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.

**Results**

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**Figure x.** Tandem running stability in *Hodotermopsis sjostedti*. A) Comparison of the duration of each tandem running event until separation between tandem runs led by females (n = 129) and males (n = 90). B) The duration of each pair separation event until each pair reunited (n = 219). Kaplan-meier survivorship curves were generated to depict the data. Shaded regions show 95% confidence intervals.

When tandems are female-led, the likelihood of separation at any given moment decreases by 36.6% (Cox mixed model, df = 1, X2 = 21.25, P<.001).