Beyond mistakes: same-sex partner acceptance and broad mating filters coexist in termite pairing

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Abstract

Same-sex sexual behavior is often interpreted either as a mistake arising from indiscriminate attempts or as an adaptive behavior directed towards same-sex partners. These explanations are typically considered mutually exclusive. Here we challenge this assumption using an adaptive same-sex pairing system in *Reticulitermes* termites. Long-term male-male pairings originate from tandem running, in which one male follows another. We found that males discriminate partner sex and rarely form male-male tandems upon first encounter, whereas male-female tandems form immediately. However, male-male pairing frequency increases over time, indicating that males progressively accept male partners despite recognizing their sex. At the same time, males frequently follow non-mating individuals, including workers and soldiers, which partially share cues with females. Such interactions are costly because workers attack following males. Males subsequently refine their decisions, reducing these interactions and ceasing them entirely after experiencing an attack. Our results show that adaptive same-sex pairing and mistaken partner choice arise from different stages of the same behavioral sequence. Rather than alternative explanations, adaptation and mistakes can coexist because partner recognition and acceptance occur sequentially in the pairing process. These findings highlight the importance of behavioral sequences when interpreting the causes of same-sex sexual behavior and other apparently misdirected behaviors.

Keywords

Indiscriminate mating; signal detection theory; broader filter; social insects; homosexual behavior; movement coordination; mate choice; sexual recognition; acceptance threshold; mistaken identity

Introduction

Interpreting animal behavior requires distinguishing between target recognition and subsequent behavioral responses through careful observations and reasoning [1]. When an animal accepts unusual targets, it may either fail to recognize them correctly or respond in an unexpected way (to the observer) despite accurate recognition. The distinction of recognition and behavior is critical in the studies of same-sex sexual behavior (SSB) [2], as the interpretation of SSB depends on the recognition status of mate searchers.

Different recognition and response processes can explain the occurrence of SSB (Table 1). Especially in arthropods, SSB has often been considered the result of mistaken identity, in which mate searchers mistake same-sex individuals for the opposite sex [3,4]. A similar, but conceptually different, idea is the indiscriminate mating [5–7], where mate searchers do not attempt to discern the sex of the partner (Table 1). Indiscriminate mating can be adaptive, but SSB should occur as an inevitable consequence [6,8]. In real animals, recognition is not discrete but often lies on a quantitative continuum. The mating filter, adapted from signal detection theory (or acceptance threshold theory) [9,10], predicts that recognition by mate searchers is not perfect but can be tuned to the conditions [11,12], with SSB occurring when the mating filter is broader [13,14]. Finally, if mate searchers recognize that the partner is a same-sex individual, but still engage in mating activity, we consider this to represent adaptive SSB with known or unknown functions [2,15–18]. These explanations are typically treated as mutually exclusive alternatives, despite differing in both recognition and response processes (Table 1). Testing this idea requires a system in which adaptive same-sex interactions occur, while opportunities for recognition errors can be independently evaluated.

Table 1. Recognition and response of mate searchers when encountering non-mating targets (e.g., same-sex, immature, dead, or inanimate objects).

Recognition	Response	Interpretation
Mistake as the other sex	Accept	Mistaken identity
No assessment	Accept	Indiscriminate mating
Recognized as same-sex	Reject	Discriminate mating
Recognized as same-sex	Accept	Adaptive SSB
Uncertain recognition	Accept/Reject	Mating filter/signal detection

Mate pairing in *Reticulitermes* termites provides an ideal system for investigating the roles of recognition and response in SSB. When females and males encounter each other during mating season, males follow females in tandem, and a pair shows coordinated motion as they travel together to find a suitable nest site [19,20]. In addition to monogamous pairing, termites also exhibit same-sex tandem running [18,21], as well as same-sex long-term nest establishment [15,22]. Such a same-sex pairing has adaptive value. Same-sex tandem running has a predator avoidance function [21,23], and same-sex nest establishments enable them to survive for years, waiting for mating opportunities during colony fusion [15,24]. The stable same-sex pairing must happen after the correct sexual recognition. In same-sex tandem running, one individual shows plastic behavioral changes to exhibit the behavior of the other sex [18], and male-male pairs often compete for the follower position [18,21,25]. Also, the selection of correct sexual recognition should have been high, given that termites have been monogamous for 150 million years [26]. However, it remains unclear if termites have precise recognition of a partner upon encounters. Because same-sex pairing is adaptive for termites, males may be indiscriminate or use a broader filter, starting by following any objects that seem like termites and then refining their decision.

Non-mating targets that look similar to the mating object provide an interesting opportunity to study the mating decisions [14,27]. Termite females produce sex pheromones that help males locate them [28,29], especially when the partners are accidentally separated [30,31]. Termites reuse the same chemical for sex pheromones of females as the trail pheromones of workers and soldiers [32]. Workers and soldiers are clearly the wrong choice. It is possible for mate searchers to encounter workers and soldiers because during swarming flights workers and soldiers also exit the nests to defend and facilitate swarming [19,33]. Encounters with these workers and soldiers are risky for dealates as they could be attacked as intruders of the nests [34]. So, if males use strict partner recognition before pairing, they should avoid following these non-mating termites. Alternatively, if the mating filter is involved, they will follow workers or soldiers. Yet, once they realize it is not a female, they should stop.

In this study, we examined partner recognition and subsequent behavioral responses in males of the termite *Reticulitermes amamianus* Morimoto 1968 (Blattodea: Heterotermitidae). We quantified pair interactions in female-male, male-male, and male-worker (or soldier) pairs using deep-learning automated posture-tracking software [35], especially focusing on the time development of partner-following activities. Overall, our observations confirm that males can immediately recognize whether the partner is the same sex in a mating context but can also be confused by workers or soldiers as partners (Fig. 1). We also found that males refine their decision according to time (Fig. 1), where males are more accepting of the same sex partner while cease following non-mating partners. Our results demonstrate that both mistakes and acceptance after recognition coexist in the termite pairing system.

