**Wasted efforts impair random search efficiency and reduce the level of choosiness in mate-pairing termites.**

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**Abstract**

Random search theories predict that animals employ movement patterns that optimize encounter rates with target resources. However, animals are not always able to achieve the optimal random search. Energy depletion, for example, limits searchers’ movement activities, forcing them to adjust their behaviors before and after the encounters. Here we quantified the cost of mate search in a termite, *Reticulitermes speratus*, and revealed that the cost reduces the selectivity of mating partners. After a dispersal flight, termites keep searching for a mating partner; otherwise, they die. We found that their movement activity and diffusiveness progressively declined over observational days. Our data-based simulations qualitatively confirmed that this reduced movement diffusiveness decreased the searching efficiency. Also, termites with prolonged search periods had lower colony foundation success and fewer offspring. Thus, mate search imposes doubled costs on termites. Finally, we found that termites with an extended mate search reduced the selectivity of mating partners, where males immediately paired with any encountering females and even frequently exhibited same-sex pairing. Thus, termites dramatically changed their mate search behavior depending on their physiological conditions. It is essential to account for the searchers’ internal states to fill the gap between random search theories and empirical behavioral observations.

**Keywords:** Random walk; Tandem runs; Sexual selection; Social insects; Movement ecology

**Introduction**

Random search strategy. What is the best movement for encounters?

Upon encounters, animals make decision. I’m mate search, there is a mate choice.

Enhancing mating encounter is critical before encounter, while evaluation of mating partner will be involved after encounter. Because movement patterns influence the frequency of mating encounter, it also has a strong influence on the subsequent decision. However, the relationships between moving patterns and mate choice remains totally unknown.

However, animals cannot always achieve the movement patterns

However, in random search theories, indigenous conditions of searching agents have rarely been taken into account. This limits the connectivity between theories of random search and empirical observations of animal searching behaviors.

To fill this gap,

Reticulitermes. Termites are ideal system to study this. During certain season of the year, alates fly to disperse for mating. After dispersal, termites shed their wings and walk to search for mating partners. Termites perform random walk to search for partners and their searching strategy is optimized to entrance encounters according to the conditions. If termites failed in finding partners, this mate search can last over multiple days. Because termites are social animals, being alone incurs a cost. This, extended mate search is expected to change their both searching strategies and mate choice decisions.

Movement patterns. Diffusiveness is important parameters. Pausing is too. All of them can be obtained using servosphere.

In this study

**Results**

**Change of searching activity over time**

**探索時間の経過に伴い、各個体の探索距離は有意に減少した（GLMM, LRT, day: χ2**

**1 = 89.2541, P < 0.0001, 図 9-2）。また性間でも探索距離に有意な違いが見られ、メスの方が長い距離歩行した（GLMM, LRT, sex: χ21 = 4.5546, P = 0.03283, 図 9-2B）。一方で、時間に伴う探索距離の変化の傾向は、性間で違いが見られなかった（GLMM, LRT, sex:day: χ21 = 0.3393, P = 0.56026, 図 9-2B）。**

