

1 **Coordination of movement via complementary interactions of leaders and followers in**
2 **termite mating pairs.**

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9

10 **Abstract**

11 In collective animal motion, coordination is often achieved by feedback between leaders and followers. For
12 stable coordination, a leader's signals and a follower's responses are hypothesized to be attuned to each
13 other. However, their roles are difficult to disentangle in species with highly coordinated movements,
14 hiding potential diversity of behavioral mechanisms for collective behavior. Here we show that two
15 *Coptotermes* termite species achieve a similar level of coordination via distinct sets of complementary
16 leader-follower interactions. Even though *C. gestroi* females produce less pheromone than *C. formosanus*,
17 tandem runs of both species were stable. Heterospecific pairs with *C. gestroi* males were also stable, but
18 not those with *C. formosanus* males. We attributed this to the males' adaptation to the conspecific females;
19 *C. gestroi* males have a unique capacity to follow females with small amounts of pheromone, while *C.*
20 *formosanus* males reject *C. gestroi* females as unsuitable but are competitive over females with large
21 amounts of pheromone. An information-theoretic analysis supported this conclusion by detecting
22 information flow from female to male only in stable tandems. Our study highlights cryptic interspecific
23 variation in movement coordination, a source of novelty for the evolution of social interactions.

24

25 **Introduction**

26 Animals often move as a group while searching for a safe place or feeding site. Coordinated group
27 movements are achieved by rules for interactions among group members, with individuals often playing
28 different roles [1,2]. One or a few individuals initiate movement, and other members follow the leader [3,4].
29 Such leadership strongly affects the collective outcome of group movements [5]. When a pair of individuals
30 explore the environment together, a leader-follower relationship is almost inevitable; the first to move is
31 the leader, and the other has no option but to follow [6]. Thus, many studies on pairs have focused on how
32 partners respond to each other to control movement speed and turning angle [7–11]. As successful
33 coordination results from social feedback, innate behavioral differences between partners can promote or
34 hinder coordination [12]. Especially if the pair shares a common goal, leader phenotypes should
35 complement follower phenotypes to maintain stable coordination, resulting in a species-specific manner
36 of social interaction.

37 Tandem running in termites is among the simplest leader-follower relationships. Unlike ants, where
38 tandem runs recruit colony members to specific resource locations [13,14], termite mating pairs perform
39 tandems after dispersal, while seeking sites for colony foundation [15]. The female leads the tandem and
40 releases a short-range sex pheromone to guide the male [16,17], and the male touches the female's
41 abdomen with its antennae and mouthparts, indicating its continued presence [15,18]. As the sex
42 pheromone varies among species [16], female behavior can also vary in order to transmit species-specific
43 signals efficiently [19]. We predict that males coevolved species-specific following capacity to form stable
44 tandems with conspecific females.

45 To disentangle the contributions of leaders and followers to behavioral coordination, we made
46 heterospecific pairings between related species, *Coptotermes gestroi* (Wasmann) and *C. formosanus*
47 (Shiraki). These two termites evolved in allopatry in the course of 18 million years of evolution [20], but
48 both are now invasive and found in sympatry in some coastal cities, including Taiwan, Hainan, and
49 Southeast Florida [21–23]. In south Florida, USA, heterospecific mating events have been observed,
50 resulting in hybrid colonies [24]. Heterospecific pairing can occur because the species share the same
51 pairing (sex) pheromone, (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol, emitted from tergal glands at the tip of the
52 abdomen of females [25] (Fig. 1A). The main difference is the quantity of pheromone, and thus the strength
53 of the transmitted signal; *C. formosanus* females produce ~10x more pheromone than *C. gestroi* females
54 [25]. Based on this difference, we hypothesized that males of these species evolved different tandem
55 following capacities matched to their conspecific female's signal strength.

56 Here we study the heterospecific tandem runs of *C. formosanus* and *C. gestroi*. We show that *C.*
57 *formosanus* males do not maintain heterospecific tandem runs because they only follow females that
58 release large amounts of sex pheromone, while *C. gestroi* males are accustomed to small quantities and are
59 not challenged in following the larger amount released by *C. formosanus* females. During this process,
60 males of *C. formosanus* may have difficulty detecting small amounts of pheromone, or they may instead
61 lack the motivation to follow weak pheromone signals. Thus, we next investigate their motivation by
62 observing males' responses when they become separated from their leader. After separation, the female
63 pauses while the male engages in active local search, and this dimorphism enhances re-encounter rates
64 [19]. By moving slowly just after separation, males increase their re-encounter rate with the same partner.
65 By instead moving quickly, they can more efficiently search for a new partner [26]. The former tactic is
66 often used in lower density conditions, while the latter is used in higher density conditions where the
67 availability of alternative mates reduces the relative value of a separated partner [26]. Thus, the male's
68 movement speed right after separation can reflect a male's evaluation of the female; slow movement
69 indicates a relatively positive evaluation of the separated female and preference to reunite; fast movement
70 means a lower rating and a preference for finding a new partner. Finally, we explore the outcome of
71 competition between *C. formosanus* male and *C. gestroi* male over *C. formosanus* female, as *C. formosanus*
72 females are attractive to males of both species. Combined, we show that partner selection has shaped the
73 species-specific association of termite mating pairs.

74 **Materials and Methods**

75 *Termites and experimental arena*

76 We collected alates of *C. formosanus* and *C. gestroi* using a light-trapping system at dusk between
77 Apr 18th and 20th in 2020 in Broward County (Florida, USA) during synchronized dispersal flights. All
78 alates were collected at a single site. We brought the alates to the laboratory and maintained them on wet
79 cardboard at 28°C. We used individuals who shed their wings by themselves and observed their behavior
80 within 12 hours after the flight. Each individual was used only once.

81 We performed all observations in an experimental arena made by filling a petri dish ($\varnothing=140\text{mm}$) with
82 moistened plaster. The petri dish had a clear lid during observations. A video camera above the arena was
83 adjusted so that the arena filled the camera frame. We extracted the coordinates of termite movements
84 from all obtained video, using the video-tracking system UMATracker [27]. All data analyses were
85 performed using R v4.0.1 [28].

86

87 *Comparing tandem run stability across different pair combinations*

88 To explore interspecies differences in tandem running behavior, we introduced one female and one
89 male to the experimental arena and recorded their behavior for 30 minutes. We tested four different
90 species combinations: conspecific pairs of *C. formosanus* (Cf-Cf), conspecific pairs of *C. gestroi* (Cg-Cg),
91 heterospecific pairs of female *C. gestroi* and male *C. formosanus* (Cg-Cf), and heterospecific pairs of female
92 *C. formosanus* and male *C. gestroi* (Cf-Cg). We prepared ten replicates for each combination.