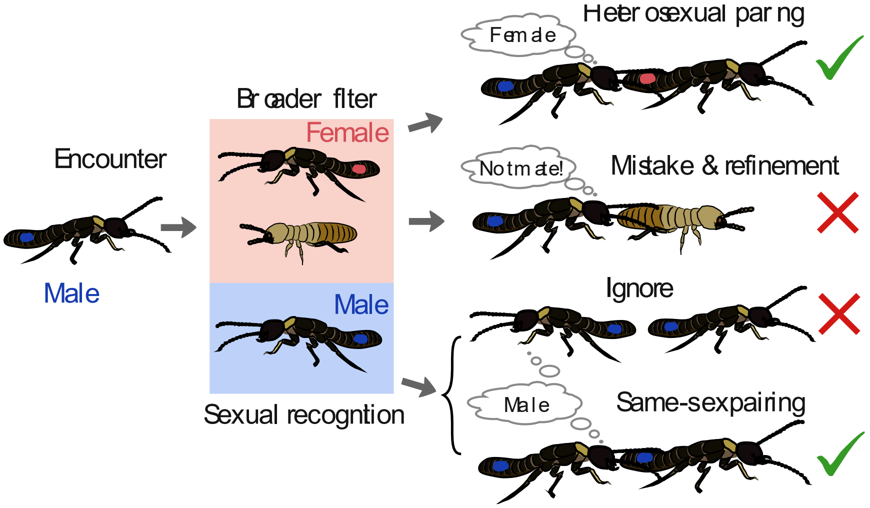

Figure 1. Termite mate pairing involves both adaptive same sex pairing after accurate recognition and maladaptive mistakes due to a broader mating filter. When a male encounters a partner, it distinguishes whether the partner is male or female. After correctly recognizing the partner is the same sex (male), males either accept or ignore that as a tandem running partner. On the other hand, males use a broader mating filter. When males encounter workers that share some chemical cues with females, males start following them as well. But later, they refine their previous decision to stop following workers.

Methods

(a) Behavior recording

We collected eight colonies of *Reticulitermes amamianus* containing alates with the nesting wood pieces from the field in Tokunoshima Island, Kagoshima Prefecture, Japan, between April 4 and 5 in 2023 (colonies A: NM23-045; B: NM23-036; C: NM23-050; D: NM23-034; E: NM23-048; G: NM23-047; H: NM23-051; I: NM23-031). All nesting wood pieces were maintained at 17-22°C until the experiments. Before each experiment, we transferred the nests to a room at 27°C, which promoted alate emergence and flight. After the swarming, we opened the nests using hatchets and extracted workers and soldiers. Alates were then separated by sex and color-marked with one dot of paint (PX-20; Mitsubishi) on the abdomen to distinguish sex. These termites were isolated individually for more than 30 min before the experiments. We used only individuals that had shed their wings voluntarily within 12 h. Each individual was used only once. The experimental arena described below was illuminated by white LED lights and maintained at 27°C. We recorded termite movements in the arena for 30 min using a video camera (HC-X1500-K, Panasonic) with a resolution of 3,840 × 2,160 px at 30 frames per second. The camera was mounted vertically above the arena. All experiments were conducted on April 7, 10, 11, and 12 in 2023.

For female-male and male-male comparisons, we introduced a female-male or male-male pair into a petri dish (Φ = 90 mm) with plaster. We only used nest-mate pairing, which does not affect either same-sex or heterosexual tandem running behaviors [36]. We observed 25 pairs for each combination (5 colonies A-E x 5 replicates). All the videos were cropped to 2,000 × 2,000 px to include only the arena in the frame before video analysis.

To record the response for non-mating targets, we introduced a female–male, female-worker, male-worker, or male-soldier pair into every cell of a 6-well culture plate (Φ = 34.5 mm) with plaster. For the male-worker pair, we used both nestmate and non-nestmate pairs across four colonies

(B, G, H, I). We recorded 48 female–male pairs, 38 female-worker pairs, 46 male-worker pairs from the same colony, 42 male-worker pairs from different colonies, and 35 male-soldier pairs. All the videos were cropped to 2,048 × 1,536 px to include only the arena in the frame before video analysis.

(b) Quantitative trajectory analysis

All videos were analyzed using SLEAP v1.3.0 [35] to obtain trajectories of body parts for each individual using a multi-animal top-down pose estimation framework. Although caste-specific annotation schemes were used during model training, all downstream analyses were based on a reduced set of three biologically homologous landmarks: head, body center, and abdomen tip. For dealates, we used a 16-node skeleton including antenna tips and bases (left and right), head center, head–pronotum boundary, pronotum–mesonotum boundary, mesonotum–metanotum boundary, metanotum–abdomen boundary, abdomen tip, foreleg, midleg, and hindleg joints (left and right), and a colored abdominal marker, following a previously used skeleton [31]. For soldiers and workers, we used simplified skeletons consisting of four nodes (head center, head–pronotum boundary, abdominal center, and abdomen tip), consistent with a previously used framework (Mizumoto et al., in review). For dealates, 37 frames (355 individuals from 13 videos) were labeled for training in 6-well experiments and then transfer trained to petri-dish experiments with additional 18 frames for labeling; for soldiers, 45 frames were labeled, and a model was trained from scratch using a top-down approach anchored at the head–pronotum junction; and for workers, 127 frames were labeled, and model training was initialized from the soldier model. All models were trained using a U-Net-based architecture on an NVIDIA GeForce RTX 4090 GPU, with data augmentation including random rotations of ±180 °. Ten percent of labeled frames were used for validation (validation fraction = 0.1). Instance identity tracking was performed using appearance-based similarity with greedy bipartite matching. The prediction performance is summarized in Table S1.

All pose estimation outputs were exported as HDF5 files. We used a Python script to perform linear interpolation to address missing values in the dataset and to apply a median filter with a kernel size of 5 to reduce noise. We also corrected tracking errors caused by identity swaps between individuals in different wells. After data processing, we converted all HDF5 files into FEATHER files for analysis in R v 4.5.2 [37]. For each pair, we standardized the body length unit (bl) to the average of the two individuals. The body length of each individual was computed as the sum of the Euclidean distances from the abdomen tip to the body center and from the body center to the head. Body length was measured across all frames, and the average was calculated for each individual.

For each frame, we determined whether males were following their partners. We regarded males as following when the distance from the male head to the partner's abdomen was within 1.5 bl, and the distance from the male abdomen to the partner's head was greater than 1.5 bl. The displacements (frame-to-frame changes in position as the Euclidean distance) of the body center positions were computed for every frame to obtain the instantaneous movement speed. The changes in movement speed (acceleration/deceleration) and direction were also computed from the displacements.

