**Observation of tandem running behavior in mating pairs of Asian dampwood termite, *Hodotermopsis sjostedti***

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**Abstract**

As a social insect, termite colonies can grow to a group of millions of individuals, yet all colonies start from a single mating pair. Recent studies indicate that the pair formation process shows a large diversity among species, especially in basal lineages. Thus, comparative information is integral to estimating the ancestral state of this essential stage of the termite life cycle. The Asian dampwood termite, *Hodotermopsis sjostedti*, has been well-studied as a model basal termite of caste differentiation processes. Yet, their pair formation remains undocumented. In this study, we found that mating pairs of *H. sjostedti* show clear tandem running behavior. Both females and males played a leading role, and they switched their leading roles even within the same pair. We also found that dish size affected tandem movement coordination; pairs showed faster and more stable tandem running in a larger dish. We also provide a tracking dataset of 17 body parts, including antennae movement and leg moments during tandem runs, which can be utilized in future comparative studies. This study supports the idea that tandem running existed in the early ancestor of termites and sheds light on the origin of termite mate pairing.

**Introduction**

Social insects play a dominant role in ecosystems, either as predators, pollinators, or decomposers, contributing to global biomass (Bar-On et al., 2018; Eggleton, 2020; Tuma et al., 2020). The ecological success of social insects is often owed to the large size of their colony, ranging from hundreds to millions of individuals. Thus, extensive research efforts have focused on colony functions, regulated by their caste systems, where parents monopolize reproduction, and offspring will either develop into working castes that are responsible for colony tasks or alates that disperse to start a new colony (Noirot, 1991; Oster and Wilson, 1978). However, highlighting mature colonies of social insects often obscures the fact that most colonies need to start from one or a few reproductive individuals dispersed from their original colonies, except for a few species (Cronin et al., 2013). The first critical task of these dispersers is finding a mating partner; such pairing behavior is as important as sophisticated social behaviors to complete their colony life cycles.

Termites are one of the major lineages of eusocial insects and have evolved from subsocial wood-feeding cockroach ancestors (Bell et al., 2007). Termite colonies usually start with a monogamous mating pair, which will be a king and a queen in the mature colony (Chouvenc, 2022; Nutting, 1969). Termite mate pairing is often described as follows; in a short period of the year, numerous alates fly off to disperse. Once they land on the ground, they shed their wings to walk to search for a mating partner. Upon encounter, the pair performed tandem running, with the males following the females while searching for a nest site. However, this description is biased toward the observation of several neoisopteran termites, and pairing processes are actually documented to be diverse, especially in other lineages (Mizumoto et al., 2022). Some do not show tandem running, but females and males separately come to the nest sites (Sugio et al., 2020; Wilkinson, 1962). Some show tandem running, but the leader role is more flexible (Grasse, 1942; Lüscher, 1951; Mizumoto et al., 2022). Furthermore, *Cryptocercus* woodroach, a sister group of termites, should adopt a distinct pairing process from termites, as they are socially monogamous but genetically not (Yaguchi et al., 2021). Therefore, it is important to study the diversity of tandem running behavior, especially in basal lineages, which are often cryptic.

Asian dampwood termite, *Hodotermopsis sjostedti*,is an extensively studied species for their caste development system (e.g., (Kobayashi et al., 2023; Koshikawa et al., 2005; Miura et al., 2004, 2000; Nii et al., 2019; Oguchi et al., 2016; Oguchi and Miura, 2023; Shimoji et al., 2019)). However, their basic biology is not well understood. For example, termite nesting strategies can be classified based on how they utilize their food and nest resources (Abe, 1987; Korb, 2008; Mizumoto and Bourguignon, 2020), and *H. sjostedtri* was originally classified as a one-piece nester whose entire colony is completed within a single piece of wood (Abe, 1987). However, a later field study clearly demonstrated that this species is actually multiple-piece nesters that nest across multiple wood pieces by interconnecting them with underground tunnels (Kitade et al., 2012). In terms of mate pairing, although there are several observations on swarming flight in nature (Ohmura and Makihara, 2005) or studies on developmental mechanisms (Kobayashi et al., 2023; Miura et al., 2000; Oguchi et al., 2016; Oguchi and Miura, 2023), yet no information about tandem running behavior.