93 During observations, termite pairs were in one of three states: (i) tandem running, (ii) interacting
94 but not tandem running, and (iii) searching (two are in a distance). Following a previous study [26], we
95 classified the pairing states based on the coordination of a female and a male. We defined them as
96 interacting (or tandem running) when the distance between their centroids was less than $1.3 \times$ mean body
97 length. This value was 11.57 mm for *C. formosanus*, 9.75 mm for *C. gestroi*, and 10.65 mm for heterospecific
98 pairs, respectively. We selected this distance to slightly exceed the average body length because termites
99 in a tandem run are nearly in physical contact [19]. An interacting pair was considered to be performing a
100 tandem run only if they met the following criteria [26]. First, the interaction needed to last for more than
101 5 seconds; a very short separation (< 2 seconds) was not regarded as a separation event unless the distance
102 between individuals was greater than 20 mm. Second, both termites needed to move more than 30 mm
103 while interacting. After separation, we considered that individuals engage in separation search until they
104 interact with an individual again for more than 1 second. We down-sampled all videos to a rate of five
105 frames per second (= every 0.2002 sec) for this analysis.

106 We obtained 103, 110, 120, and 132 tandem run events for Cf-Cf, Cg-Cg, Cg-Cf, and Cf-Cg,
107 respectively. We compared tandem duration between the two conspecific pairs and between heterospecific
108 and conspecific pairs for each male species. We used the mixed-effects Cox model (coxme() function in the
109 coxme package in R [29]), with female species as a fixed effect and video id as a random effect. The random
110 effect accounted for the inclusion of multiple tandem events for each pair of termites. The likelihood ratio

111 test was used to determine the statistical significance of each explanatory variable (type II test).
112 Observations interrupted by the end of the video were treated as censored data. We also fitted a variety of
113 distributions to the tandem survival curves, including exponential, Weibull, gamma, and power-law
114 distribution. After calculating the maximum log-likelihood for each model, we determined the best fitting
115 model using Akaike's Information Criterion (AIC). For model fitting, we removed censored data.

116

117 *Moving speed during tandem runs*

118 We compared moving speed during tandem runs across different pair combinations to further
119 explore the role of females and males for heterospecific behavioral coordination. We first calculated the
120 moving step length between two successive frames at 5FPS. The step length distribution was bimodal, with
121 two peaks around 0 and 3mm (Fig. S2). The two peaks can be regarded as representing pauses and moves,
122 respectively. Based on the histogram of each pairing combination using 0.1mm bins, we obtained the value
123 representing the second peak of moving speed (Fig. S2). Then, we defined thresholds to distinguish
124 movements from pauses by multiplying the value of the 2nd peak by a factor of 0.2 (Cf-Cf: 3.4mm, Cg-Cg
125 2.9mm, Cf-Cg: 3.5mm, and Cg-Cf: 2.9mm) [19]. A pause was defined as a step length shorter than or equal
126 to the threshold. By removing data for pause durations, we obtained a dataset only including moving speed.
127 Finally, we used a linear mixed model to analyze moving speed, where the species of female and male were
128 included as fixed effects and video id as a random effect. The likelihood ratio test was used to determine
129 the statistical significance of each explanatory variable (type II test). Note that, although we present
130 results applying species-specific thresholds, we reached the same conclusions when we used one identical
131 threshold (=2.9mm, obtained from the histogram of a pooled dataset).

132

133 *Information transfer between females and males*

134 We used transfer entropy to quantify the degree to which the female leader's motion predicts that
135 of the male follower, a measure of coupling strength within the pair. Transfer entropy quantifies how well
136 knowledge of present behavior of the sender reduces uncertainty about the future of behavior of the
137 receiver, after taking account of the receiver's history [30,31]. This value can be determined for both
138 directions, with the difference giving a measure of the net direction and amount of information flow. We
139 calculated transfer entropy by coarse-graining their movement trajectories into a sequence of discrete
140 behaviors. During tandem runs, the female explores the environment to look for a potential nest site with
141 the following male [15]. In a random search, both move/pause patterns and turning patterns link to search
142 efficiency [32]. We discretized trajectories of each runner to obtain time-series describing the pausing and
143 rotation pattern [9]. The behavior of each runner was classified into three states: pause (P), motion with
144 clockwise rotation (M-CW), and motion with counterclockwise rotation (M-CCW). The pause state was
145 distinguished from others using the threshold obtained in the moving speed analysis. As this threshold
146 was computed on the basis of data sampled at 5FPS (sampling period = 0.2002s), we simply rescaled this
147 threshold by the ratio of sampling periods to obtain that for other sampling periods. If the step length

148 between successive frames was shorter than the threshold, the state of the frame was recorded as a pause
 149 P. Otherwise, the state was either M-CW or M-CCW depending on the direction of motion computed as
 150 the cross product of movement vectors between successive time steps. If no rotation was detected (i.e.,
 151 cross-product equal to 0), the rotation direction was copied from the previous time step.

152 We employed transfer entropy to investigate the coupling between female leaders and male
 153 followers during tandem runs (refer to [9] for a detailed description of this methodology). Transfer entropy
 154 is an information-theoretic measure that quantifies the predictive power given by knowledge of the
 155 present state of an individual about the future state of a different individual. In other words, it measures
 156 causal interactions between a sender and a receiver in terms of Granger causality [33]. If L and F are
 157 behavioral sequences representing the leading female and the following male, then transfer entropy from
 158 L to F is defined as

$$159 \quad T_{L \rightarrow F} = \sum_{f_{i+1}, f_i^{(k)}, l_i} p(f_{i+1}, f_i^{(k)} | l_i) \log_2 \frac{p(f_{i+1} | f_i^{(k)}, l_i)}{p(f_{i+1} | f_i^{(k)})},$$

160 where l_i is the value of sequence L at time i , f_{i+1} is the value of sequence F at time $i+1$, and $f_i^{(k)}$ is the k -
 161 history of F at time i (i.e., the last k states in the sequence). As range of values of transfer entropy is
 162 determined by the encoding of states in the sequences, we can normalize its value to obtain a coupling
 163 measure in the range of $[0;1]$ by dividing it for its maximum [9,34]. Normalized transfer entropy indicates
 164 the proportion of the follower's future behavior that is predicted by the leader's present behavior: it is 1
 165 when the follower behavior is entirely determined by the behavior of the leader, and 0 when the two are
 166 independent from each other. Furthermore, transfer entropy can be computed in both directions, from
 167 leader to follower and from follower to leader. By comparing these values, $T_{L \rightarrow F}$ and $T_{F \rightarrow L}$, we can obtain
 168 the predominant direction of information flow. The difference in transfer entropy between the two
 169 directions, $T_{L \rightarrow F} - T_{F \rightarrow L}$, is called net transfer entropy [9,34]. The value is positive when information flow
 170 from leader to follower is predominant ($T_{L \rightarrow F} > T_{F \rightarrow L}$) and negative when flow from follower to leader
 171 ($T_{L \rightarrow F} < T_{F \rightarrow L}$) predominates.

