of tunnel branching patterns (Su et al. 2004; Buhl et al. 2006; Toffin et al. 2009), little is known about the behaviors responsible for branching. Termite species can vary in tunneling patterns, reflecting species-specific foraging strategies and differences in the distribution of wood resources experienced by each species (Grace et al. 2004; Su et al. 2004; Hapukotuwa and Grace 2011). Moreover, our phylogenetic analysis indicates that tunneling behavior has evolved independently several times in termites (figs. 1, S1; figs. S1–S7 are available online). This provides an opportunity to explore how tunneling patterns emerge from individual behavior in a range of species with different degrees of relatedness.

To observe tunneling behaviors of termites in distinct phylogenetic groups, we used three subterranean termite species. *Paraneotermes simplicicornis* (Kalotermitidae) evolved tunneling independently from *Reticulitermes tibialis* and *Heterotermes aureus* (Rhinotermitidae; figs. 1, S1). We observed tunnel development at two different scales: the patterns of tunnel branching and the behavior of each termite. We empirically demonstrate that there is no simple one-to-one relationship between individual behaviors and group-level patterns. We find that *R. tibialis* and *H. aureus* build tunnels with branching patterns that are distinct from each other by using the same behavioral repertoire. We further show, using a combination of empirical observations and data-based simulations, that different branching patterns between these species result from parameter tuning of the same behavioral rules. In contrast, we also find that *P. simplicicornis* builds tunneling patterns similar to those of *R. tibialis* by using a distinct behavioral repertoire. Thus, interspecific variation in tunnel structure can emerge both via quantitative modification of shared behaviors and via reliance on distinct individual behaviors.

Material and Methods

Termites

We used four colonies of *Paraneotermes simplicicornis* (Kalotermitidae) and five colonies of *Heterotermes aureus* (Rhinotermitidae) collected from cholla and mesquite desert in Gila and Maricopa Counties, Arizona, and five colonies of *Reticulitermes tibialis* (Rhinotermitidae) collected from a pine forest in Pinal County, Arizona. *Paraneotermes simplicicornis* is the sole subterranean species in Kalotermitidae (Light 1937), while all species in *Heterotermes* and *Reticulitermes* are subterranean. Colonies were maintained at 22°C in plastic boxes with wood or cactus pieces and the soil in which they were nesting in the field. For *H. aureus* and *R. tibialis*, our experiments used workers; for *P. simplicicornis*, we used pseudergates or nymphs, which play the role of the worker caste in Kalotermitidae (Noirot and Pasteels 1987). Each *P. simplicicornis* group contained either all pseudergates or all nymphs. Each individual was used only once.
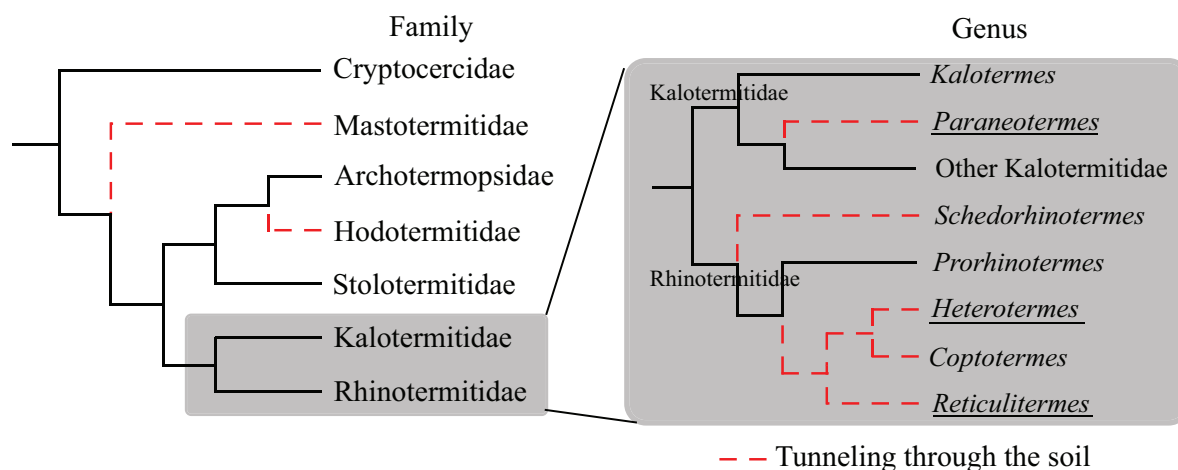

Figure 1: Simplified phylogeny of lower termites (modified from Krishna 1961; Bourguignon et al. 2015) with information on tunneling behavior. Ancestral states were reconstructed with maximum parsimony (detailed in the "SI text" section of the supplemental PDF, available online, and fig. S1). Tunneling through the soil has evolved independently several times in Mastotermitidae, Hodotermitidae, *Paraneotermes*, and Rhinotermitidae. In this study, we used three species from the three underlined genera: *Paraneotermes* (Kalotermitidae), *Heterotermes*, and *Reticulitermes* (Rhinotermitidae).