In a pairing treatment pairing a male with a worker from a different colony, we observed workers attacking males by biting, frequently appendages such as antennae and legs. The attack often resulted in the amputation of appendages of males or defecation by workers (Video S1). We first checked the endpoints of all videos to see if attacks had occurred by confirming the traces of amputation or defecation, where males were typically dying with both traces. Then, for each pair with clear attack traces, we examined when the first attack (biting) and the first amputation attack occurred. We also investigated whether tandem running behavior was observed before and after the attack. Manual observation was independently confirmed by two authors to account for inter-observer variability, and agreement was examined using Cohen's Kappa and the Intraclass correlation coefficient (ICC) with the 'Irr' package [38]. The two observers showed perfect agreement for alate damage (Cohen's κ = 1.00, *n* = 42) and tandem after the attack (*n* = 17). For tandem behavior before the attack, agreement was slightly lower due to a single discrepant case (κ = 0.85, n = 14). Inter-observer reliability was also high for the timing of the first attack (ICC = 0.6) and nearly perfect for the amputation attack (ICC = 1.0) (Fig. S6).

(c) Statistical analysis

We measured the proportion of the time spent in tandem running every minute for each individual. We applied a logit transformation for this proportional measurement [39], using a small adjustment constant (0.003) to handle boundary values. We then used linear mixed-effects models (LMMs) with treatment (pairing types), time (in min), and their interaction as fixed effects, and pair identity as a random intercept. The model was fit for dish experiments and 6-well experiments separately, using the lmer() function of the 'lme4' package [40]. Fixed-effect significance was assessed using Type II tests with the Anova() function of the package 'car' (same for the rest of the analysis). To evaluate whether temporal slopes differed among treatments, we estimated marginal slopes per treatment and conducted pairwise contrasts using the emtrends() function of the package 'emmeans' [41] (same for the rest of the analysis).

Next, we computed all continuous tandem running events observed for each pair. Tandem running events were classified as short (< 1.5 s) or long (≥ 1.5 s) based on an antimodal distribution (Fig. 2C). The probability of having a long tandem was modeled using generalized linear mixed models (GLMMs) with a binomial distribution, where treatment, start time of tandem running, and their interaction were included as fixed effects and pair identity as a random intercept. We used the function glmer() in the package 'lme4' [40]. Also, the durations of long tandem running were compared using mixed-effects Cox models between (or among) treatments, with the coxme() function in the 'coxme' package [42], with the pair ID as a random effect. The proportional hazards assumptions were verified by the cox.zph() function [43] (so as in the Cox models below).

Finally, for each pair, we extracted the longest tandem-running event as the most successful mating interaction. The start times of the longest tandem running event were compared between (or among) treatments using Cox proportional hazards models, with the coxph() function of the 'survival' package. We also compared the ordinal position of the longest tandem using general linear models (GLM) with a negative binomial error distribution to account for overdispersion. We used the glm.nb() function of the package 'MASS'.

Results

(a) Male same-sex pairing happens after sexual recognition

We found a clear difference in male response to a female and a male. Overall, tandem running was less frequently observed in male-male pairs compared with female-male pairs (Fig. 2A), where the proportion of tandem running was high from the beginning in female-male pairs (LMM; effect of treatment: $\chi^2_1 = 80.8$, $P < 0.001$). On the other hand, in male-male pairs, the proportion of tandem running was initially low but increased according to time (effect of interactions: $\chi^2_1 = 37.8$, $P < 0.001$; Fig. 2A). The slope had no trend in female-male pairs ($t_{1651} = 0.03$, $P = 0.98$), while a positive relationship in male-male pairs ($t_{1651} = 8.11$, $P < 0.001$). The longest tandem running events were observed earlier in female-male pairs compared with male-male pairs (time: Cox model, $\chi^2_1 = 18.8$, $P < 0.001$; ordinary number: GLM, $\chi^2_1 = 46.9$, $P < 0.001$; Fig. S1). Also, the duration of the longest tandem running was longer for female-male pairs compared with male-male pairs (Cox model, $\chi^2_1 = 23.6$, $P < 0.001$; Fig. 2B). That said, the duration of the longest tandem run well overlapped between female-male and male-male pairs (overlap of the density plot in Fig. 2B was 52%).

We observed two distinct following-behaviors by males: a short tandem running that lasted less than 1.5 seconds and a long tandem running that lasted more than 1.5 seconds (Fig. 2C). Female-male and male-male pairs differed in the proportion of the tandem running types, with female-male pairs showing more long tandem runnings (GLMM, effect of treatment: $\chi^2_1 = 120$, $P < 0.001$). Also, the time development of the probability of long tandem running was different between female-male and male-male pairs (GLMM, effect of interaction: $\chi^2_1 = 32.4$, $P < 0.001$). The female-male pair did not change over time, remaining high throughout the observations ($z = 0.00$, $P = 0.99$; Fig. 2D). On the other hand, in male-male pairs, the proportion of long tandem running greatly increased over time ($z = 9.30$, $P < 0.001$; Fig. 2D).

