**Loss of pair formation predates the evolution of male-less society in *Glyptotermes* termites**

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**Abstract**

Although males contribute to sexual reproduction, they have been lost repeatedly in diverse lineages, which further modifies behaviors of asexual species. In social animals with biparental care, male loss requires reorganizing pairing processes for parental care. Termite society starts from a mating pair, where many species form a tandem running courtship pair while looking for a nest site. However, this raises the question of how a male-less society could evolve in termites, where the colony started with only females. Here we compare the tandem running behaviors of *Glyptotermes* termites and find that they lost tandem runs and used aggregation before the evolutionary male loss in *G. nakajimai*. We found that *G. fuscus* and *G. satsumensis*, exhibited both female-leader and male-leader tandem runs, which should be the ancestral state in this genus. On the other hand, tandem running was rare and ephemeral in both sexual and asexual lineages of *G. nakajimai*. Instead, mating pairs of *G. nakajimai* often aggregate in one place. These results are consistent with the idea that *G. nakajimai* may start a nest as a group, which facilitated the evolution of asexuality. Our study highlights the unique aspects of the evolution of asexuality in social animals, contributing to our understanding of complex behavioral phenotypes.

**Keywords**

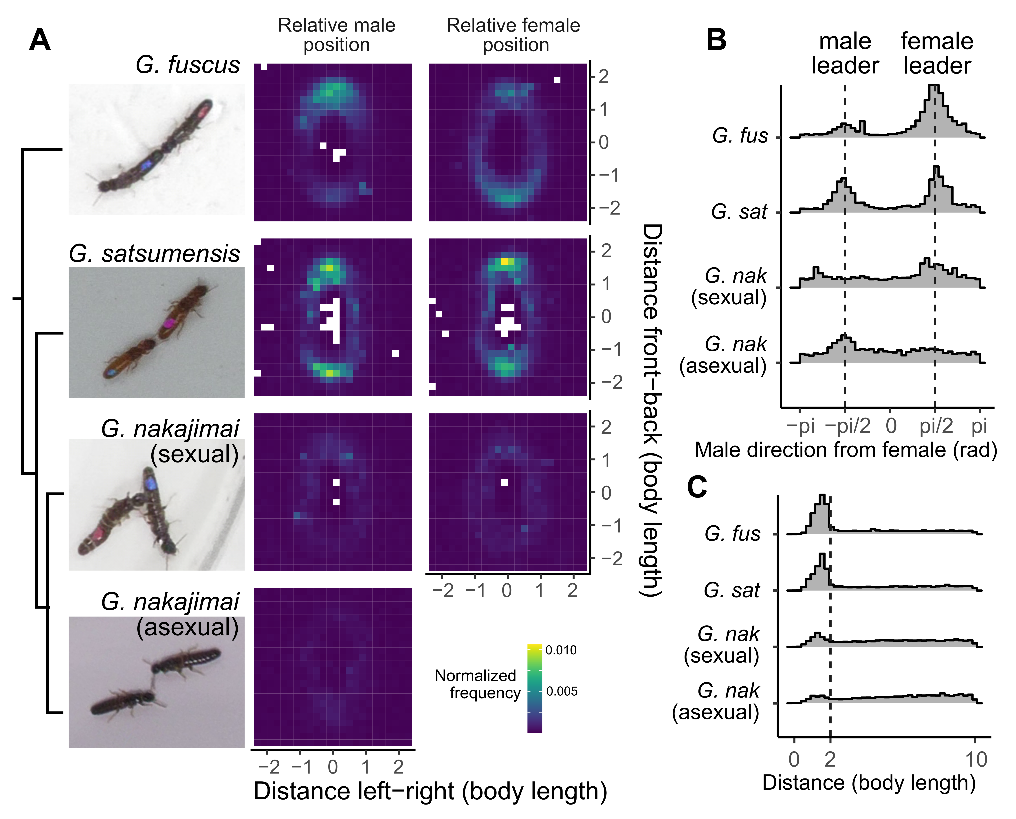
Furthermore, we found that another species, G. nakajimai, did not show tandem running behavior.

1. Same-sex tandem runs, female-female tandem runs

2. meeting at the nest sites

Tandem running is the simplest movement coordination in a pairing animal. In many species of termites, tandem running occurs between mating pairs, where female leaders search for nesting sites while male followers maintain coordination by following her. However, the leader-follower role is not static rather flexible across social contexts and across species. Here we study of tandem run of Glyptotermes termites.

In biology, common knowledge is formed



**Figure 1.** Spatial positioning between partners in *Glyptotermes* termites. (A) Comparison of relative position of the partner, given that female (left) or male (right) heading towards the top.