A screenshot of a computer

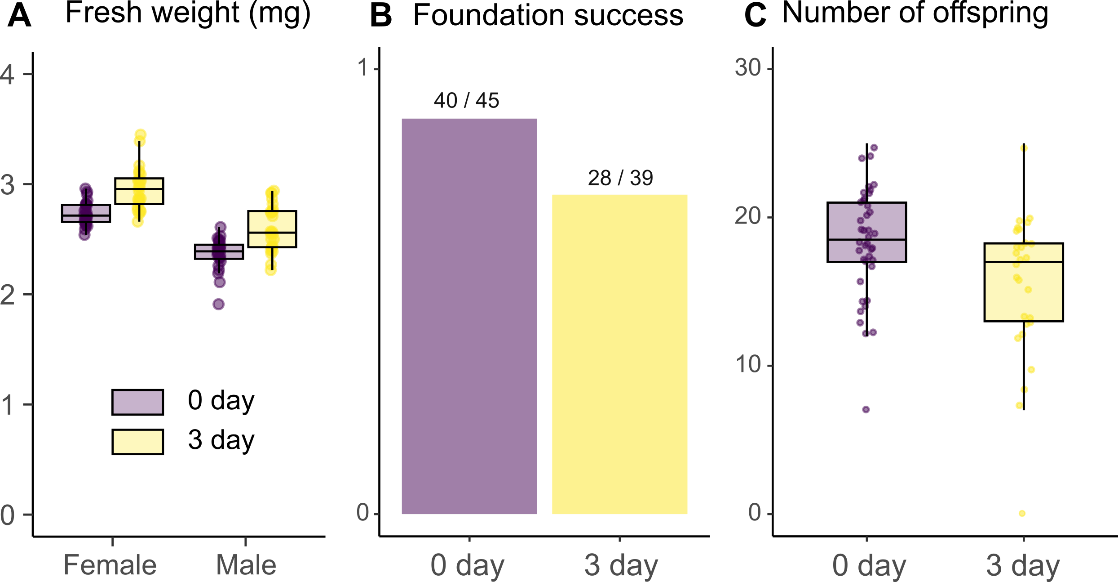
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**Figure 1.** Change of movement patterns according to extended mate search in a termite *Reticulitermes speratus*. (A) Overview of the mating biology and experimental setup. After swarming flights, termites shed their wings and walk to search for a partner. After successful encounter, they form a tandem running pair and found a colony. Mate search usually ends in a day, otherwise it can last until they find a partner. We observed their movement patterns every 24 hours for 30 minutes over 4 days (day 0-3). (B) Representative trajectories of a termite. Each trajectory corresponds to 25 minutes, where a termite moved less and less distances across days. (C-D) Comparison of traveled distances (C) and pausing duration (D) of termites across days after swarming. Bars indicate mean ± s.e. (E) Mean squared displacements (MSD) of the trajectories on the servosphere across days after swarming. Thick lines indicate mean for each day’s observations, where data of females and males were pooled. (F) Simulation results for comparing searching efficiencies of movement patterns observed in termites across days after swarming (*L* = 223.6, φ = 7). The results were obtained from means of 100,000 simulations.

**Fitness cost of extended mate starch**

**We found that extended mating search incurs the cost on colony foundation success. The colony foundation success was significantly reduced in 3 days extended search individuals than fresh individuals（GLMM, LRT, sex: χ21 = 4.0153, P = 0.04509, 図 9-3A). Also the number of offspring is smaller in extended searching individuals（GLMM, LRT, sex: χ2**

**1 = 4.1447, P = 0.04176, 図 9-3B）.**



**Figure 2.** Comparison of colony foundation success between alates just after swarming and after 3 days mate search.

**更に、3 日間探索を経験した個体では、タンデム行動にも変化があった。異性ペアのタンデムの観察数は、群飛直後と比べ、3 日探索後では有意に大きくなった（Wilcoxon Signed rank test, V = 0, P = 0.03125; 図 9-4B）。また 3 個体以上のタンデムの数もまた有意に大きくなった（Wilcoxon signed rank test, V = 0, P = 0.03125; 図 9-4B）。3 個体以上のタンデムはそのほとんどは、メス 1 個体に対しオス 2 個体が追いかけているものであった**

**（84/95; 88.4%）。一方、オス同士やメス同士の同性タンデムの数は、3 日間の探索の前**

**後で変化はなかった（Wilcoxon signed rank test, male: V = 14, P = 0.5625; female: V = 1.5, P**

**= 0.375; 図 9-4B）。これらのタンデムペアの組み合わせは、群飛直後、3 日探索後とも**

**にネストメイトか非ネストメイトかは関係がなかった（Wilcoxon signed rank test, 0 day:**

**V = 10, P = 1; 3 day: V = 8, P = 0.6875）。また群飛直後の個体では、時間の経過に伴って**

**観測されるタンデムの数が増加したが（Spearman’s rank correlation, S = 3.5474, P =**

**0.01489; 図 9-4A）、3 日間探索では増加の傾向はなかった（Spearman’s rank correlation, S**

**= 26, P = 0.6583; 図 9-4A）。**

In total, we observed 166 heterosexual tandem runs (76 with nestmates and 90 with non-

