February 2024

Dear Editors,

We are pleased to submit our manuscript, " **The strength of sexual signals predicts same-sex paring in termites**," for consideration for publication in *Biology Letters*.

The enigma of same-sex sexual behavior (SSB) is increasingly studied in evolutionary biology and behavioral ecology, because such behavior cannot directly result in reproduction. Theoretical papers predicted that the loss of sexual communication signal is an evolutionary driver of SSB, calling for empirical works to test the hypothesis. Here we report findings from a suite of empirical works on two different termite species that illustrate the clear association between sex pheromones and the frequency of same-sex pairing.

To investigate how the strength of sexual signals relates to the SSB, we took advantages of the two unique termite species that share the same chemicals for sex pheromone but with distinct quantities. Using the automated video tracking and trajectory analysis, we found that female-female pairing was more stable in the species with more sex pheromones, while male-male pairing was more frequent in the species with a smaller quantity of pheromones. We clearly demonstrated that the evolution of SSB in termites is inseparable from the evolution of sex-attracting signals, which provides empirical evidence to the previous theoretical papers (e.g., Monk et al. 2019 *Nature Ecol Evol*; Lerch and Servedio 2023 *Am Nat*) and encourages further empirical tests in other animal lineages.

Controversy about the evolutionary origins and maintenance of SSB is not only important to those who perform basic scientific research – the topic also attracts widespread societal interest. It is therefore important to approach this research question with diverse animal groups. Most previous studies have only focused on one species, leaving questions about the diversity of SSB. We designed our study to compare the two related termite species, as well as relate our finding with other termite lineages as discussed. Such comparative perspectives open up new directions of research by informing works on the diversity of animal mating systems, evolution of alternative reproductive tactics, and the regulation of collective animal behavior.

The present manuscript is original and is not under consideration for publication elsewhere (preprint is available in bioRxiv: https://doi.org/XXXXXXXXXXXXX). We appreciate your consideration.

Yours sincerely,

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