172 To test that our results were significant, and not an artifact of finite sample size, we artificially
 173 created surrogate datasets by pairing time series obtained from leaders and followers, not tandem running
 174 together; then, we computed transfer entropy for these datasets and compared it with the experimental
 175 results [9,34]. To produce a surrogate dataset, we paired randomly selected leaders and followers belonging
 176 to different tandem runs. Although females and males from different tandems are still influenced by the
 177 same environmental cues of the experimental arena, this randomization process ensures that there are no
 178 causal interactions within the surrogate pair. For each pairing combination and parameter configuration,
 179 we repeated the randomization process and obtained 100 surrogate datasets. We used these to estimate
 180 the mean and standard error of transfer entropy for surrogate datasets with the same size of the
 181 experimental ones. Finally, measurements of transfer entropy for the experimental data were discounted
 182 by a correction factor given by the mean values estimated from surrogate datasets to account for their
 183 finite sample sizes.

184 Our information-theoretic analysis depended on setting the values of two parameters: the sampling
185 period of continuous spatial trajectories and the history length of transfer entropy, k . The optimal choice
186 of these parameters varies for different pairing combinations and focal behavioral patterns due to
187 behavioral, morphological, and cognitive differences manifesting at different time scales [9]. To find good
188 parameterizations, we computed net transfer entropy for 900 different parameter configurations for each
189 species (history length $k \in \{1, \dots, 20\}$ and sampling period $\{0.0334s, \dots, 1.5015s\}$). The resulting landscapes
190 of net transfer entropy show robustness to different parameter values over most of the tested range (Fig.
191 S3). We selected the parameter configurations that maximize the net transfer of information (Table S1).

192 For the chosen parameter configurations, we performed two statistical tests. First, we tested if the
193 experimental data showed significantly greater values of transfer entropy with respect to the surrogate
194 data. We used one-sided two-sample Wilcoxon rank-sum tests with continuity correction. Second, we
195 tested differences in the flows of information between the two possible directions (from leaders to
196 followers and from followers to leaders) to determine which among the leader and the follower was the
197 predominant source of information. We used one-sided paired Wilcoxon signed-rank tests with continuity
198 correction. All information-theoretic measures were computed using the `rinform-1.0.1` package for R [35].

199

200 *Moving speed after separation*

201 When termites in a pair are accidentally separated, females pause while males move to enhance the
202 chances to reunite [19]. As moving speed is related to reunion efficiency [26], we measured the change in
203 movement speed, focusing on a time window around separation events. We compared movement speed
204 between the last 2 seconds before separation and the first 2 seconds after separation. For each separation
205 event, we measured the mean movement speed for both time windows. Then, we used linear mixed models
206 (LMM), with the time window treated as a fixed effect and video ID included as a random effect. The model
207 was fit for each combination of pairs. The likelihood ratio test was used to determine the statistical
208 significance of each explanatory variable (type II test). Finally, we examined if re-encounter after
209 separation resulted in a tandem run or not.

210

211 *Interspecific competition over a female*

212 Because males of both species show stable tandem runs with *C. formosanus* females, we introduced
213 one female *C. formosanus*, one male *C. formosanus*, and one male *C. gestroi* to the experimental arena to
214 study interspecific competitions. We prepared 13 replicates and recorded their behavior for 30 minutes.
215 Tandem runs were identified using the method described above. By doing so, we obtained the time series
216 of states observed among three individuals. There were four different states: (i) no tandem run is observed,
217 (ii) tandem run between female *C. formosanus* and male *C. formosanus*, (iii) tandem run between female *C.*
218 *formosanus* and male *C. gestroi*, (iv) tandem run involving three individuals. We counted tandem runs of
219 three individuals when both males were concurrently interacting with the female. When three individuals
220 were in a straight line, we regarded it as a tandem run of heading female and the male just after her. Then,

221 we counted the number of transitions from one state to another. Usually, state (i) can transit to (ii) or (iii),
 222 state (ii) or (iii) to (i) or (iv), state (iv) to (ii) or (iii) (Fig. 4A). Then we compared the tendency of state
 223 transition using binomial tests. We also checked if there is a different state transition trend from (ii) or
 224 (iii), using Fisher's exact test.

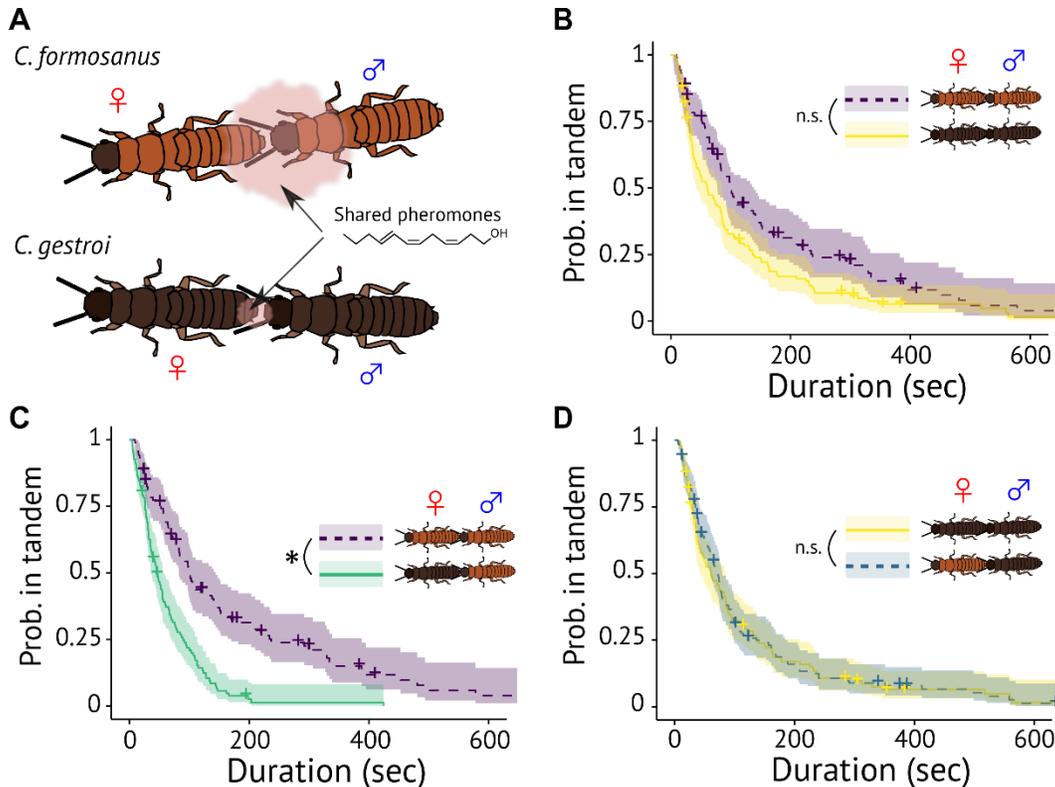
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226 Results