nestmates), 36 male-male tandem runs (seven with nestmates and 29 with non-nestmates), one

female-female tandem run (with non-nestmates), and 16 tandems with >3 individuals. We found no

significant differences in the number of tandem runs between nestmate and non-nestmates in

heterosexual tandems (GLM with Poisson distribution, χ21 = 1.182, P = 0.277), while non-nestmate

tandems were more frequent than nestmate tandems in male-male tandems (GLM with Poisson

distribution, χ21 = 14.439, P < 0.001, effect size: non-nestmate - nestmate = 2.035).

These results show the followings. First, there was no clear inbreeding avoidance in R. speratus

heterosexual pairing. Second, we found more non-nestmate tandems than nestmate tandems in

male-male pairing, and thus our results in the main text (and Fig. S2) may underestimate the

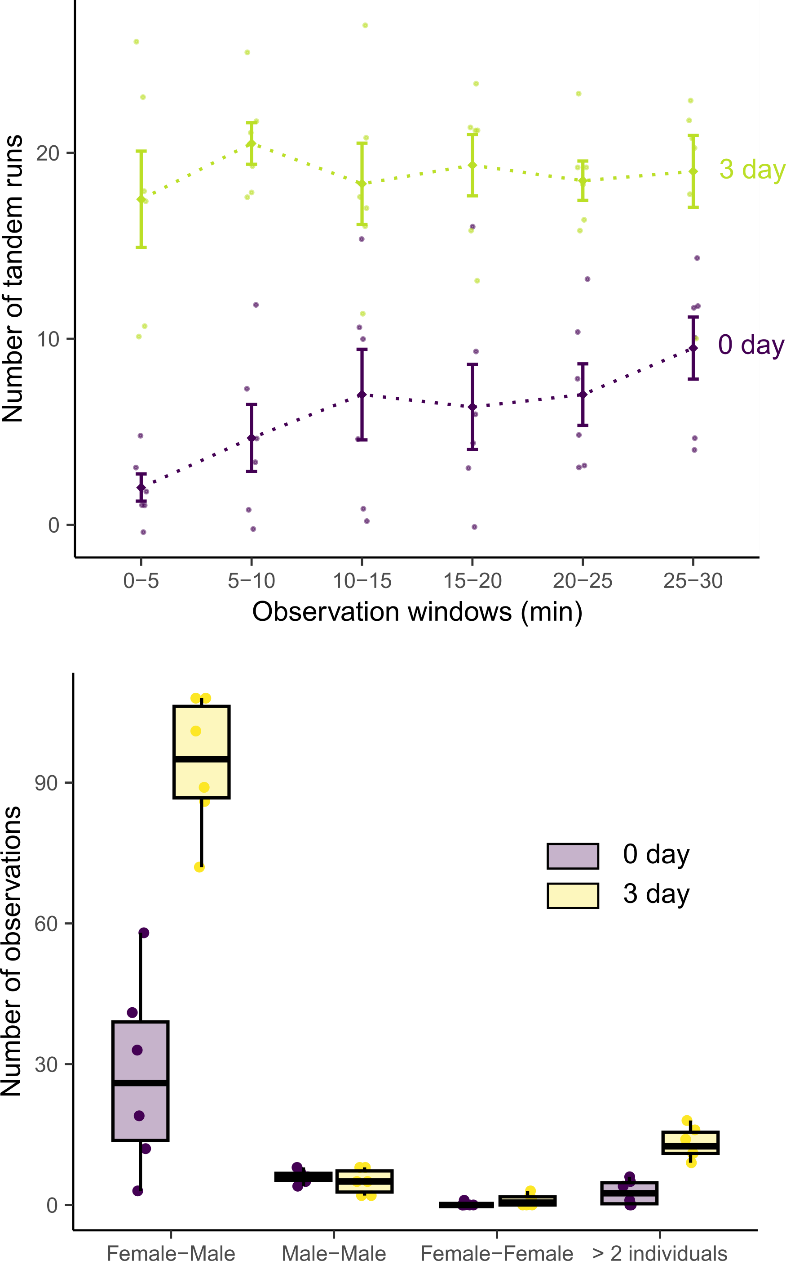
stability of male-male tandem runs. Males of male-male tandems compete over the follower