Macroscale Observation of Tunneling Patterns

To compare the branching patterns of tunnels, we built a two-dimensional experimental arena, which is known to reflect many characteristics of tunneling patterns, such as tunnel number and branching angles, found in three-dimensional and natural settings (Bardunias and Su 2005). Our arena consisted of three layers. The middle layer, with a thickness adjusted to each species (1 mm for *H. aureus* and *R. tibialis* and 2 mm for *P. simplicicornis*), had a round area filled with white sand (Marble White Sand, National Geographic) moistened with distilled water (10% by volume). At the edge of the round area was a teardrop-shaped entry area where termites could be introduced (fig. 2*A*). Sand particles were homogenized to a size ranging from ~0.15 to ~0.25 mm using two screens with 60 and 100 mesh. The top layer had an opening only above the entry area, which was covered by a glass plate. We observed 15 groups (three colonies × five replicates) for

H. aureus and *R. tibialis* and 16 groups (eight, three, and five replicates from three different colonies) for *P simplicicornis*. Each group had 20 termites. After placing termites in the entry area, we recorded tunnel development for 24 h. Snapshots were imported into ImageJ (National Institutes of Health, Bethesda, MD), and measurements were taken by tracing the length of each branch after calibration. We defined the beginning of the tunnel structure as a single point connected to the entry area. Tunnels longer than one body length (6.2, 4.4, and 3.9 mm for *P. simplicicornis*, *R. tibialis*, and *H. aureus*, respectively) were counted as unique branches.

Overall, *P. simplicicornis* formed tunnels much more slowly than *R. tibialis* and *H. aureus* (fig. S2). To avoid an effect of environmental heterogeneity arising from the wall at the boundary (Lima and Costa-Leonardo 2012), we compared the structures of tunnels at the time the first group in each species reached the wall (14 h for *P. simplicicornis* and 5 h for *R. tibialis* and *H. aureus*; fig. S2). We
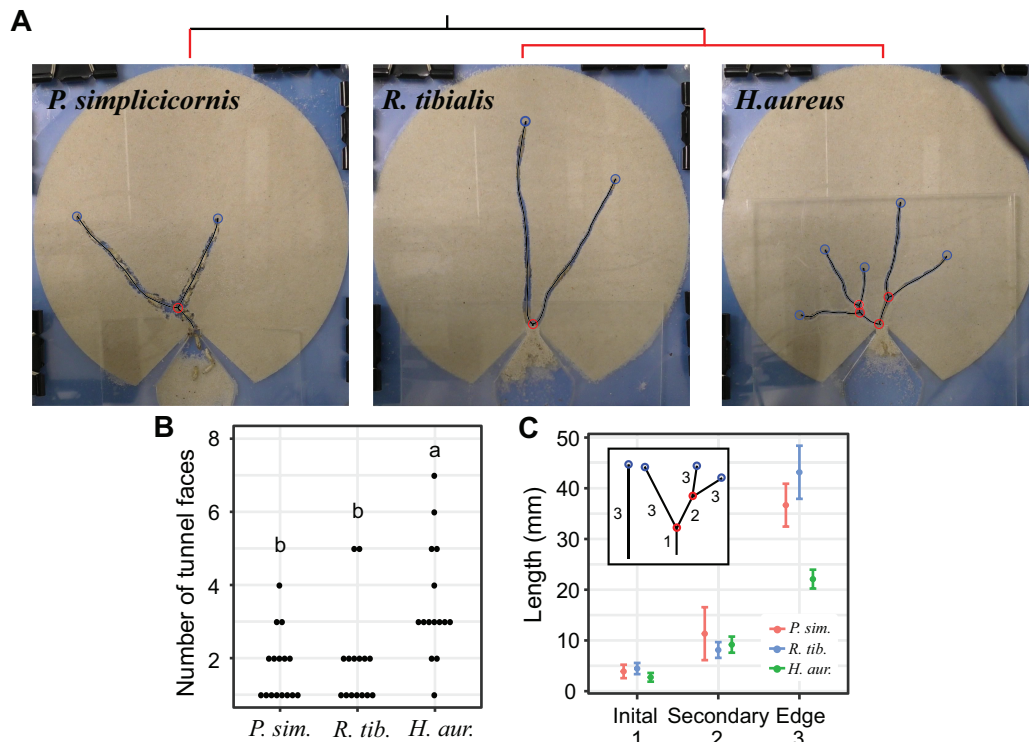

Figure 2: Interspecific comparison of termite tunneling patterns. *A*, Typical tunneling patterns of each species. The red lines in the simplified phylogeny above the photos indicate the independent evolution of tunneling. Red circles indicate branching points, and blue circles indicate the faces of the tunnels. *B*, Comparison of the number of tunnel faces among species. Different lowercase letters indicate significant differences (*P* < .05). *C*, Comparison of the tunnel length when divided into segments. Initial tunnel is a segment from the entrance to the first branch. Secondary tunnel is a segment between two branches. Edge tunnel is the segment reaching the faces of the tunnels. When a tunnel has no branch, it contains only an edge tunnel.