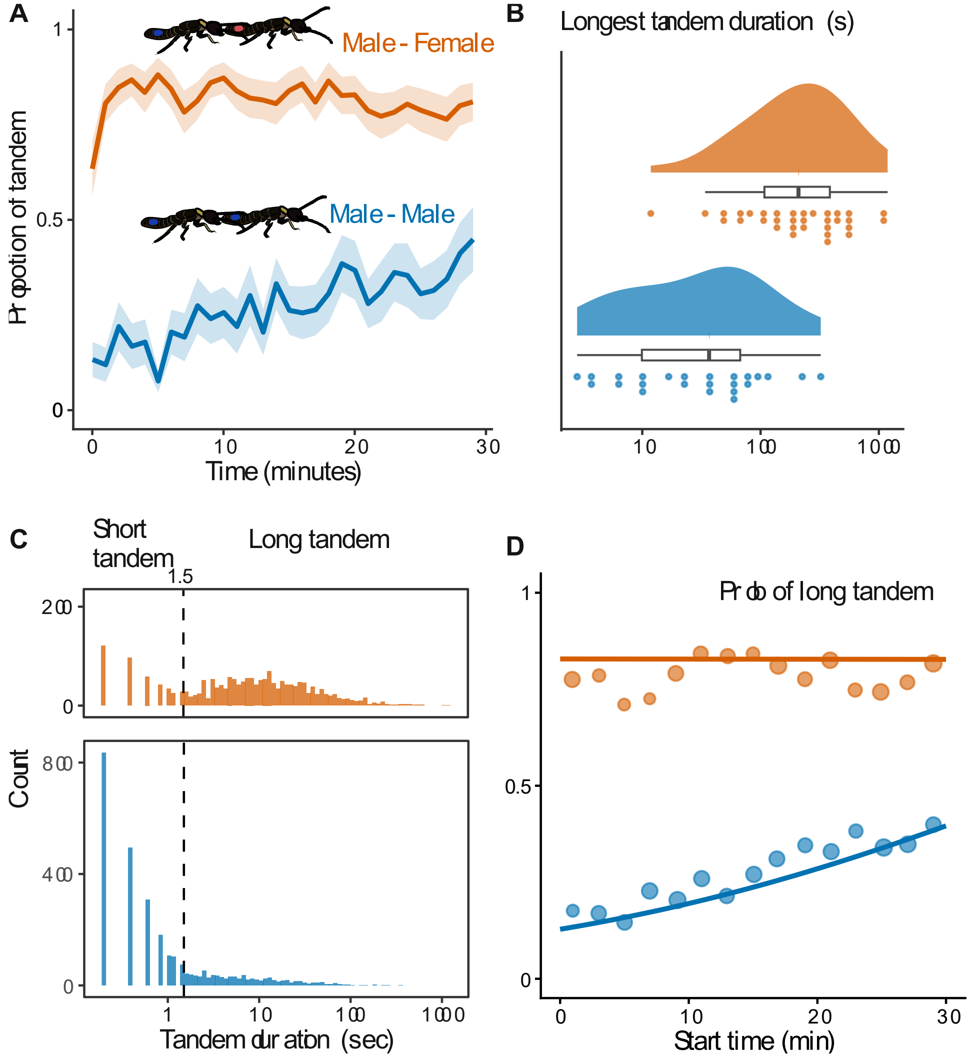

Figure 2. Termite males recognize the sexual identity of partners but participate in same-sex pairing according to time. (A) The time development of the proportion of time spent in tandem running. Shaded areas represent mean ± s.e. (B) Comparison of the duration of the longest tandem running events for each pair. (C) The distribution of the duration of the tandem runnings. The short and long tandem running events were separated by a threshold of 1.5. (D) The time development of the probability of starting long tandem running events. Regression lines are created by a GLMM. The point size indicates the number of observations in each 2-minute bin.

(b) Males follow non-mating targets

We found that males also followed non-mating targets as well. We observed male following behaviors towards workers (originating from both the same and different colonies) and soldiers (Fig. 3AB). Still, the female-male pair had the highest proportion among others (LMM, effect of treatment, $\chi^2_3 = 46.5$, $P < 0.001$). The reference treatment (female-male pair) showed a significant negative slope (effect of time, $\chi^2_1 = 290$, $P < 0.001$, $t = -4.88$). Pairwise slope contrasts showed that male-worker pairs (different colony) and male-soldier pairs declined significantly faster than female-male pairs ($P < 0.001$), while the male-worker pairs (same colony) slope also declined faster but not significantly from female-male pair ($P = 0.368$).

Tandem running events were also classified as short and long, as in the dish experiments (Fig. S2). We found that the proportion of long tandem was the highest for the female-male pair (LMM, treatment: $\chi^2_3 = 44.4$, $P < 0.001$), where there was a declining trend of the proportion of the long tandem running in female-male pairs (effect of time, $\chi^2_1 = 122$, $P = 0.003$). Pairwise slope contrasts showed that all other partner types also showed decline, where male-worker pairs (different colony) declined significantly faster than others ($P < 0.01$).

The duration of long tandem running events was significantly longer in female-male pairs than in other non-mating targets (mixed-effect Cox model, $\chi^2_3 = 54.8$, $P < 0.001$; pairwise comparison $P < 0.05$; Fig. 3D), while there were no significant differences among workers and soldiers ($P > 0.28$). Cumulative hazard functions confirmed this pattern, showing that female-male tandem running has consistently lower separation risk than other pairings. That said, we observed substantial overlap in the distribution of the longest tandem running events (Fig. 3C). Furthermore, the start timing of the longest tandem running events was mostly overlapped across treatments (time: Cox model; $\chi^2_3 = 6.76$, $P = 0.080$; ordinary number: GLM; $\chi^2_3 = 6.76$, $P = 0.007$; Fig S3).