positions (Video S1), and the larger male generally wins (1). In non-nestmate male-male pairing,

the body size difference between partners is greater than in nestmate male-male pairing.

Differences in body size may facilitate the stability of same-sex tandem runs in males, which should

be confirmed in future studies. Finally, female-female tandem is much rarer than ma

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**Figure 3.**

**Discussion**

本研究で、シロアリが単独で配偶者相手を探す行動には、適応度上のコストが存在

することが明らかとなった。まず探索する時間が長くなれば長くなるほど、歩行する距

離が減少した（図 9-2）。相手がどこにいるか全く分からないランダム探索の状況では

（第 4 章における完全ランダム探索の状況）、可能な限り早く、また直進的に動くこと

が遭遇効率を上昇させる上で重要な要因となる (James et al. 2008)。そのため、シロアリ

が探索する時間が長くなればなるほど、移動能力が下がることは、探索効率の減少につ

ながる。群飛したシロアリは、巣にいたときに蓄えた脂肪の貯蓄のみを用いてその後の

行動を行い、コロニー創設をしワーカーが生じるまでは外からエネルギーを得ることは

ない (Vargo and Husseneder 2009)。そのため、このような移動量の減少は、探索にかか

るエネルギーのコストによって引き起こされた可能性がある (Wickman and Jansson

1997)。さらにこの探索にかかるエネルギーの消費は、その後のコロニー創設の成功に

も大きな影響を与えることも明らかとなった（図 9-3）。このように、通常は社会性で集

団の中に生きるシロアリにとって、単独で他個体を探索するという行動が長引くことは、

大きな適応度上のコストである。

このような探索にかかる適応度上のコストは、配偶者選択に大きな影響を与えた。

アリーナ内でタンデム行動の観察を行ったところ、群飛直後の個体と比べ、3 日間探索

した個体では、多くのタンデム歩行が見られた（図 9-4）。また 2 個体のオスが 1 個体の

メスを巡って争う 3 個体以上のタンデムも多く観察できた（図 9-4）。このような配偶者

選択の変化は、複数回交尾をよく行う種でもみられ、メスによるオスの配偶者選択が行

われる種において、この時メスの残り寿命が短い (Wilson et al. 2010; Wilgers and Hebets

2012)、オスの密度が低い (Alatalo et al. 1988)、繁殖可能な期間が短い (Gotthard et al.