compared the number of tunnel faces among species using a generalized linear model (GLM) with Poisson error and a log link function. The likelihood ratio test was used to test for statistical significance of the explanatory variable (type II test). We pooled the data of three colonies for each species because we did not find any significant colony variation (GLM, likelihood ratio test: for *P. simplicicornis*, $\chi_2^2 = 1.35$, $P = .51$; for *H. aureus*, $\chi_2^2 = 2.05$, $P = .36$; for *R. tibialis*, $\chi_2^2 = 4.14$, $P = .13$). In case of a significant effect of species, we ran Tukey's post hoc test.

Microscale Observation of Digging Behavior

To compare individual digging behavior among the three species, we prepared experimental arenas of two different sizes depending on species (small for *H. aureus* and *R tibialis*; large for *P. simplicicornis*). The experimental arenas consisted of three layers: bottom and middle layers made of acrylic board and a top layer made of glass plates. The middle layer was L shaped and included a square entry area for introducing termites (small: 15 mm × 15 mm × 1 mm; large: 20 mm × 20 mm × 2 mm) and a narrow passage (small: 3 mm × 100 mm × 1 mm; large: 4 mm × 100 mm × 2 mm) filled with white sand (fig. S3).

We used 10 termites for each trial. All termites were marked with one dot on the head and two dots on the abdomen (Racing Finish, Pactra, Testors, Rockford, IL). We used the marking on the head for tracking, and those on the abdomen for individual identification. After installing termites in the entry area, we recorded their behaviors until they dug a tunnel 50 mm long; as in the two-dimensional experiment, *P. simplicicornis* took longer to reach this milestone (fig. S2). A video camera was mounted above the arena to record the square entry area and the first 50 mm of the passage. We observed three groups from two colonies for each species. For one replicate using *P. simplicicornis*, recording ended at 24 h after introduction of the termites, when the tunnel had reached 47.60 mm in length.

All videos were split into 30-min segments. We then identified the segment in which the termites started excavation. Starting with the segment immediately following, we observed their behavior for 10 min every 60 min. During observations, we extracted the coordinates of each termite's head at a rate of one frame per second from each video using the video-tracking system UMATracker (Yamanaka and Takeuchi 2018). We also measured the length of the tunnel at the beginning and end of each observation.

Because the tunnel paralleled the *X*-axis in each video, we were able to use the *X*-axis position to characterize the locations of termites and the tunnel face through time. This enabled an automated approach using R (R

script has been deposited in the Dryad Digital Repository [https://doi.org/10.5061/dryad.3bk3j9kfm; Mizumoto et al. 2020]). For each frame, we identified the position rank of individuals relative to the tunnel face: the first individual is the one closest to the tunnel face, the second individual is the second closest, and so on. Because of the narrow tunnel, only the first individuals can access the tunnel face. We considered the first individual to have visited the tunnel face when its position came within 1.5 mm of the tunnel tip (the boundary between sand and excavated area at the farthest point from the tunnel entrance) and then backed away more than 2 mm (or 3 mm for *P. simplicicornis*). We also manually confirmed that using this criterion at a sampling rate of one frame per second was accurate enough to identify all excavation events within focal 10-min time frames. We then calculated the number of visits to the tunnel face by first individuals and the number of changes in position between the first and second individuals. We estimated the mean numbers of these behaviors performed during the digging of a 1-mm length of tunnel. Then we compared the mean frequency of these behaviors among species using one-way analysis of variance (ANOVA). We pooled the data of two different colonies for each species because we did not find significant colony differences for any species, although sample size is small (*t*-test; number of visits: for *P. simplicicornis*, $t_{2.91} = -1.82$, $P = .17$; for *H. aureus*, $t_{2.99} = -1.07$, $P = .36$; for *R. tibialis*, $t_{2.81} = 0.20$, $P = .85$; number of changes: for *P. simplicicornis*, $t_{4.00} = -1.63$, $P = .18$; for *H. aureus*, $t_{2.41} = -0.39$, $P = .73$; for *R. tibialis*, $t_{2.00} = -2.80$, $P = .11$).

In addition to the automated analysis, we also performed a manual inspection to determine the behavioral repertoires of first individuals, who can visit the tunnel face, excavate sand, and transport sand particles away. According to the result of the above analysis, we observed the behavior of the first individual that visited the tunnel face when the tunnel was longer than 40 mm. We observed these visits to check whether the termites excavated sand, how they carried sand particles, and where and how they deposited them. Next, we examined the interaction patterns between the first and second individuals at the tunnel face. We automatically determined the individuals for the manual inspection, where we considered only second individuals that were within a minimum distance of the first termite. This distance was 6.5, 4.5, and 4 mm for *P. simplicicornis*, *R. tibialis*, and *H. aureus*, respectively (i.e., a little longer than a body length for each species). The frequency of observed behaviors was compared among species using Fisher's exact test.