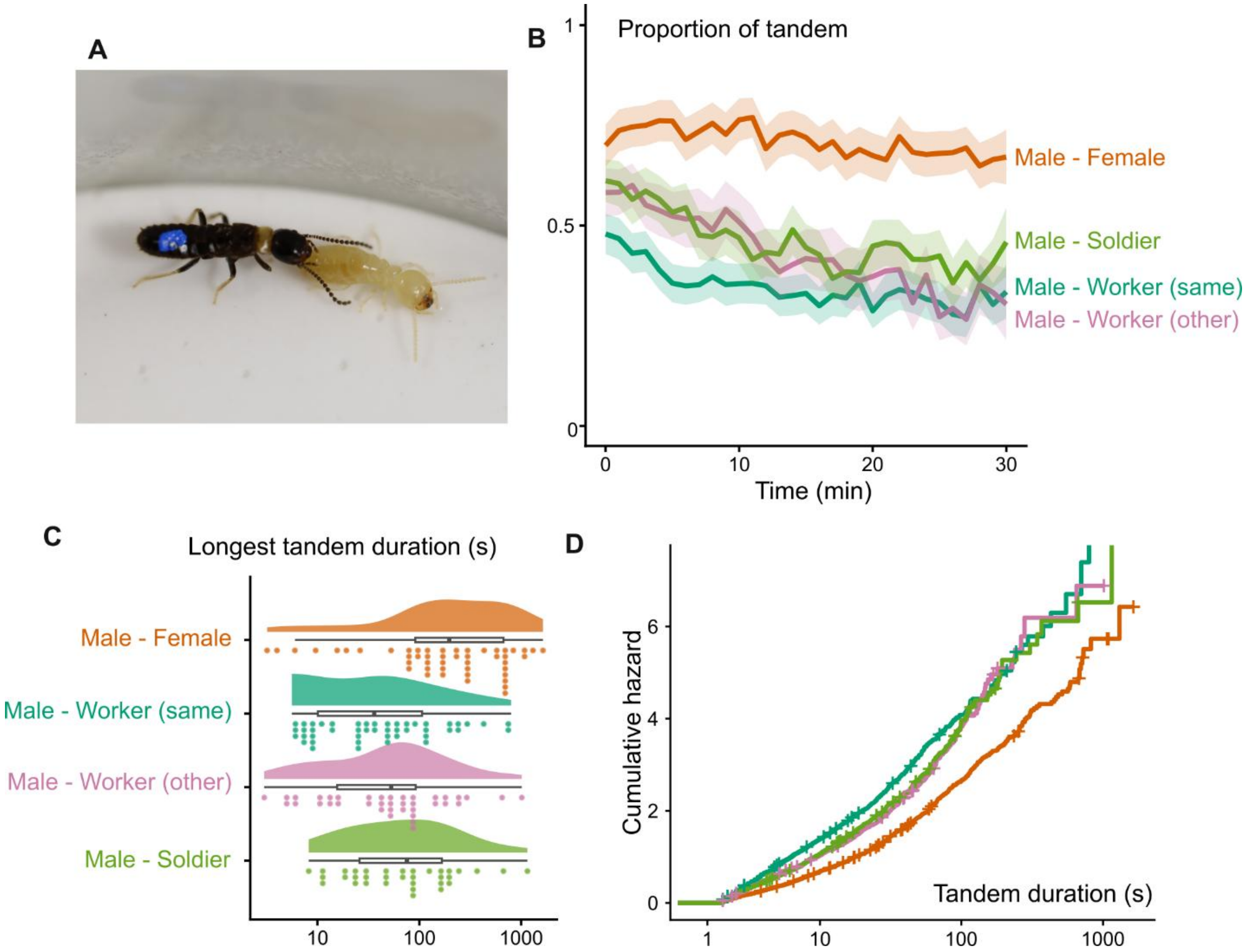

Figure 3. Termite males follow non-mating targets, such as workers and soldiers. (A) A photo of a male following a worker. (B) The time development of the proportion of time spent in tandem running. Shaded areas represent mean ± s.e. (C) Comparison of the duration of the longest

tandem running events for each pair. (D) Cumulative hazard of the interruptions of tandem running behaviors. + indicates the censored observations.

(c) Mistakes are costly for males

In a treatment involving workers from different colonies, we observed that workers attacked males (Video S1). Among 42 pairs observed, worker attacks were observed in 17 pairs. This attack by workers changed the males' worker-following behaviors. Among these 14 pairs, where the attack happened after more than 1 minute of recording start, we observed tandem running behaviors in 5 pairs. However, none of these males showed continued tandem running after experiencing attacks from workers (Fig. 4B).

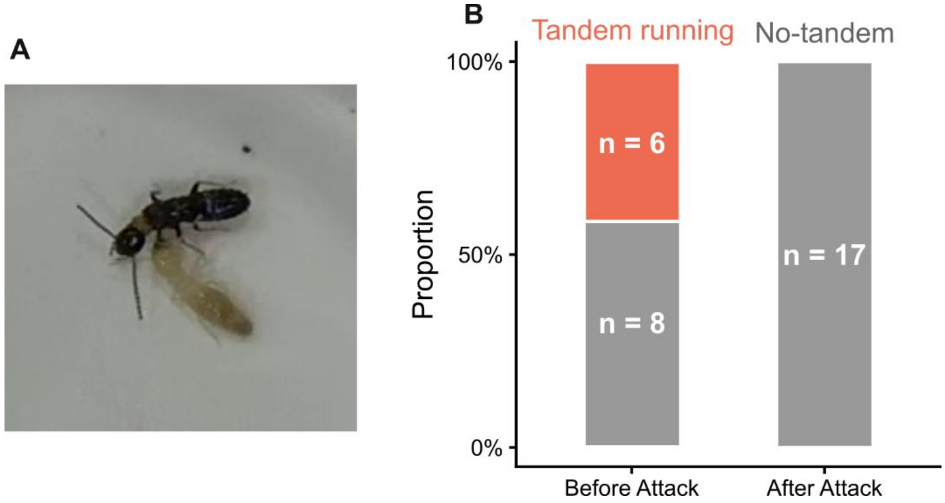

Figure 4. Mistakes are costly for termite mating attempts. (A) A snapshot of a worker attacking a male (see Video S1). (B) In the treatment with workers from a different colony, the proportion of males that exhibited tandem running behavior towards the worker before and after being attacked by the worker. Results are shown for observer 1 (Fig. S6).

Discussion

By observing the temporal patterns of tandem running behaviors initiated by males, our results clarified that mate recognition and acceptance are different during the termite pairing process. In male-male pairs, tandem running did not occur immediately after encounters, whereas it did in female-male pairs (Fig. 2A). This indicates that termite males distinguish male and female partners upon encounter and do not accept males as partners at the beginning (discriminate mating; Table 1, Fig. 1). However, over time, males started to accept other males as partners for tandem running (Fig. 2A). This is consistent with the interpretation of adaptive explanation, where males recognize the partner as the same sex but still engage in pairing (adaptive SSB; Table 1, Fig. 1). For males, the best option is always pairing with females, and long-term same-sex pairing in termites functions as a "making the best of a bad job" when no other sex partner is available [15]. Thus, same-sex pairing in *Reticulitermes* termites is the result of updated decision-making after recognition [36], rather than the instance recognition errors or indiscriminate following.

Importantly, the males exhibited multiple recognition-response pathways depending on the target encountered. In addition to being able to immediately recognize whether a partner is the same sex, males also adopt broad mating filters for uncertain targets [13,14]. When males encounter workers or soldiers, they start following these non-female targets as well (Fig. 3), although pheromone abundance is lower in workers than in females [32]. Males further refined these interactions following negative feedback, particularly after worker attacks (Fig. 4). Importantly, males responded differently to workers and soldiers than to females, suggesting that these targets were not treated equally throughout the interactions (tandem running with them happened less frequently and ended earlier). Such quantitatively different responses to various targets align with the interpretation of mating filters (mating filter/signal detection; Table 1, Fig. 1). Termite mating happens within a very short period of time (up to a few hours), where failing to find a partner results in death [15,19,44]. Under such constraints, signal detection theory predicts a broader filter [13], where termites should follow any individuals similar to conspecific females. This also explains why heterospecific tandem pairing and subsequent hybridization are common in termite mating systems [33,45–47] (also see Video S5 for tandem running between termites from different families). Thus, multiple recognition-reaction processes coexist within the termite mating system (Table 1), highlighting the complexity of mating decisions in general.