227 Comparison of movement coordination across pairs

228 Despite the relatively small quantity of sex pheromone involved, *C. gestroi* conspecific tandem runs
 229 lasted as long as those of *C. formosanus* (mixed-effects Cox model, $\chi^2_1 = 0.942$, $P = 0.332$, Figure 1B). As for
 230 heterospecific tandem runs, their durations were asymmetric. When the male was *C. gestroi*, heterospecific
 231 tandems lasted as long as conspecific ones (mixed-effects Cox model, $\chi^2_1 = 0.01$, $P = 0.91$, Figure 1D). When
 232 the male was *C. formosanus*, heterospecific tandems ended sooner than conspecific ones (mixed-effects
 233 Cox model, $\chi^2_1 = 19.52$, $P < 0.001$, Figure 1C). Thus, tandem runs were unstable only for the combination
 234 of a *C. gestroi* female and a *C. formosanus* male, as predicted in [24,25]. For the stable combinations, tandem
 235 breakups appeared to happen accidentally, as their durations were best fit by exponential distributions
 236 (i.e., a smaller AIC comparing to gamma, Weibull, and power-law distributions). On the other hand,
 237 tandem durations of the unstable combination of a *C. gestroi* female and a *C. formosanus* male followed a
 238 gamma distribution.

239



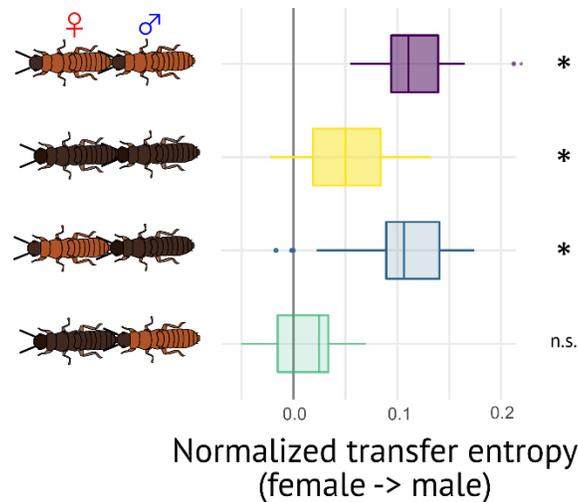
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241 **Figure 1.** Interspecific variation of tandem running in *Coptotermes* termites. (A) During tandem
242 runs, the female leader releases a short-range pheromone that attracts the male follower. The
243 chemical is shared between *C. formosanus* and *C. gestroi*, but the quantity is much larger in *C.*
244 *formosanus*. (B-D) Comparison of the duration of tandem running until separation across different
245 combinations. Kaplan-Meier survival curves were generated for each pairing combination. *
246 indicates significant difference (mixed effect Cox model, $P < 0.05$). + indicates censored data due
247 to the end of observations. Shaded regions show 95% confidence intervals.

248
249 Moving speeds of females and males were highly correlated across all pairing combinations (Fig. S1).
250 However, modal moving speed was higher for conspecific *C. formosanus* pairs (17.0 mm/sec) than for
251 conspecific *C. gestroi* pairs (14.5 mm/sec, Fig. S2). Thus, for heterospecific pairs to synchronize their
252 movement, one or both partners need to adjust their speed. We found evidence that males make this speed
253 adjustment; the modal speed of heterospecific pairs was similar to that of the female's conspecific tandem
254 runs (*C. formosanus* female-*C. gestroi* male: 17.5mm/sec, *C. gestroi* female-*C. formosanus* male: 14.5mm/sec,
255 Fig. S2). Also, across all tandem runs, speed depended on the female species (LMM; female moving speed,
256 female species: $\chi^2_1 = 14.888$, $P < 0.001$, male species: $\chi^2_1 = 2.0802$, $P = 0.1492$; male moving speed, female
257 species: $\chi^2_1 = 12.2442$, $P < 0.001$, male species: $\chi^2_1 = 1.5145$, $P = 0.21845$).

258 The asymmetry between heterospecific pairings was further supported by an information-theoretic
259 analysis. The future behavior of males was significantly predicted by the present behavior of females in all
260 stable combinations—that is, conspecific pairs or heterospecific pairs of *C. formosanus* females and *C.*
261 *gestroi* males (Wilcoxon rank-sum test, $P < 0.001$) (Fig. 2, Table S2). In these pairs, information flow from
262 females to males was significantly stronger than in the opposite direction (Wilcoxon signed-rank test, $P <$
263 0.05) (Table S2). However, in the unstable heterospecific pairs of *C. gestroi* females and *C. formosanus* males,
264 neither female nor male behavior was significantly predicted by their partner's behavior (Wilcoxon rank-
265 sum test, $P > 0.05$); thus, there was no predominant direction of information flow (Wilcoxon signed-rank
266 test, $P > 0.05$) (Fig. 2, Table S2). The lack of predictive power by female behavior of male behavior indicates
267 deficient following by males in this combination.