All statistical analyses were performed using R version 3.5.3 (R Development Core Team 2017). All data and R scripts for the analysis have been deposited in the Dryad

Results

Tunneling Patterns

Tunnel structures in a two-dimensional arena showed both convergence and divergence of patterns (fig. 2A). The most striking tunnel feature was the number of branches. *Heterotermes aureus* built a significantly larger number of branches than *Paraneotermes simplicicornis* and *Reticulitermes tibialis*, while there was no significant difference between *P. simplicicornis* and *R. tibialis* (GLM, likelihood ratio test: $\chi^2_2 = 11.568$, $P = .00308$; Tukey contrasts: for *R. tibialis* and *P. simplicicornis*, $z = 0.376$, $P = .925$; for *H. aureus* and *P. simplicicornis*, $z = 3.007$, $P = .0073$; for *H. aureus* and *P. simplicicornis*, $z = 2.611$, $P = .0245$; fig. 2B). Branches arose mainly near the tunnel entrance (fig. 2A), where the length of the tunnel before the branches is much shorter than that after the branches (fig. 2C).

Individual Digging Behavior

Individual behavior did not correspond directly to group-level patterns; instead, *P. simplicicornis* used a distinct transporting behavior unlike that of either *R. tibialis* or *H. aureus*. Each *P. simplicicornis* excavator dug sand with its mandibles, formed the sand into a ball with its legs, and kicked the ball backward to the individual behind it (fig. 3A; video 1; videos 1–3 are available online). This behavior was observed only in this species (Fisher's exact test, $P < .0001$; fig. 3B) and contrasts with the well-recognized behavior of rhinotermitid termites, which excavate and carry particles using their mandibles (fig. 3A; video 2). These different behaviors were associated with different tactics for removing the sand: *R. tibialis* and *H. aureus* individually carry sand out of the tunnel, while *P. simplicicornis* instead forms a bucket brigade of multiple individuals. This was apparent in the fact that *R. tibialis* and *H. aureus*, but not *P. simplicicornis*, finish transportation by compressing clumps of sand particles against the sidewall (Fisher's exact test, $P < .0001$; fig. 3B). *Paraneotermes simplicicornis* kicked sand balls into the tunnel passage, where they were taken over by another individual. In addition, the order of individuals inside a tunnel was maintained in *P. simplicicornis*, with the first individual at the tunnel face being less likely to move back and change positions with the second individual (ANOVA, $F_2 = 24.307$, $P < .0001$; fig. 3C). Moreover, *P. simplicicornis* visited the tunnel face fewer times (ANOVA, $F_2 = 24.892$, $P < .0001$; fig. 3C), indicating that they transport a large

amount of sand at once using the bucket brigade. Finally, after visiting the tunnel face, first individuals of *P. simplicicornis* moved back only a short distance, as another individual can take over the sand ball. This trend was prominent when the tunnel became longer, with *P. simplicicornis* typically moving back only about 10 mm regardless of the length of the tunnels while *R. tibialis* and *H. aureus* moved back increasingly long distances as the tunnel lengthened (fig. 3D).

We further investigated interactions among individuals to specify individual-level differences that might account for the different tunnel patterns of *R. tibialis* and *H. aureus*. During excavation, direct interaction between termites can occur in a clogged tunnel. We observed the behavior of second individuals when they were immediately behind first individuals who were excavating at the tunnel face (fig. 4A). We found that *R. tibialis* and *H. aureus* chose from the same behavioral repertoire, either excavating the sidewall or waiting until the first individual had finished excavation. However, the two species differed in the frequency of these behaviors (Fisher's exact test, $P = .0035$; fig. 4A). The second individuals in *R. tibialis* waited more often, while those in *H. aureus* had a higher probability of beginning to excavate the sidewall (fig. 4A). In contrast, *P. simplicicornis* showed a distinct behavior not present in the other two species, with the second individual taking over the transportation of the sand ball kicked back by the first individual (figs. 3A, 4A). Thus, the phylogenetically distinct species studied here have different behavioral repertoires, while the closely related species share the same repertoire but quantitatively modify their use of it, indicating the existence of parameter tuning.

Individual-Based Model

We hypothesized that the observed quantitative difference in sidewall excavation between *R. tibialis* and *H. aureus* is the mechanism of branching pattern variation. Such sidewall excavation is known to widen the tunnel and eventually result in a new branch (Bardunias and Su 2010). To test our hypothesis, we developed a cellular automaton model simplifying the tunneling process (fig. S4). We modeled the digging arena as a two-dimensional discrete space (tunnel length and tunnel number) composed of cells with two possible states (empty and sand filled; video 3, *left*). Termites were modeled as mobile agents, each one occupying a single empty cell. All termites were initially placed in the entry area, which can contain all individuals.