1999)などにより、探索にかかるコストが大きくなる場合には、メスの選り好みが緩和

されることが知られている。本研究では、このような探索コストを被ることに伴う配偶

者選択の緩和が、一夫一妻の種において見られることを示した。これはシロアリの配偶

者選択においても性選択が重要な役割を果たしていることを示唆する。

タンデム行動の観測数の時間変化においても、群飛直後と 3 日間の探索後で大きな

違いが見られた。群飛直後の個体では、観察時間の経過に伴って観測されるタンデムの

数が増加した一方、3 日経過ではそのような増加は見られず、最初からタンデムの観測

数は多かった（図 9-4）。これは次の様に解釈できる。群飛直後のオスは、まだ自身の探

索行動に余裕があるため、複数のメスを評価してから質の高いメスを選択する。一方で、

3 日間探索したオスは、自身の探索行動に余裕がないため、質にかかわらず出会ったメ

スを選択する。というものである。このような現象は、最適停止問題の 1 つであるおみ

あい問題（秘書問題）を適用することで統合的に理解できるだろう。おみあい問題では、

N 人と順番にお見合いするとして、何人目の相手と交際すると最も好みである人と交際

できるかを考える問題である (Ferguson 1989)。この解は k = n/e 人目までを無条件で断

り、それ以降で一番条件の良いパートナーで選ぶものである。群飛直後は、これからま

だ多くの相手と出会うことがあできると予測されるまで、k が大きな値となり、初期に

はタンデムを行わず相手を評価するだけである。そして、出会った個体の数が増えてく

ると、タンデムを行うことを決め始める個体が出てくるため、時間の経過に応じてタン

デムの数は増加する。一方で、3 日間他個体と出会うことができなかった個体にとって

は、k はかなり小さい値となる。そのため、無条件で断ることがなくなり、初期段階か

ら多くのタンデム行動がみられる。このようにシロアリは、配偶者探索における

Sequential choice model の実証に適した材料である (Real 1990; Real 1991)。

探索効率の最適化について、空間的、時間的な最適戦略を探る研究では、本章で検

討したような個体の状態が考慮されることはほとんどない。本研究で示した、探索活動

を行うことそのものが、活動量やその後の配偶者選択にも影響を及ぼすという視点を組

み合わせると、生物の移動パターンの進化についてより深い議論が可能になるだろう

Cross species variation. Some species should be less choosy. While others can be somewhat choosy. However, everything could be secondary because most important is to make a pair. This, it is surprising that termites might refrain pairing with a partner for the first several minutes. This indicates choosing better partners could provide enough strong advantages for colony foundation success. Not that several studies show that termites with some characters are strong candidate of mating pairs, larger body size, without leaking body parts, microbial community, heterozygosity, and so on. However, it is questionable how termites can distinguish these partners, given that they even do not care the species of their partners. Their interindividual interactions are mostly limited in touching with antenna. Passive selection, e.g., strong candidate are better at tandem run, may be a cause of assistive mating, rather than active mate choice.

**Material and Methods**

*Termite*

We collected *R. speratus* alates with a piece of nesting wood from seven colonies in Kyoto in May 2016 and 2017. May is the swarming season of this species in Kyoto. To control flight timing, all nesting wood pieces were maintained at 20°C until experiments. Before each experiment, we transferred nests to a room at 25°C, which promoted alates to emerge and fly. Alates were then collected and separated by sex. We used individuals who shed their wings by themselves. We stayed all experiments within 24 hours after the swarming flight.

*Change of searching activity over time*

We investigated how searching activities change according to time after swarming flight using three colonies collected in 2016. We investigated the search activity of nine females and nine males (three from three colonies). Each individual was placed on an omnidirectional servosphere (Nagaya et al., 2017) and freely walked on an infinite two-dimensional surface for 30 minutes. The observation was performed four times every 24 hours, i.e., just after swarming (= day 0), day 1, day 2, and day 3. Termites were individually maintained in a Petri dish (φ = 90 mm) with a moistened piece of filter paper (a quarter of 70 mm). The bottom of the dish was polished so that termites could walk smoothly on the dish. We maintained each termite under the light condition of 16L8D and at 25°C during the interval of each observation. The observations were performed during the time of light conditions.

Because the sampling rates of data acquisition were not constant in our servosphere, we smoothed the coordinates data with a median filter (k =5) and interpolated them to obtain the coordinates every 0.2 seconds (5Hz). We removed data for the first five minutes and used the rest for 25 minutes for further analysis.

First, we obtained the moved distance every 0.2 seconds (steplength). By summing up these steplengths, we calculated the total distances termites walked during 25 minutes. Also, we examinied pausing duration during this period. In *Reticulitermes speratus*, the previous study estimated that the threshold for moving and pausing was 0.7mm (Mizumoto and Dobata, 2019); termites are regarded as moving if they moved more than 0.7mm in 0.2 seconds, while they are pausing if less than 0.7mm. We measured the total pausing duration during 25 minutes observations. Then, we investigated the effect of time after swarming on walking distracts and pausing durations, using linear mixed models (LLM) that includes time after swarming (0, 1, 2, and 3 days after swarming), sex, and their interactions as fixed effects, and the original colony as the random effect (random intercept). The statistical significance of each variable was tested using the chi-square test (type II ANOVA), herein, and the following statistical analysis.