Here, we study the tandem running behavior of *H. sjostedti*. We observe their tandem running in the same methodological framework as previous studies in other genera (Mizumoto et al., 2021; Mizumoto and Dobata, 2019). Also, we qualify their behavior using deep learning posture tracking to compare the female leader and the male leader. Finally, given the large body size of this species, we compare the observation between two different-sized dishes.

**Methods**

*Behavioral observation*

The colony of *Hodotermopsis sjostedti* was collected at Yakushima Island, Kagoshima prefecture in when?, 2023. The colony included nymphs. The colony was maintained in the laboratory.

In July, we confirmed that these nymphs are differentiated into alates. The plastic containers with nests were moved to 27°C, and alates flew to come out of the nests. Alates were then collected, separated by sex, introduced to be dealated by manually pinching wings with forceps, and color-marked with one dot of paint (PX-20; Mitsubishi) on the abdomen to distinguish sex identities. These termites were isolated individually more than 30 min before the experiments.

We introduced a female-male pair into the experimental arena. The experimental arenas consist of a petri dish (φ = 90 and 140 mm, respectively) covered with a layer of moistened plaster that was polished before each trial. We recorded termite movements in the arena for 30 minutes using a video camera (HC-X1500-K, Panasonic) with a resolution of 3840x2160 pix at 59.96 frames per second (FPS). In total, we obtained the videos of X pairs for 90 mm dish and Y pairs fir 140 mm dish. Because only one colony was available, all pairs were from nest mate. All the videos were cropped to 2000x2000 pix to only include the arena in the frame before the video analysis.

*Data processing*

All videos were analyzed using SLEAP v 1.4.0 (Pereira et al., 2022) to estimate the movement of body parts of each individual. The model was based on the that developed for *Reticulitermes speratus* and *Coptotermes formosanus* in a previous study (Mizumoto and Reiter, 2025), with a 17-node skeleton: antenna tips (LR), antenna middle (LR), antenna base (LR), head (middle of mouth parts), head-pronotum boundary, pronotum-mesonotum boundary, metanotum-abdomen boundary, abdomen-tip, fore legs (LR), mid legs (LR), and the hind legs (LR). We labeled X individuals from Y videos for training. We trained a U-Net-based model with a multi-animal top-down approach, with a receptive field size of X pixels for the centroid and Y pixels for the centered instance, on Nvidia GeForce RTX 4090, where augmentation was done by rotating images from -180 to 180 degrees. The mean Average Precision (mAP) and mean Average Recall (mAR) of this model were 0.687 and 0.766, respectively. While tracking after the inference, we used the instance similarity method with the greedy matching method. All pose estimation data were converted to HDF5 files for further analysis.

We used Python to format all HDF5 files for further analysis and converted them into FEATHER files for analysis in R (R Core Team, 2023). We employed a linear interpolation method to address missing values in the dataset after down-sampling data into 30 FPS. After scaling all data from pixels to mm (2000 pixels = arena size), we used a median filter with a kernel size of 5 to reduce noise.

*Data analysis*

To compare tandem running behaviors among species, we automatically determined whether pairs were in tandem based on the postures and spatial position of partners. First, we regarded as two individuals being in interaction when the distance between the body centers of partners was less than two body lengths, based on the frequency distribution of this distance (Fig. 1C). In this process, we ignored the short interaction events or non-interaction events less than 2 seconds to smooth the data. Second, during interactions, we classified termite heading orientation as female-leader and male-leader. We obtained the heading directions of females and males as vectors from the abdomen tips to the head front. Then, a pair was in the female leader when the male was behind relative to the female heading direction, and the female was front relative to the male heading direction, and vice versa (Fig. 1AB). If a pair spent more than half of the time in a female-leader position during an interaction event, we regarded the interaction event as a female-leader tandem run. This classified all frames into female-leader tandem, male-leader tandem, other interactions (including tandem runs where they switch leader-follower roles), and non-interactions. We obtained the traveled distance for which the leader walked during each tandem running event. Then, we compared this traveled distance, using mixed-effects Cox models, with species being treated as a fixed effect and each pair id as a random effect. We used the coxme() function in the coxme package in R (Therneau, 2015). Note that we used distance instead of duration to evaluate how much tandem running pair could explore the environments by removing pausing time during interactions.

**Results**

From visual examinations, we observed tandem running behavior in Hodotermopsis sjostedti. In 90 mm dish condition, among X pairs, we observed tandem running behavior in Y pairs, among which XX pairs showed female-led, YY male-led, and ZZ both tandem running (Video 1 and 2, Figure 1). Similarly, in 140 mm dish condition, ~.