Termites have five different states: moving forward (advancing), excavating, backing with loading, backing without loading, and waiting. Inside a tunnel, termites determine
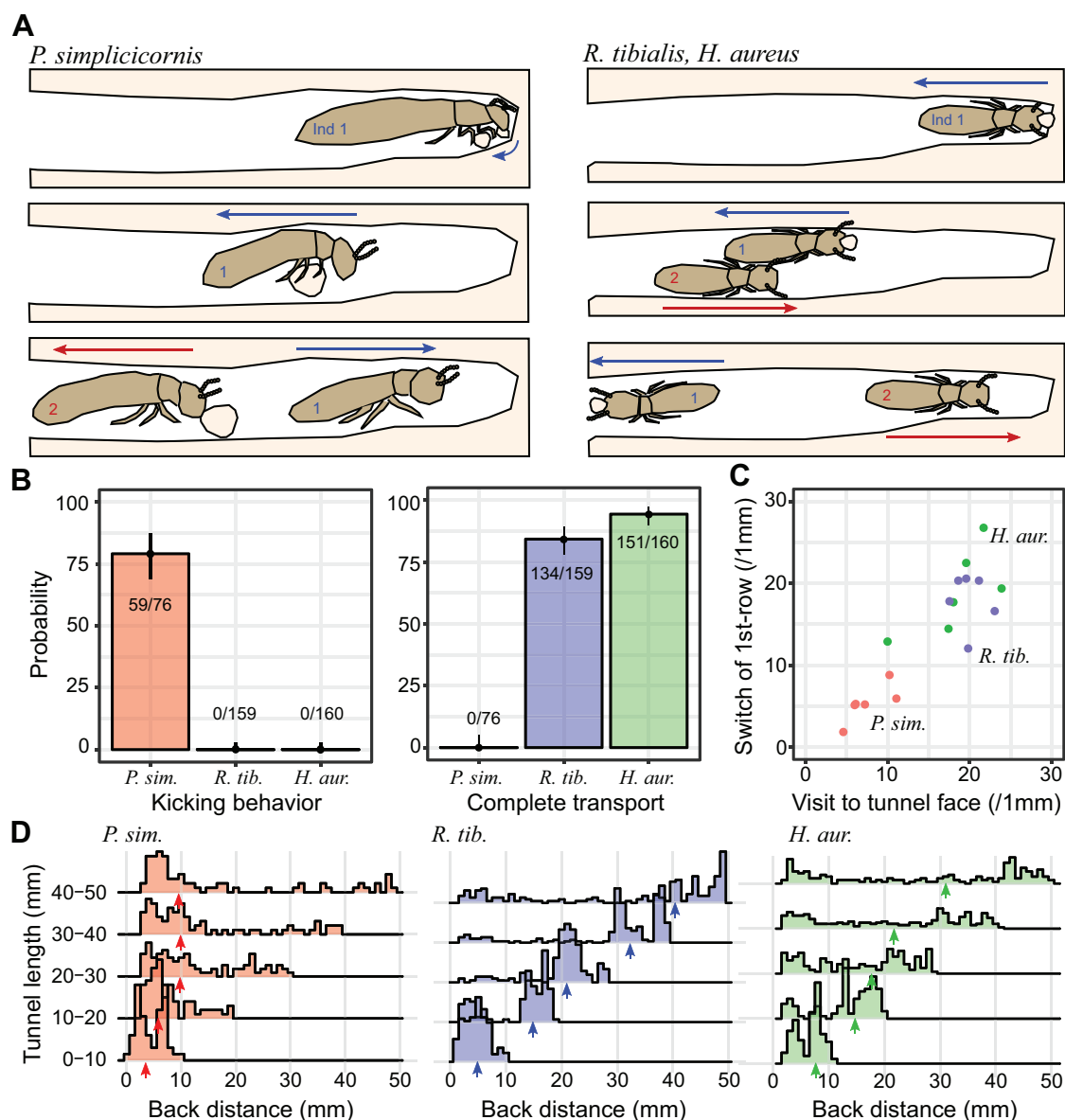
A

P. simplicicornis

R. tibialis, H. aureus

Figure 3: Comparison of individual behaviors during tunneling. *A,* Illustration of sand excavation behavior. After excavating sand at the tunnel face, *Paraneotermes simplicicornis* use their legs to form a ball of sand particles and kick it back behind, where it is taken over by the second individual. *Reticulitermes tibialis* and *Heterotermes aureus* instead carry sand particles with their mandibles, and the individual in the first position often changes. *B,* Interspecific comparison of the probability to show kicking behavior and complete transportation. Completion of transportation is defined as compressing or attaching sand particles, which are then not used by other individuals. Error bars show 95% confidence intervals for binomial proportions. *C,* Interspecific variation of the number of visits to the tunnel face and the number of position switches between first and second individuals. *D,* Comparison of the distance of backward movements after visiting the tunnel face. Arrows indicate median values.