268

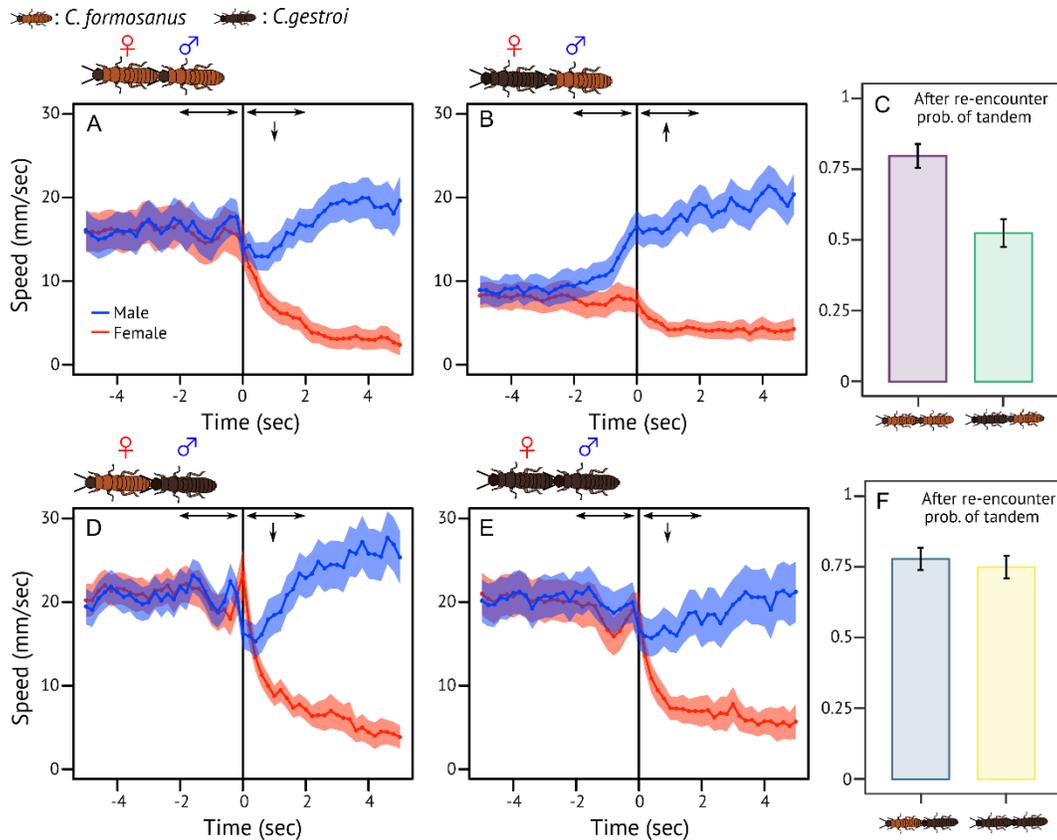


 : *C. formosanus*  : *C. gestroi*

Figure 2. Comparison of the strength of information flow during tandem runs. The predominant direction of predictive information is given by the proportion of uncertainty reduction explained by the interaction between leading females and following males. * indicates the combination with significant information flow from female to male.

Evaluation of leader females by follower males

After separation, females of both species slowed down significantly irrespective of partner species (comparison of mean speed two seconds before and two seconds after separation, LMM, $P < 0.01$, Fig. 3ABDE). Males of *C. gestroi* evaluated both conspecific and *C. formosanus* females as good leaders because they slowed down just after the separation to enhance re-encounter rates (LMM, $P < 0.05$, Fig. 3DE). On the other hand, *C. formosanus* males slowed down upon separation from conspecific females (LMM, estimate \pm s.e. = -0.4409 ± 0.2217 , $\chi^2_1 = 3.9546$, $P = 0.04674$, Fig. 3A), whereas they increased their speed after separating from *C. gestroi* females (LMM, estimate \pm s.e. = 1.1553 ± 0.1981 , $\chi^2_1 = 34.003$, $P < 0.0001$, Fig. 3B). Moreover, when the original partners did re-encounter each other, their probability of resuming a tandem run was lower for pairings of a *C. formosanus* male and a *C. gestroi* female than for other pairing combinations (GLMM, Tukey's test, $P < 0.01$, Fig. 3CF). These results suggest that a *C. formosanus* male evaluates a *C. gestroi* female as a poor leader and begins to search for another partner upon separation. Thus, male preference plays an important role in the success of heterospecific tandem runs.

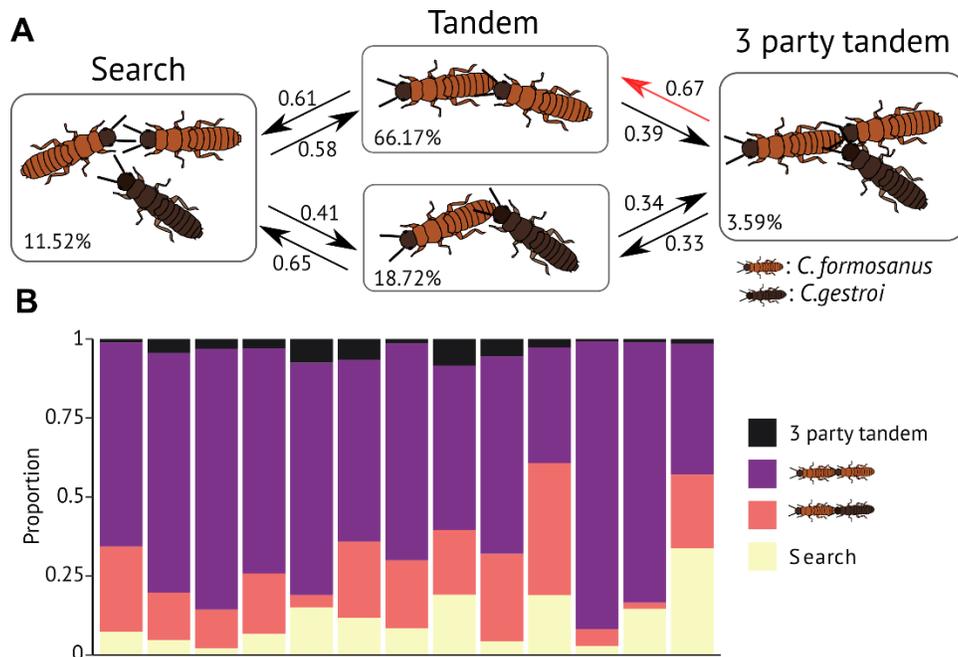


289
 290 **Figure 3.** Behavioral change of tandem runners before and after separation events. (A,B,D,E) The
 291 time development of speed across different combinations of mating pairs. Pair separation occurs
 292 at 0 sec. Shaded regions indicate mean speed $\pm 2SE$. Arrows indicate significant increase (upwards)
 293 or decrease (downwards) of male speed compared with before separation. (C, F) The probability of
 294 resumption of a tandem run upon re-encounter following a separation event. We obtained this
 295 probability by observing if a separated female and a male started a tandem run (= 1) or not (= 0)
 296 when they first came close enough for interaction after the separation event. Bars indicate the
 297 mean $\pm SE$.