**Discussion**

Our observations clearly showed that dealates of *H. sjostedti* show tandem running behavior, with both females and males playing both leader and follower roles. In many species of termites, females play a leader role in tandem running (Mizumoto et al., 2022). Still, documentation of tandem running with both female and male leaders has been limited in several Kalotermitidae species (summarized in (Mizumoto et al., 2022)), including *Kalotermes flavicollis* (Grasse, 1942; Lüscher, 1951), *Cryptotermes havilandi* (Lüscher, 1951) (but suspected as *dudleyi*. See (Mizumoto et al., 2022)), *Paraneotermes simplicicornis* (Carr, 1972), and *Glyptotermes* *fuscus* and *satsumensis* (Mizumoto et al., 2022). In addition, the fossil record indicates that the extinct kalotermitid termite, *Electrotermes affinis*, shows male-led tandem running behavior (Mizumoto et al., 2024a). However, even though the previous ancestral state reconstruction estimated that the ancestor of termites exhibited tandem running with both females and males being leaders (Mizumoto et al., 2022), there was no record of that behavior in other lineages than Kalotermitidie. Thus, our observation on *H. sjostedti* is critical as this species is Hodotermopsidae in a distinct clade of Teletisoptera (Hodotermopsidae, Stolotermitidae, Hodotermitidae, and Archotermopsidae) (Wang et al., 2022), highlighting the diversity of pairing process of this group (Mizumoto et al., 2022).

Although the biology of *Hodotermopsis* is often compared with that of *Zootermopsis* as a related species (refs), our observations suggest that these two groups use distinct mate-pairing processes. The pairing process of *Zootermopsis* species has been documented in several papers (Castle, 1934; Howse, 1970; Shellman-Reeve, 2001, 1999, 1994; Stuart, 1969), their use of tandem running behavior is less clear (summarized in Fig. S12 in (Mizumoto et al., 2022)). A previous study treated the tandem running status of *Zootermopsis* as a female-led tandem (Mizumoto et al., 2022), yet original descriptions clearly mention that the tandem pairing is weaker than other termite species with static tandem running behavior (Castle, 1934; Howse, 1970). By using the same experimental setup as the current study, we could not observe the clear tandem running in *Zootermopsis nevadensis*, collected in Hyogo Prefecture, Japan (although small sample size; *n* = 3 pairs, on Jan 4th 2022). These observations indicate that the pairing process of *H. sjostedti* is distinct from *Zootermopsis* species. It is reasonable that these species exhibit different nesting habitats, with *Hodotermopsis* being a multiple-piece nester and *Zootermopsis* being a one-piece nester. One future direction is to study the relationship between nesting habitat and the pairing process in a phylogenetic comparative framework.

One limitation of the current study is that our observation is limited to one colony. In other species, it is known that tandem running behavior can be affected by the individual conditions, such as body size (Husseneder and Simms, 2008; Matsuura et al., 2002) and time after swarming (Mizumoto et al., 2024b). Thus, reflecting individual status, there should be a quantitative variation of tandem running propensity across different colonies. However, it is unrealistic to suppose the colony variation in the pairing mode, with some colonies exhibiting tandem running while others using different pairing methods. For example, in *Marginitermes hubbardi*, a laboratory observation demonstrates that this species does not usually show tandem running behavior except for one pair (Carr, 1972). The author observed a tandem running behavior of *M. hubbardi* on the tree trunk in the field condition (one personal observation by N. Mizumoto on July 31, 2019, in Tempe, Arizona), implying that there might be a specific condition for this species to exhibit tandem running behavior. Thus, it might be difficult to prove the lack of tandem running only from the laboratory observations. However, even with limitations, our study provides a positive observation of the clear tandem running behavior of *H. sjostedti*, which should be valid in field environments.

Effect of dish size.

In conclusion, our study contributes to the understanding of the diversity and evolution of mate-pairing behavior in termites. Even though mate pairing plays a crucial role in the life cycle of termites, little attention has been paid compared to other social behaviors. One challenge is that mate pairing is a seasonal event, which can be observed in a limited period of the year for each species. Yet, given the cryptic diversity of the tandem running behavior in non-neoisoptera termites, species-specific descriptive efforts are essential.

**Author contributions**

N.M.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualization, writing-original draft.

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