their behaviors depending on their state and their interactions with other individuals. Each behavior takes one time step (fig. S4). If individuals do not encounter others, advancing termites move forward as long as the cell in front is empty. When the front is sand filled, advancing termites change to the excavating state, then they excavate sand and

change to the state of backing while loaded with sand. Backing termites move back as long as the cell behind them is empty, until they have moved a given backing distance or have left the tunnel. As the backing distance increased proportionally to the tunnel length (fig. 3*D*), we determined the backing distance by multiplying the tunnel
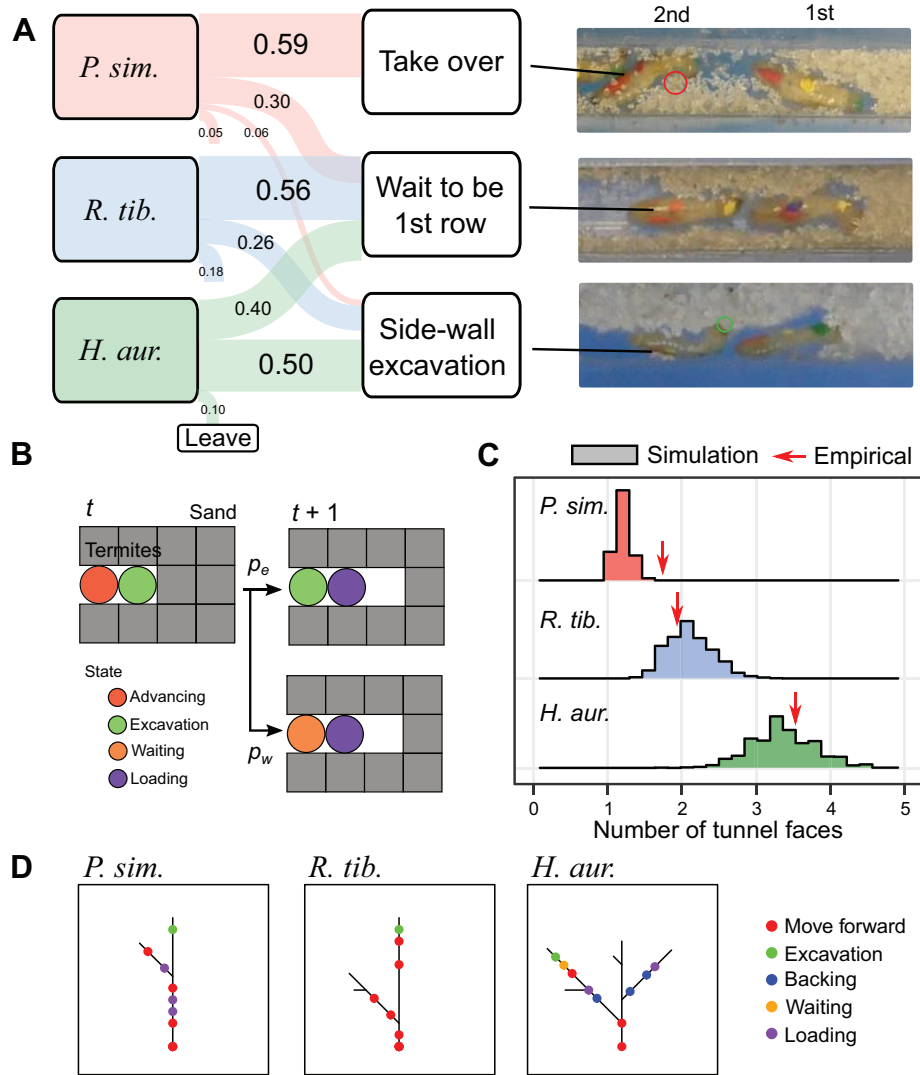
Figure 4: Parameter-tuning mechanism for interspecific variation of tunneling patterns. *A*, Interaction rules between individuals within a clogged tunnel. In *Paraneotermes simplicicornis*, the second individual often takes over the sand ball that the first individual has kicked back. On the other hand, *Reticulitermes tibialis* and *Heterotermes aureus* wait or excavate the sidewall, and these two species differ in the frequency of these behaviors. "Leave" indicates that the second individual left the tunnel without transporting sand particles. *B*, Behavioral rules governing the simulated interactions for *R. tibialis* and *H. aureus*. If a termite finds an excavating individual in front of it, it will choose either to wait or to excavate the sidewall, depending on the probability obtained in the experiments. *C*, Comparison of the results of experiments and simulations. Histograms indicate the mean number of tunnel faces for every 15 or 16 simulations (*N* = 1,000), while red arrows indicate the mean values observed in experiments. *D*, Representative branching patterns with the location of individuals at the end of simulations. In our simulations, we fixed the branching angles at 45° for visualization purposes. See also video 3 for developmental processes.