Our findings emphasize the importance of temporal aspects of animal mating decisions. Termite males showed opposite decision-making trajectories with different non-female partners: they initially rejected same-sex partners (males) but later accepted them, whereas they initially followed non-mating partners (workers) but later rejected them. These opposite trajectories indicate that mating decisions are not fixed at encounter but are continuously updated through interactions. Such temporal aspects of decision-making have been repeatedly emphasized in mate-choice contexts. For example, the sequential choice model, where a decision-maker seeks to select the best option from a sequentially presented set of options [48,49], predicts that mate searchers ignore the first several options in any way, which can affect our interpretation of single behavioral observations with same-sex or opposite-sex interactions. Also, the Bayesian updating model assumes that mate searchers change their decision-making according to the information collected from the environment by updating their prior expectations [50,51], which could explain the initiation of male-male pairing observed in this study. Signal detection theory approaches also found that mate searchers adjust their mating filters in response to their experienced socio-environmental contexts, resulting in different frequencies of SSBs [11–13,52] . With improvements in current behavioral quantification methods, recording the temporal dynamics of mating decisions is a promising direction for the study of SSBs.

The recognition cues remain unknown, requiring further holistic approaches. First, chemical cues should have played a role. In *Reticulitermes* termites, mating communications heavily depend on contact through antennae and palps [31], without clear visual cues [53], and thus, cuticular hydrocarbons could be an important candidate [54]. Although behavioral observations suggest that the use of male-attracting sex pheromones is limited to reunion after a tandem is accidentally separated [30,31], it is also a strong candidate [32]. In addition to the chemicals themselves, male chemical perception could have been modified to enable mate recognition, similar to the divergence in neural circuit organization observed in *Drosophila* courtship [55]. Second, we suggest that interactive behavioral feedback can be another important cue. Non-following after worker attacks suggests that behavioral feedback contributes to partner assessment (Fig. 4). Also, the movement patterns of workers and soldiers are distinct from those of dealates, with slower speeds in general and a lack of clear motion coordination during acceleration and deceleration (Figs. 4-5). Male-male encounters often result in competition for the follower positions, with specific postures, indicating that the partner is not female [18,21,25].

In conclusion, using termite pairing as a model system, we challenged the assumption that the mistaken-identity hypothesis and adaptive explanations are exclusive alternatives and instead showed that they can coexist within the same mating process. Although existing explanations typically focus on a single recognition-response pathway, we demonstrate that multiple pathways can coexist within the same mating system (Table 1, Fig. 1). In the SSB context, these two are often considered automatically linked; if mate searchers recognize that the partner is the same sex, they avoid mating engagements. Same-sex recognition and rejection could actually be linked in many animal mating systems [3,56] (even males of another termite genus, *Coptotermes,* avoid same-sex tandem after mate recognition [36]), or truly indiscriminative without an assessment [57,58]. Yet, such assumptions hinder our understanding of the cryptic behavioral diversity and hidden functions produced by evolution [2]. More broadly, observers' assumptions about recognition and response might have influenced research on animal misdirected behaviors in general. For example, social insects are almost always assumed to be aggressive towards the non-nestmate intruders [9], they can ignore or accept others even after recognizing them as non-nestmates if the cost of fighting is high enough [59]. In this situation, accepting non-nestmates is an adaptive decision-making after accurate recognition, analogous to the adaptive SSBs. By highlighting the importance of the temporal dynamics of sexual interactions between same-sex or opposite-sex pairings, these findings suggest potential research directions across behaviors considered to be "mistakes".

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Ethics

Termites were collected from public land, where no specific collection permit is required for non-protected invertebrate species. All data collection and experiments in this study were conducted in accordance with institutional guidelines on the ethical treatment of invertebrates

Data accessibility

The data and codes for this study are available in Zenodo: 10.5281/zenodo.20820371.
The codes are also available in the GitHub repository: https://github.com/nobuaki-mzmt/worker_tandem_filter

Declaration of AI use

We used AI-assisted technologies, including ChatGPT (OpenAI), Claude (Anthropic), Gemini (Google), and Grammarly, during manuscript preparation. These tools were used to improve language and readability, obtain feedback on the soundness of analysis methods, and assist with coding of data-processing and analysis scripts. No AI system was used to generate scientific hypotheses, perform data analysis, interpret results, or draw scientific conclusions. All analyses, interpretations, and conclusions were conducted and verified by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

Author contributions

NM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - Original Draft; EPC: Data curation, Formal analysis, Validation, Writing - Review & Editing

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Supplementary materials for

Beyond mistakes: same-sex partner acceptance and broad mating filters coexist in termite pairing

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This file contains

Table S1
Figure S1-6
Legends for Video S1-5

Table S1. Pixel errors for each body part in models trained in this study.

Termites	Body parts	Median	p70	p90
dealate	Head	2.45	3.05	4.42
	Body center	2.23	4.10	5.79
	Abdomen tip	1.50	1.98	7.74
worker	Head	1.65	1.96	2.79
	Body center	1.85	2.59	3.84
	Abdomen tip	1.61	2.28	3.17
soldier	Head	1.17	1.63	2.41
	Body center	1.93	2.59	3.02
	Abdomen tip	1.04	1.47	2.53

p70: 70th percentile. P90: 90th percentile.