298
 299 *Interspecific competition over females*

300 When one *C. formosanus* male and one *C. gestroi* male competed over one *C. formosanus* female, the
 301 termites could be in one of four different states (Fig. 4A): search (no tandem run), conspecific tandem run
 302 of *C. formosanus*, heterospecific tandem run of *C. formosanus* female and *C. gestroi* male, and three-partner
 303 tandem run with the two competing males side by side behind the female. When two individuals were in
 304 tandem, there was no interspecific difference in the probability to return to the search state (Fisher's exact
 305 test, $P = 0.5174$). However, when three individuals were in tandem, the probability of state transition was
 306 asymmetrical; competitions were twice as likely to end with victory by the *C. formosanus* male than by the
 307 *C. gestroi* male (Binomial test, $P < 0.001$). As a result, the most frequently observed state was conspecific
 308 tandem running by *C. formosanus* (Fig. 4B). These results demonstrate that *C. formosanus* males are

309 superior to *C. gestroi* when competing over a female, possibly because of their better-matched moving
 310 speed and body size.
 311



312 **Figure 4.** Competition between *C. formosanus* and *C. gestroi* males to follow one *C. formosanus*
 313 female. (A) State transition diagram for a 3-party tandem run with a *C. formosanus* female, a *C.*
 314 *formosanus* male, and a *C. gestroi* male. The transition from search to 3-party tandem is not shown
 315 (~ 0.01). The percentage in each box indicates overall time in that state. (B) Proportion of time in
 316 each state during observation. Each bar indicates replicate. Overall, the conspecific tandem of *C.*
 317 *formosanus* was the most frequent state.
 318

319
 320 **Discussion**

321 Our study has demonstrated that tandem coordination depends on a close association between the
 322 behavior of male followers and the signal strength of female leaders. Males of *C. gestroi*, whose females
 323 produce only a tenth of the amount of sex pheromone as *C. formosanus* [25], are adapted to follow a weak
 324 signal and so can maintain stable tandem runs with *C. formosanus* females. On the other hand, *C.*
 325 *formosanus* males are adapted to follow females with stronger signals and display a poor ability to maintain
 326 tandem runs with *C. gestroi* females. When they become separated from a female with a weak signal, *C.*
 327 *formosanus* males search for an alternative leader. This result demonstrates active behavioral mate choice
 328 in termites, hypothesized in previous studies but not shown empirically [36,37]. Note that females behaved
 329 the same regardless of male species, implying that they maintain the tandem passively through pheromone
 330 production, rather than actively choosing their potential mate. In summary, our results suggest that
 331 behavioral coordination in termite tandem runs is a product of coevolution between females and males.
 332 The species-specific association of leader and follower phenotypes may explain previous observations on

333 the collective behavior of mixed-species groups; some function as well as conspecific groups, while others
334 show a loss of coordination [38–40].

335 Leadership may be more likely in some individuals, due to traits like body size or personality (reviewed
336 in, e.g., [6,41,42]). In such a group, coordination may be difficult when there is a conflict of interest among
337 members (e.g., about where to go). However, this is not the case in termite tandem runs. There is no
338 conflict over leadership because roles are usually fixed by sex, with females leading and males following,
339 although this is flexible in occasional same-sex pairs [43]. Additionally, it is reasonable to assume that the
340 pair share the same goal of successful colony foundation. Predation risk is high during tandem [44], so
341 pairs must establish a nest as soon as possible regardless of combinations [45]. Their highest priority is not
342 who they found a colony with but simply to found as soon as possible [46]. Indeed, *C. formosanus* males
343 were willing to approach *C. gestroi* females and formed tandem pairs with them, although they evaluated
344 conspecific females more highly upon separation events (Fig. 3). Thus, we conclude that unstable tandem
345 runs result not from conflicts of interest but from a communication mismatch between *C. gestroi* females
346 and *C. formosanus* males, where female signaling is not sufficient for the males to follow.

347 Laboratory experiments have shown that individual behavior underlying group coordination can
348 evolve in just a few generations [47,48]; however, such changes have yet to be documented in the field.
349 Species invasions provide opportunities to observe evolutionary changes in behavior [49,50]. In our study
350 of an invasive population, we found less stable tandem runs than those previously observed in a native
351 population of *C. formosanus* in Japan ([19], Supplemental text, Fig. S4). This suggests modification of
352 tandem coordination following invasion, although such differences may have already existed between the
353 source population and other native ranges [51]. Further investigation is needed to determine what is the
354 cause of evolutionary change in behavior of *C. formosanus* from Florida, as invasive populations often have
355 different populational structures from native populations (e.g., high density or relatively low genetic
356 diversity [51]). Moreover, colony foundation experiments have confirmed that hybrid colonies can last > 2
357 years [52]. Research on female pheromone production and male following ability of hybrid adults will tell
358 us the inheritance mechanism of movement coordination in termites.

359 Evolutionary theory predicts that the development of conserved morphological structures can involve
360 significant modifications in their regulatory mechanisms [53,54]. We argue that this is possible even in
361 behavioral systems. For example, similar group-level patterns can emerge from different individual-level
362 behavioral rules regulating social interactions [55,56]. In this study, we show that a similar level of
363 behavioral coordination can be achieved from different leader/follower combinations. In *C. formosanus*,
364 the leader produces an abundant signal tracked by a competitive follower; while in *C. gestroi*, the leader
365 produces a weaker signal, but the follower has enhanced tracking ability. Tandem runs are seen across
366 most termite taxonomic groups [16]. However, our results imply that their underlying mechanisms for
367 coordination can vary, because the adaptiveness of a tandem run is not determined by how they coordinate
368 but by how well they maintain contact during nest site search in a vulnerable period. By emphasizing that

369 there are multiple solutions for the same coordination problem, our study has implications beyond pair
370 coordination and gives insight into the convergent evolution of collective behavior across different taxa.

371

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380

381 **Authors contributions**

382 NM: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing-
383 original draft preparation, Writing-review and editing, Visualization, Funding acquisition; SBL: Data
384 collection, Methodology, Investigation, Writing-review and editing; GV: Methodology, Validation, Formal
385 analysis, Writing-review and editing; TC: Conceptualization, Methodology, Writing-review and editing,
386 Funding acquisition; SCP: Conceptualization, Writing-review and editing, Supervision

387

388 **Data accessibility statement**

389 All data is available in dryad: [https://datadryad.org/stash/share/PxLMS43iwX4GAgjQYPd0_0Q-](https://datadryad.org/stash/share/PxLMS43iwX4GAgjQYPd0_0Q-VQAIjL3qoxjT3H7XhQ4)
390 [VQAIjL3qoxjT3H7XhQ4](https://datadryad.org/stash/share/PxLMS43iwX4GAgjQYPd0_0Q-VQAIjL3qoxjT3H7XhQ4).