length by a random number generated from a beta distribution with parameters α and β (table S1, available online), which are variable among species and obtained by fitting to observed transport distances (data are shown in fig. S5). The beta probability density distribution is $P(x) = Cx^{\alpha-1}(1-x)^{\beta-1}$, where C is a normalization constant equal to $1/(\int_0^1 x^{\alpha-1}(1-x)^{\beta-1}dx)$. We used a beta distribution be-

cause it can describe the observed bimodal shape, especially in *H. aureus*. We observed short- and long-distance transportation, even when only one individual was present within a tunnel. Individual termites probably alternated two different transportation behaviors: bringing sand particles out of the tunnel, like in *R. tibialis*, and depositing sand particles inside the tunnel to minimize moving distance, as observed

in a different species (Lee et al. 2020). After backing, individuals unload the sand and enter the advancing state. Once unloaded, the sand is no longer available in simulations of *R. tibialis* and *H. aureus* but remains available in simulations of *P. simplicicornis*, where other individuals can pick it up in a time step.

When there is an excavating individual in front of an advancing termite, the advancing termite chooses between the waiting state, waiting for the current excavator to finish, and the excavating state, excavating the sidewall and thus starting a new branch tunnel (figs. 4*B*, S4). The simulation used species-specific probabilities of these behaviors based on the experimental results for *R. tibialis* and *H. aureus* (fig. 4*A*, 4*B*). Waiting individuals will swap positions with the individual in front once its state changes to backing; excavating individuals will excavate the sidewall to create a new branch. When there is a backing individual in front of an advancing termite, the latter changes to the backing state because of the confined space. We also simulated building by *P. simplicicornis* to predict whether the same mechanism can explain the branching patterns of this species. In *P. simplicicornis*, when an advancing individual encounters a backing individual who is loaded with sand, the advancing termite takes over the sand particles. After this, the backing individual changes to the advancing state, while the advancing individual changes to the backing state. Similarly, individuals of *P. simplicicornis* can also choose to take over a sand load when they encounter excavating individuals (fig. S4).

We tested whether the simulation could replicate the macroscale characters (branching patterns) observed in the experiments. In each trial, we modeled 20 individuals, as in the experiments. These individuals act sequentially in random order at each time step except for swapping or taking over where we need to compute the action of two individuals at the same time. The side length of a single cell is 10 mm. The spatial domain accommodates up to 40 tunnels, each as long as 100 mm (video 3). As in the experiments, we ran the simulation 15 times for *R. tibialis* and *H. aureus* and 16 times for *P. simplicicornis*. We then counted the number of tunnel faces and measured the mean values. We repeated this process 1,000 times to estimate the expected distribution of the mean value. Simulations were implemented in R, and the script has been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.3bk3j9kfm; Mizumoto et al. 2020).

The model effectively reproduced the interspecific variation among rhinotermitid termites, with *R. tibialis* building fewer branching tunnels than *H. aureus* (fig. 4*C*). This corresponded to how they used their behavioral repertoire: the number of tunnel faces increased with the individual probability of sidewall excavation. However, the model underestimated the branching rates of *P. simplicicornis*, suggesting that this species may have another behavioral mechanism for branching in addition to sidewall excavation within a clogged tunnel. For example, in ants, branching patterns can be species specific: *Lasius niger* produces branches at the tunnel face (Toffin et al. 2009), while *Messor sancta* often initiates a new tunnel along an existing tunnel wall (Buhl et al. 2006). From visual inspection, both branching patterns can be observed in *P. simplicicornis*, suggesting avenues for further investigation of the branching mechanism in this species.

Moreover, our model revealed that high local density causes branching in termite tunnels. The simulation reproduced our experimental finding that the branching of termite tunnels is concentrated near the tunnel start (figs. 2*C*, S5). This is because, at the beginning of the excavation, the area of the tunnels is smaller, which increases the local density of termites and the probability of sidewall excavation. As the tunnels grow, the area increases and local density declines, which results in lower branching rates in later stages of excavation. It is well-known that group size and density affect the tunneling structures in many social insects (Buhl et al. 2004; Su and Lee 2009; Toffin et al. 2009). But our results indicate that even with the same group size, changes in local density will greatly affect pattern formation.

Discussion

Our comparative study revealed a complex relationship between behavioral mechanisms and group-level patterns. We found that *Reticulitermes tibialis* and *Heterotermes aureus* share the same behavioral repertoire, but quantitative differences in the frequency of different actions result in divergent branching patterns (fig. 4). This result shows that parameter tuning of the same rule set can explain interspecific variation of pattern formation, without requiring a dramatic change of behavioral repertoire. Note that theoretical studies that have predicted a similar mechanism can explain other spatial patterns in animal groups, beyond nest construction by social insects (e.g., Couzin et al. 2002). In contrast, we also found that two phylogenetically divergent species (*Paraneotermes simplicicornis* and *R. tibialis*) possess different behavioral repertoires for collective excavation but create tunnels with a similar branching pattern (figs. 2, 3). Thus, similarity of patterns need not imply a shared behavioral algorithm. Altogether, we conclude that the collectively produced structures and individual behaviors observed in each species do not correspond to each other. This makes it most challenging to solve the inverse problem of inferring individual behavioral rules from the end structures that they produce

without observation of the structures' growth dynamics. Our results emphasize the importance of direct comparative studies of behavioral mechanisms of self-organizing systems.