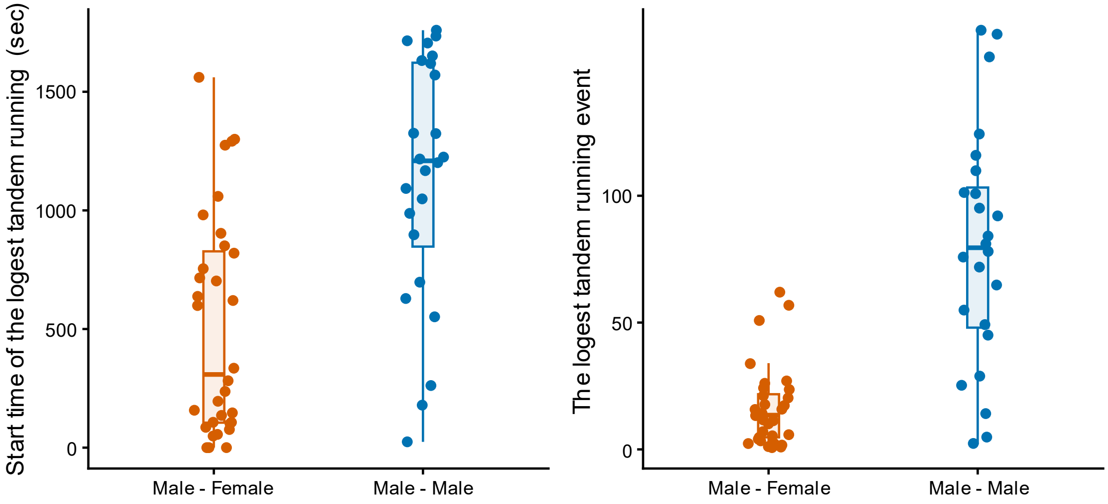

Figure S1. Comparison of the latency until the start of the longest tandem running event for each pair.

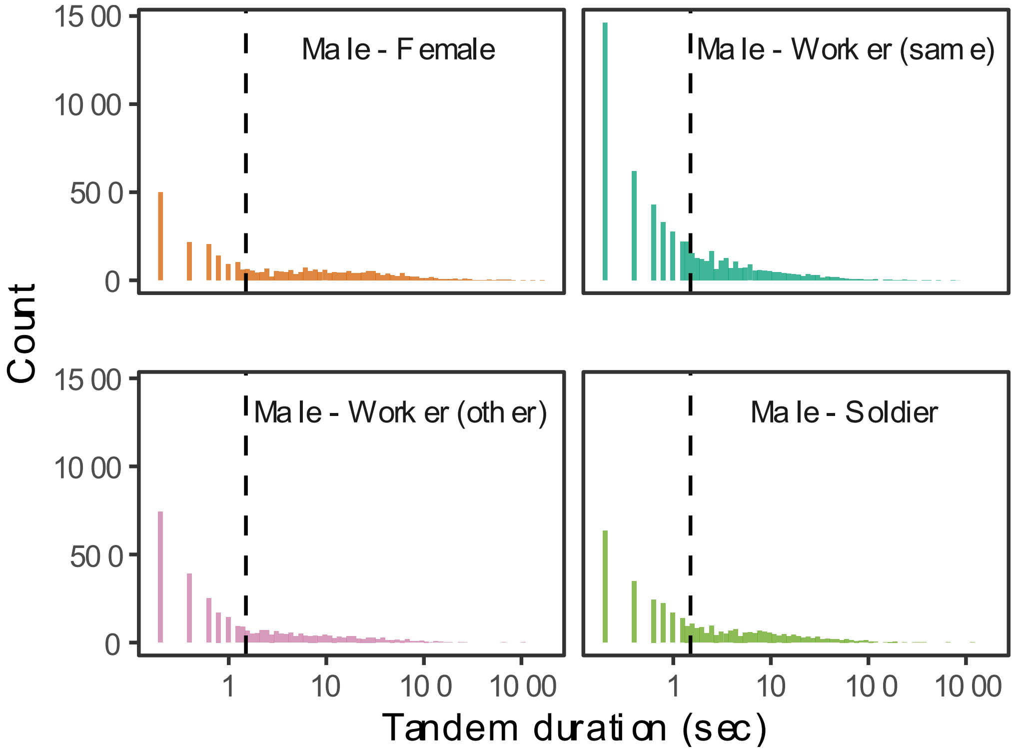

Figure S2. The distribution of the duration of the tandem runnings. The short and long tandem running events were separated by a threshold of 1.5.

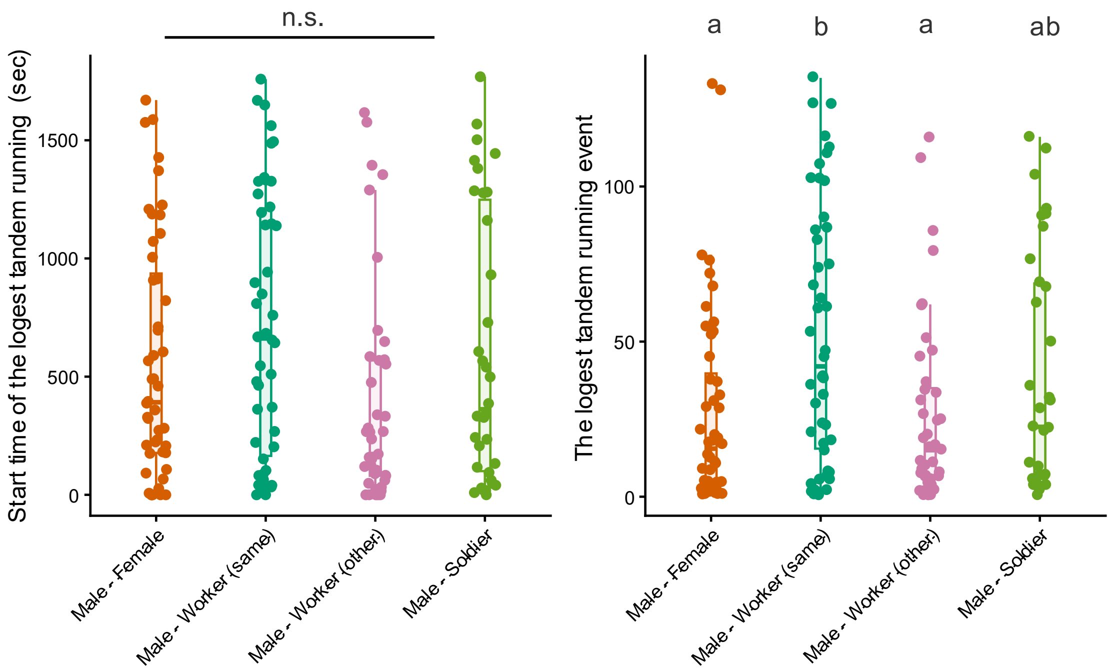

Figure S3. Comparison of the latency until the start of the longest tandem running event for each pair. n.s.: not significantly different (Cox model; $P > 0.05$). Different letters indicate significant differences (GLM, $P < 0.05$).