Second, we evaluated the diffusive properties of individual movements. High diffusiveness is critical for the efficiency of random search when the targets are randomly distributed and searchers do not have any prior information on tagets (James et al., 2008; Mizumoto et al., 2017a), which is relevant for termite mate search before encounters (Fig. 1A) (Mizumoto and Dobata, 2019). We computed the mean squared displacements (MSD) to compare the diffusive properties of individual movements across time after swarming. The MSD is defined as the mean of squared distance that an organism travels from its starting location to another point during a given time, τ. We obtained MSD in the rang of 0.2 < τ < 1500, using the function *computeMSD*() in the package “flowcatchR”. To compare the MSD between time after swarming, we used a LMM, where τ, time after swarming, sex, and their interactions were included as fixed effects, and individual ids nested within original colonies were included as a random effect (random intercept). MSD and τ were log transformed before the LMM fitting.

*Evaluation of searching efficiency*

Because termites moved less distances with less diffusive properties and a lot of pauses (Fig. 1B-E), searching efficiency is expected to reduce according to extended mate search. To quantify the searching efficiency, we used a data-based simulation approach. We projected trajectories of a female and a male after randomization and calculated if and when they encountered. When mate search of termites extended to multiple days, they synchronize their search efforts with the following swarming events (Mizumoto et al., 2017b). Given that most termites do not extend mate search for multiple days due to pairing or predation (Mizumoto et al., 2016), we expect that mating encounters of extended mate searchers are usually with new mate searchers (day 0 individuals). Thus, we investigated the encounter efficiency in the combinations of 0 day-0 day, 0 day-1 day, 0 day-2 day, and 0 day-3 day trajectories. We picked up one trajectory with 5 FPS of a female and a male and place them at a random location in a periodic boundary condition of size = *L* × *L*. Each trajectory was horizontally and vertically reversed with the probability of 1/2. Following inversion, we rotated the trajectory at random degrees from 0 to 360 around the starting point of the trajectory. After projecting two trajectories, we estimated if and when these two individuals encountered. We regarded they encountered when two are within the distance φ at the same time. We performed this process 100,000 times for eight cominations (0 day-0 day, 0 day-1 day, 0 day-2 day, and 0 day-3 day, for female-male and male-female). The parameter φ value was set as 7 mm, based on the body size of *R. speratus* (Mizumoto and Dobata, 2019), and *L* as 223.6, based on the previous studies (Kusaka and Matsuura, 2017; Mizumoto and Dobata, 2019).

*Fitness cost of extended mate starch*

We investigated the cost of extended mate search using four colonies collected in 2017. We prepared termite females and males that were isolated for 72 hours in a Petri dish described above. After 72 hours of isolated mate search, these termites were paired with each other. Similarly, we also prepared pairs of termites just after swarming. Each pair was introduced to a Petri dish (40 mm) filed with mixed sawdust in a depth of 5mm (more explanation on mixed sawdust). Termites excavated into the sawdust to establish their nests. All pairs were produced using nestmate. We prepared 12 pairs for each condition in colony A, B, And C, and 9 pairs for colony D. As several individuals were dead during 72 hours isolated mate search, we had 45 pairs for just after swarming and 39 pairs after 72 hours of matter search, in total.

All dish were maintained at 25℃ in dark condition for 60 days. After 60 days, we opened all dish and counted the number of individuals for each caste separately (female, male, larvae, and eggs). We defined that the pair succeed in colony foundation only when both female and male were surviving. We compared colony foundation success between just after swarming and 72 hours after swarming, using a generalised linear mixed model (GLMM) with binomial distribution and logic link function, in which termite condition was included as fixed effect, while original colony was included as random effect (random intercept).