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392 **References**

- 393 1. Camazine S, Deneubourg J-L, Franks NR, Sneyd J, Theraulaz G, Bonabeau E. 2001 *Self-organization*
394 *in Biological Systems*. Princeton: NJ: Princeton University Press.
- 395 2. Couzin ID, Krause J. 2003 Self-organization and collective behavior in vertebrates. *Adv. Study*
396 *Behav.* **32**, 1–75. (doi:10.1016/S0065-3454(03)01001-5)
- 397 3. Krause J, Hoare D, Krause S, Hemelrijk CK, Rubenstein DI. 2000 Leadership in fish shoals. *Fish*
398 *Fish.* **1**, 82–89. (doi:10.1111/j.1467-2979.2000.tb00001.x)
- 399 4. King AJ. 2010 Follow me! I'm a leader if you do; I'm a failed initiator if you don't? *Behav. Processes*
400 **84**, 671–674. (doi:10.1016/j.beproc.2010.03.006)
- 401 5. Couzin ID, Krause J, Franks NR, Levin SA. 2005 Effective leadership and decision-making in
402 animal groups on the move. *Nature* **433**, 513–516. (doi:10.1038/nature03236)
- 403 6. King AJ, Johnson DDP, Van Vugt M. 2009 The origins and evolution of leadership. *Curr. Biol.* **19**,
404 R911–R916. (doi:10.1016/j.cub.2009.07.027)
- 405 7. Franks NR, Richardson TO. 2006 Teaching in tandem-running ants. *Nature* **439**, 153.

- 406 (doi:10.1038/439153a)
- 407 8. Schaerf TM, Herbert-Read JE, Myerscough MR, Sumpter DJT, Ward AJW. 2016 Identifying
408 differences in the rules of interaction between individuals in moving animal groups. *arXiv*
- 409 9. Valentini G, Mizumoto N, Pratt SC, Pavlic TP, Walker SI. 2020 Revealing the structure of
410 information flows discriminates similar animal social behaviors. *Elife* **9**, e55395.
411 (doi:10.1101/765198)
- 412 10. Orange N, Abaid N. 2015 A transfer entropy analysis of leader-follower interactions in flying bats.
413 *Eur. Phys. J. Spec. Top.* **224**, 3279–3293. (doi:10.1140/epjst/e2015-50235-9)
- 414 11. Schaerf TM, Herbert-Read JE, Ward AJW. 2021 A statistical method for identifying different rules
415 of interaction between individuals in moving animal groups. *J. R. Soc. Interface* **18**, 20200925.
416 (doi:10.1098/rsif.2020.0925)
- 417 12. Harcourt JL, Ang TZ, Sweetman G, Johnstone RA, Manica A. 2009 Social feedback and the
418 emergence of leaders and followers. *Curr. Biol.* **19**, 248–252. (doi:10.1016/j.cub.2008.12.051)
- 419 13. Franklin EL. 2014 The journey of tandem running: The twists, turns and what we have learned.
420 *Insectes Soc.* **61**, 1–8. (doi:10.1007/s00040-013-0325-3)
- 421 14. Möglich M, Maschwitz U, Hölldobler B. 1974 Tandem calling: a new kind of signal in ant
422 communication. *Science* **186**, 1046–7. (doi:10.1126/science.186.4168.1046)
- 423 15. Nutting WL. 1969 Flight and colony foundation. In *Biology of termites* (eds K Krishna, FM
424 Weesner), pp. 233–282. New York: Academic Press. (doi:10.1016/B978-0-12-395529-6.50012-X)
- 425 16. Bordereau C, Pasteels JM. 2011 Pheromones and chemical ecology of dispersal and foraging in
426 termites. In *Biology of Termites: A Modern Synthesis* (eds DE Bignell, Y Roisin, N Lo), pp. 279–320.
427 Dordrecht: Springer Netherlands. (doi:10.1007/978-90-481-3977-4_11)
- 428 17. Sillam-Dussès D. 2011 *Trail pheromones and sex pheromones in termites*. New York: Nova Science
429 Publishers/Novinka.
- 430 18. Raina AK, Bland JM, Dickens JC, Park YI, Hollister B. 2003 Premating behavior of dealates of the
431 Formosan subterranean termite and evidence for the presence of a contact sex pheromone. *J.*
432 *Insect Behav.* **16**, 233–245. (doi:10.1023/A:1023967818906)
- 433 19. Mizumoto N, Dobata S. 2019 Adaptive switch to sexually dimorphic movements by partner-
434 seeking termites. *Sci. Adv.* **5**, eaau6108. (doi:10.1126/sciadv.aau6108)
- 435 20. Bourguignon T, Lo N, Šobotník J, Sillam-Dussès D, Roisin Y, Evans TA. 2016 Oceanic dispersal,
436 vicariance and human introduction shaped the modern distribution of the termites
437 *Reticulitermes*, *Heterotermes* and *Coptotermes*. *Proc. R. Soc. B Biol. Sci.* **283**, 20160179.
438 (doi:10.1098/rspb.2016.0179)
- 439 21. Rust MK, Su NY. 2012 Managing social insects of urban importance. *Annu. Rev. Entomol.* **57**, 355–
440 375. (doi:10.1146/annurev-ento-120710-100634)
- 441 22. Evans TA, Forschler BT, Kenneth Grace J. 2013 Biology of invasive termites: A worldwide review.
442 *Annu. Rev. Entomol.* **58**, 455–474. (doi:10.1146/annurev-ento-120811-153554)