Termite phylogeny shows that tunneling behavior could be present in the common ancestor of Rhinotermitidae (fig. 1), suggesting that the behavioral repertoire is shared in this whole group. The Rhinotermitidae have a wide diversity of tunneling patterns (Su et al. 2004; Su and Bardunias 2005), and our results show that the behavioral mechanism underlying this pattern diversification is simple. The tunnel geometry is sensitive to a single behavioral parameter governing interactions, namely, a threshold for individuals in a clogged tunnel to excavate a sidewall instead of waiting for access to the tunnel face. The different tunneling patterns can be either selectively neutral or the result of adaptation. In the latter case, we speculate that interspecific variation of tunneling patterns reflects different foraging strategies (Grace et al. 2004; Lee and Su 2009; Hapukotuwa and Grace 2012). For example, *H. aureus* engages in an intensive search by building highly branched tunnels, as they are in deserts with cactus resources that are small and relatively difficult to find. On the other hand, *R. tibialis*, which lives in pine forests with large wood resources, performs an extensive search by focusing on fewer tunnels. The function of tunneling patterns, including foraging efficiency and resource transportation (Lee et al. 2007), deserves further study.

On the other hand, the presence of a differentiated behavioral rule in *P. simplicicornis* indicates the importance of evolutionary contingency. This species' behavioral repertoire appears to have been shaped by the physiological and morphological traits of its family, Kalotermitidae. First, kalotermitids move more slowly than rhinotermitids, possibly because of lower metabolic rates or shorter legs (Smith and Rust 1993; Scheffrahn and Su 2014). During our observations, the maximum instantaneous moving speed of *P. simplicicornis* was significantly slower than that of *R. tibialis* or *H. aureus* (fig. S6). Kicking works well for slower-moving termites because it requires a shorter total movement distance to excavate a unit length of tunnel (fig. S6). Second, the body shape of kalotermitids is more elongated than that of rhinotermitids (fig. S7), which limits turning around inside narrow tunnels (Aguilar et al. 2018). Because of this characteristic, *P. simplicicornis* may do better with the kicking type of tunneling. Indeed, turning behavior, which often involves transportation of sand particles for a longer distance, is less frequently observed in *P. simplicicornis* (fig. S7). Thus, the kicking type of tunneling might be an adaptation to confined space for species with lower mobility.

When a group of animals moves within a narrow and confined space, they face a problem of high-density clogs, which affect task performance and collective outcomes (Toffin et al. 2009; Aguilar et al. 2018). The bucket brigade is one solution because excavators do not need to pass each other (Ratnieks and Anderson 1999). In addition to *P. simplicicornis*, there are a few observations of this behavior in social mole rats (Jarvis and Sale 1970) and army ants (Weissflog et al. 2000). Another mechanism, observed in fire ants, is individual idleness, which limits the number of excavators at the face of tunnels and reduces the frequency of clogs (Aguilar et al. 2018). The higher proportion of waiting behaviors by *R. tibialis* is consistent with this idea. Thus, there are different clog-control mechanisms behind the convergence of reduced tunnel branching in *P. simplicicornis* and *R. tibialis*. Instead of reducing local density, *H. aureus* exploits high-density clogs as a mechanism for building new branches, thus forming highly branched tunneling patterns. A similar mechanism of high density creating a new branch has also been proposed for ant nest construction (Toffin et al. 2009). Combined with previous studies, our results illustrate that collective behavior in confined spaces is variable within each animal taxon.

In summary, our comparison of three species demonstrates two possible behavioral mechanisms for interspecific variation of collective behaviors. First, related species can share the same set of behavioral rules, with parameters tuned to yield distinct patterns among species. Second, behavioral rules may be different between phylogenetically distinct species, but group-level patterns can converge. The extent of how prevalent these two mechanisms are in evolutionary history should be explored by future broader comparison, either intensively within each family or extensively from a representative set of species across multiple families (fig. S1). Thus, our findings can serve as a foundation for comparative studies of collective behaviors. Furthermore, our results challenge theoretical studies that often assume the same behavioral rules across taxa as a result of the observation of a limited number of species. Direct comparative studies promise a comprehensive view of the mechanisms of collective behavior and will give us an understanding of the origin of coordination and the general algorithms underlying it.

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Statement of Authorship

All authors conceived and designed the study. N.M. performed experiments, data analysis, and computer simulations. All authors interpreted the results. N.M. drafted the manuscript, and S.C.P. edited the manuscript. All authors gave final approval for publication.

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