Next, among pairs that succeeded in colony foundation, we compared the number of eggs and larvae between termite conditions. We used a GLMM with Poisson distribution.

The effect of extended searching on mate choice

We investigated how extended mate search affect the pairing dynamics, using colonies collected in 2016. After swarming, 10 females and 10 males of dealate were selected from two different colonies. These 40 individuals were paint marked with one colored on the abdomen for sex and colony identification (PX-20; Mitsubishi). All 40 individuals were maintained for 30 min (just after swarming) or 72 hours (extended mate search) separately in a 24 well plate before the observation. The results of 30 minutes treatment are reported in Mizumoto et al. 2022. We introduced 40 individuals to the experimental arena (ø=600mm). The experimental arena consisted of a Styrofoam board (600 x 600 mm) and a circular plastic tube (ø=600mm, height = 100mm). After being introduced in the arena, we observed termite movements for 30 minutes within a part of the experimental arena (200 x 100 mm) located at the edge of the circular arena. We did so because most individuals walked along the edge of the arena, repeatedly passing across the observational arena. We counted the number of individuals passing across the observational area with their status (single individuals, heterosexual tandems, same-sex tandems, tandems with >3 individuals). We performed the experiments six times with different colony combinations (SA-SB, SA-SC, SB-SC, SD-SE, SD-SF, SE-SF) for each treatment. The experimental arena was cleaned with 70% ethanol and distilled water before each experiment.

We compared the number of observation of pairing units, using Wilcoxon signed rank test. Also, we compared the number of observation of nestmate pairs and non-nest mate pairs. Finally, we investigated the time development of observed tandem running pairs. We binned our 30 minutes observation into 0-5 minutes, 5-10 minutes, …, 25-30 minutes and counted the number of observed tandem pairs during each time window. Then, we used spearman’s rank relation test to examine the time developments of the number of observed tandem running pairs.

All analysis were performed by R v4.3.1 with libraries of “exactRankTests”、 “lme4”、”car”.