- 443 23. Chouvenc T *et al.* 2016 Revisiting *Coptotermes* (Isoptera: Rhinotermitidae): A global taxonomic
444 road map for species validity and distribution of an economically important subterranean termite
445 genus. *Syst. Entomol.* **41**, 299–306. (doi:10.1111/syen.12157)
- 446 24. Chouvenc T, Helmick EE, Su N-Y. 2015 Hybridization of two major termite invaders as a
447 consequence of human activity. *PLoS One* **10**, e0120745. (doi:10.1371/journal.pone.0120745)
- 448 25. Chouvenc T, Sillam-Dussès D, Robert A. 2020 Courtship behavior confusion in two subterranean
449 termite species that evolved in allopatry (Blattodea, Rhinotermitidae, *Coptotermes*). *J. Chem.*
450 *Ecol.* , 1–14. (doi:10.1007/s10886-020-01178-2)
- 451 26. Mizumoto N, Rizo A, Pratt SC, Chouvenc T. 2020 Termite males enhance mating encounters by
452 changing speed according to density. *J. Anim. Ecol.* **89**, 2542–2552. (doi:10.1111/1365-2656.13320)
- 453 27. Yamanaka O, Takeuchi R. 2018 UMATracker: An intuitive image-based tracking platform. *J. Exp.*
454 *Biol.* **221**, 1–24. (doi:10.1242/jeb.182469)
- 455 28. R Core Team. 2020 R: A language and environment for statistical computing.
- 456 29. Therneau TM. 2015 coxme: mixed effects Cox models.
- 457 30. Schreiber T. 2000 Measuring information transfer. *Phys. Rev. Lett.* **85**, 461–464.
458 (doi:10.1103/PhysRevLett.85.461)
- 459 31. Lizier JT, Prokopenko M. 2010 Differentiating information transfer and causal effect. *Eur. Phys. J.*
460 *B* **73**, 605–615. (doi:10.1140/epjb/e2010-00034-5)
- 461 32. Bartumeus F, Levin SA. 2008 Fractal reorientation clocks: Linking animal behavior to statistical
462 patterns of search. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 19072–19077.
463 (doi:10.1073/pnas.0801926105)
- 464 33. Bossomaier T, Barnett L, Harré M, Lizier JT. 2016 *An introduction to transfer entropy: Information*
465 *flow in complex systems*. (doi:10.1007/978-3-319-43222-9)
- 466 34. Porfiri M. 2018 Inferring causal relationships in zebrafish-robot interactions through transfer
467 entropy: a small lure to catch a big fish. *Anim. Behav. Cogn.* **5**, 341–367.
468 (doi:10.26451/abc.05.04.03.2018)
- 469 35. Moore DG, Valentini G, Walker SI, Levin M. 2018 Inform: Efficient information-theoretic analysis
470 of collective behaviors. *Front. Robot. AI* **5**, 1–14. (doi:10.3389/frobt.2018.00060)
- 471 36. Husseneder C, Simms DM. 2008 Size and heterozygosity influence partner selection in the
472 Formosan subterranean termite. *Behav. Ecol.* **19**, 764–773. (doi:10.1093/beheco/arn041)
- 473 37. Kitade O, Hayashi Y, Kikuchi Y, Kawarasaki S. 2004 Distribution and composition of colony
474 founding associations of a subterranean termite, *Reticulitermes kanmonensis*. *Entomol. Sci.* **7**, 1–8.
475 (doi:10.1111/j.1479-8298.2003.00048.x)
- 476 38. Ward AJW, Schaerf TM, Burns ALJ, Lizier JT, Crosato E, Prokopenko M, Webster MM. 2018
477 Cohesion, order and information flow in the collective motion of mixed-species shoals. *R. Soc.*
478 *Open Sci.* **5**. (doi:10.1098/rsos.181132)
- 479 39. Boulay J, Deneubourg JL, Hédoüin V, Charabidzé D. 2016 Interspecific shared collective decision

- 480 making in two forensically important species. *Proc. R. Soc. B Biol. Sci.* **283**, 1–9.
481 (doi:10.1098/rspb.2015.2676)
- 482 40. Jolles JW, King AJ, Manica A, Thornton A. 2013 Heterogeneous structure in mixed-species corvid
483 flocks in flight. *Anim. Behav.* **85**, 743–750. (doi:10.1016/j.anbehav.2013.01.015)
- 484 41. Petit O, Bon R. 2010 Decision-making processes: The case of collective movements. *Behav.*
485 *Processes* **84**, 635–647. (doi:10.1016/j.beproc.2010.04.009)
- 486 42. Herbert-Read JE. 2016 Understanding how animal groups achieve coordinated movement. *J. Exp.*
487 *Biol.* , 2971–2983. (doi:10.1242/jeb.129411)
- 488 43. Matsuura K, Kuno E, Nishida T. 2002 Homosexual tandem running as selfish herd in *Reticulitermes*
489 *speratus*: novel antipredatory behavior in termites. *J. Theor. Biol.* **214**, 63–70.
490 (doi:10.1006/jtbi.2001.2447)
- 491 44. Deltigne J, Quennedey A, Blum M. 1981 The enemies and defense mechanisms of termites. In
492 *Social insects* (ed H Hermann), pp. 1–76. New York: Academic Press.
- 493 45. Mizumoto N, Yashiro T, Matsuura K. 2016 Male same-sex pairing as an adaptive strategy for
494 future reproduction in termites. *Anim. Behav.* **119**, 179–187. (doi:10.1016/j.anbehav.2016.07.007)
- 495 46. Chouvenc T. 2019 The relative importance of queen and king initial weights in termite colony
496 foundation success. *Insectes Soc.* **66**, 177–184. (doi:10.1007/s00040-019-00690-3)
- 497 47. Kotrschal A *et al.* 2020 Rapid evolution of coordinated and collective movement in response to
498 artificial selection. *Sci. Adv.* , 5–12. (doi:10.1101/2020.01.30.926311)
- 499 48. Ioannou CC, Guttal V, Couzin ID. 2012 Predatory fish select for coordinated collective motion in
500 virtual prey. *Science (80-.)*. **337**, 1212–1215. (doi:10.1126/science.1218919)
- 501 49. Sax DF *et al.* 2007 Ecological and evolutionary insights from species invasions. *Trends Ecol. Evol.*
502 **22**, 465–471. (doi:10.1016/j.tree.2007.06.009)
- 503 50. Ruland F, Jeschke JM, Ruland F. 2020 How biological invasions affect animal behaviour : A global ,
504 cross-taxonomic analysis. *J. Anim. Ecol.* , 2531–2541. (doi:10.1111/1365-2656.13306)
- 505 51. Blumenfeld AJ *et al.* 2021 the global invasion history of a termite. *Commun. Biol.*
506 (doi:10.1038/s42003-021-01725-x)
- 507 52. Lee S, Chouvenc T, Patel J, Su N, Lee S. 2020 Altered mobility and accumulation of inefficient
508 workers in juvenile hybrid termite colonies. *Front. Ecol. Evol.* **8**, 1–8.
509 (doi:10.3389/fevo.2020.589762)
- 510 53. True JR, Haag ES. 2001 Developmental system drift and flexibility in evolutionary trajectories.
511 *Evol. Dev.* **3**, 109–119. (doi:10.1046/j.1525-142X.2001.003002109.x)
- 512 54. Wang X, Sommer RJ. 2011 Antagonism of LIN-17/frizzled and LIN-18/RyK in nematode vulva
513 induction reveals evolutionary alterations in core developmental pathways. *PLoS Biol.* **9**,
514 e1001110. (doi:10.1371/journal.pbio.1001110)
- 515 55. Mizumoto N, Bardunias PM, Pratt SC. 2020 Complex relationship between tunneling patterns and
516 individual behaviors in termites. *Am. Nat.* **196**. (doi:10.1086/711020)

517 56. Weitz S, Blanco S, Fournier R, Gautrais J, Jost C, Theraulaz G. 2012 Modeling collective animal
518 behavior with a cognitive perspective: A methodological framework. *PLoS One* 7.
519 (doi:10.1371/journal.pone.0038588)
520