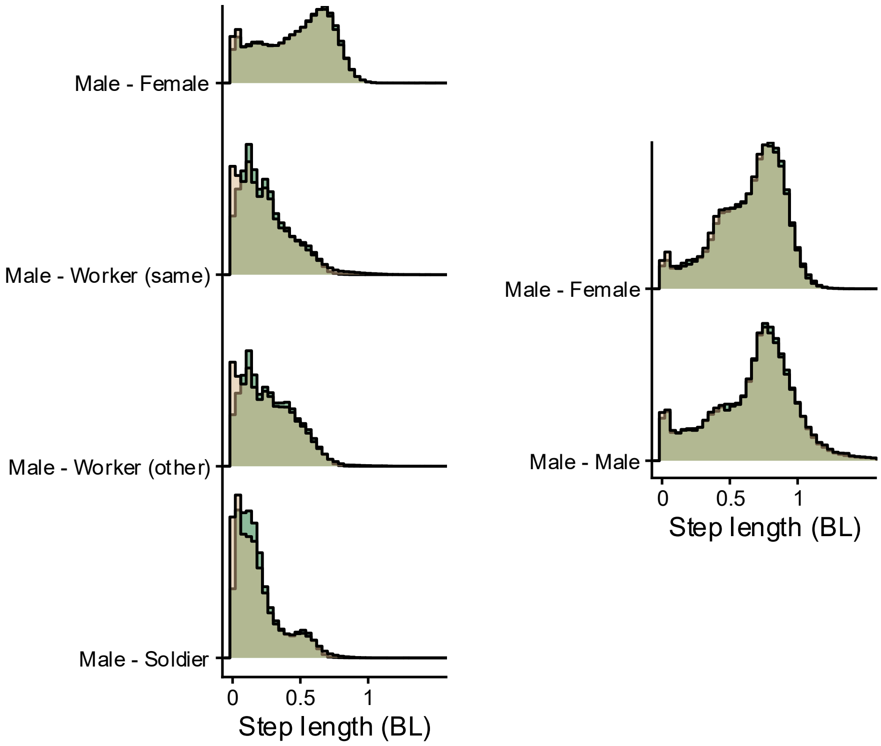

Figure S4. Distribution of the moved distance between frames (0.2 seconds) across different pairing combinations.

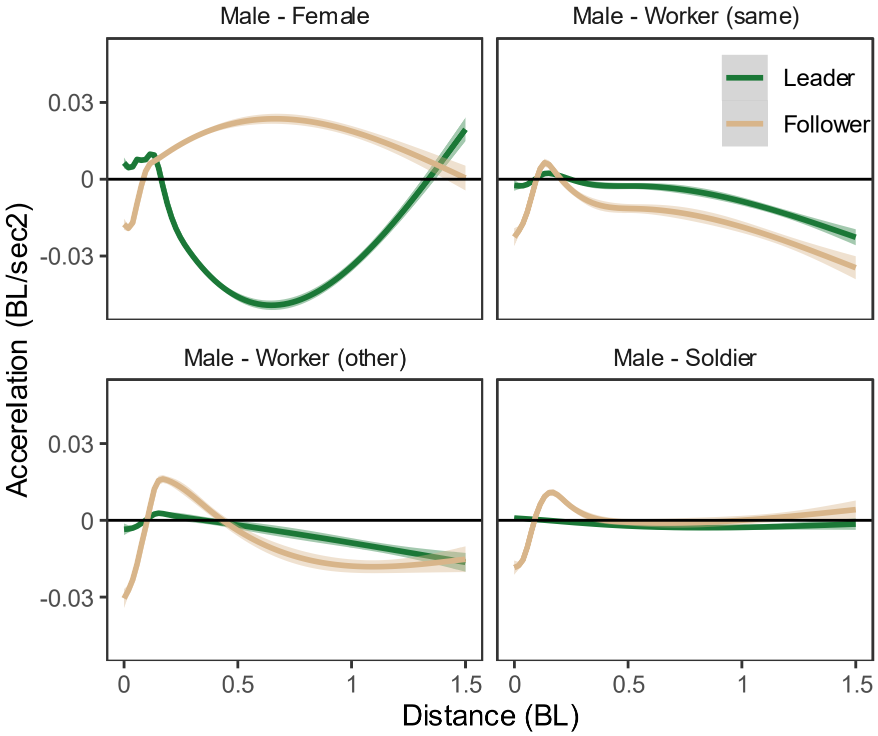

Figure S5. Acceleration of males and partners, relative to leader–follower distance. Lines and ribbons show GAM fits ± 95% CI. GAM was used to capture overall trends despite high variability.

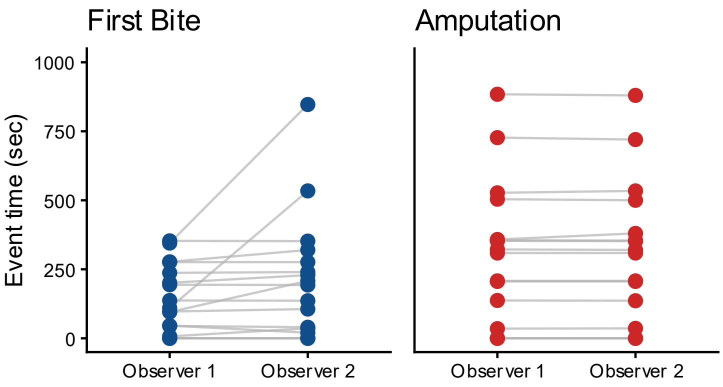

Figure S6. Interobserver variability for the timing of first bite event and amputation bite events.

Legends for videos

Video S1. An example video clip showing a male following a worker in *Reticulitermes amamianus*.

Video S2. An example video clip showing a male following a soldier in *Reticulitermes amamianus*.

Video S3. An example video clip, where a worker attacked a male after a following event in *Reticulitermes amamianus*.

Video S4. An example video clip showing a male following a worker in *Heterotermes aureus*.

Video S5. An example video clip showing an inter-family tandem running between *Pericapritermes nitobei* female (Termitidae, Mirocapritermitinae) and *Reticulitermes spratus* male (Heterotermitidae).