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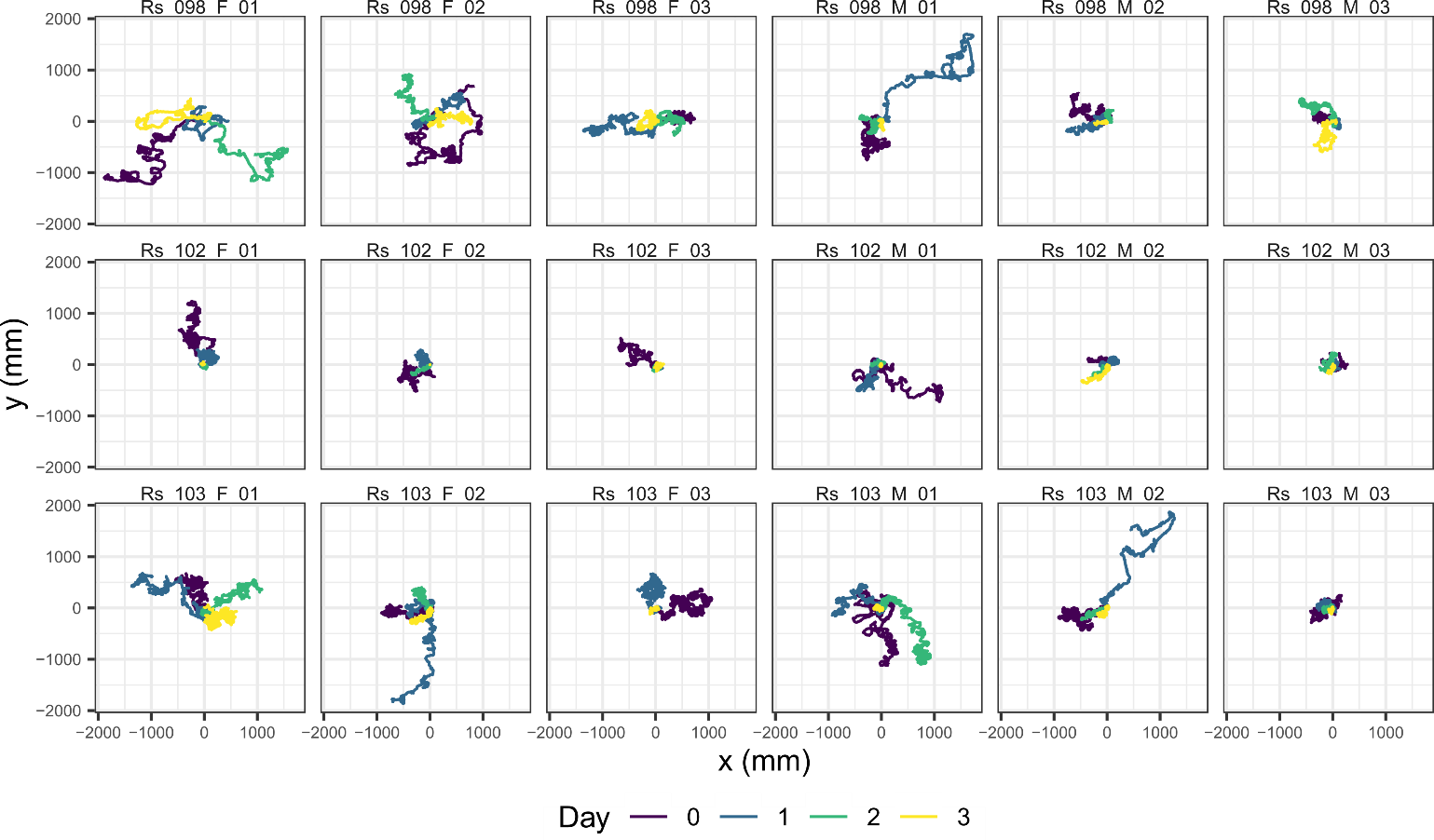
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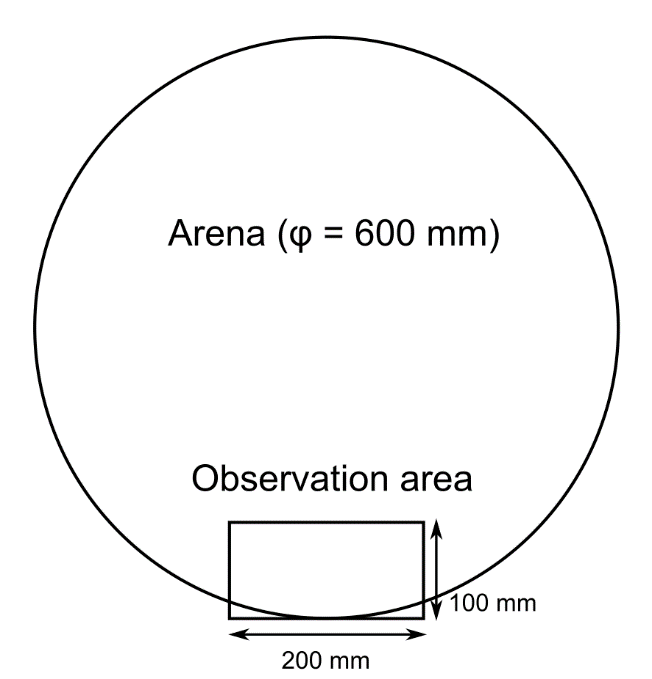
**Supplemental materials**

**Figure S1**. Trajectories on the servosphere of all observed individuals.

**Figure S2.** Observational arena for tandem pairing.



**Figure S1**. Trajectories on the servosphere of all observed individuals. Each label indicates Species name (Rs = *Reticulitermes speratus*) + Colony name + Sex + Replicates.



**Figure S2.** Observational arena for tandem pairing. A video camera was located above the observation area on a tripod. Termites were released at the center of the arena, and those who crossed the observation area were observed.