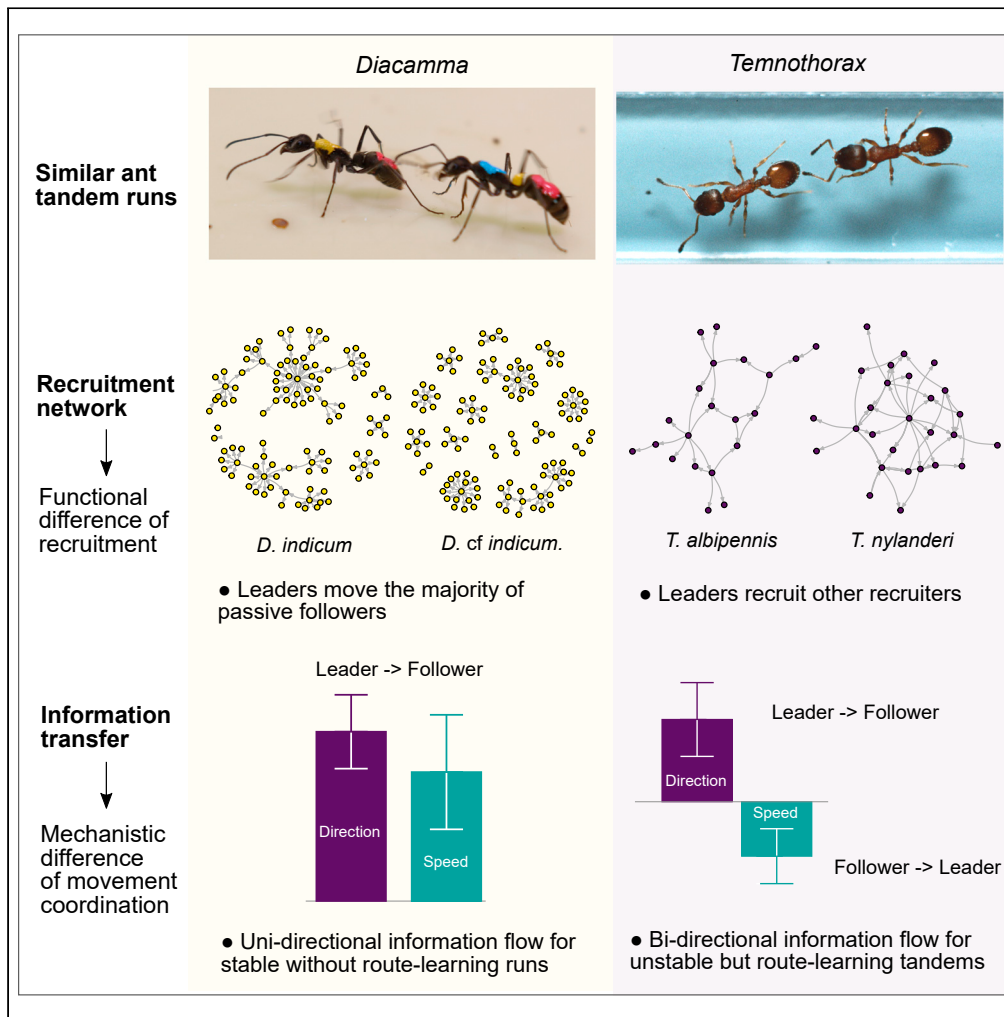


Article

# Functional and mechanistic diversity in ant tandem communication



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**Highlights**

Tandem runs of *Temnothorax* and *Diacamma* ants are fundamentally distinct

Network analysis revealed the differences in recruitment function

*Temnothorax* recruits additional recruiters, while *Diacamma* moves passive members

Functional differences appear in information transfer during coordination

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## Article

## Functional and mechanistic diversity in ant tandem communication

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## SUMMARY

Communication is fundamental to the organization of animal societies, often resulting in the convergent evolution of similar social behavior across lineages. However, this similarity may conceal underlying functional and mechanistic differences. Here we combined network and information-theoretic analysis to quantify how tandem recruitment is distinguishable between two ant genera, *Temnothorax* and *Diacamma*. We show that *Temnothorax* uses tandem running to recruit additional recruiters, while *Diacamma* uses it principally to move the passive majority of their colony, a task that *Temnothorax* accomplishes with a different behavior, social carrying. Accordingly, the network structure of *Diacamma* tandems was dissimilar to that of *Temnothorax*, instead resembling the social-carrying networks in *Temnothorax*. Furthermore, our information-theoretical analysis on movement trajectories revealed that *Diacamma* tandem runs lack bidirectional information transfer, the signature of route learning in *Temnothorax*. By quantifying the diversity of similar communication systems, this study increases the resolution of our understanding of animal societies.

## INTRODUCTION

Social animals cooperatively accomplish an impressive array of collective tasks, including group emigration with movement coordination, construction of complex nest structures, and flexible division of labor.<sup>1,2</sup> These collective behaviors are underpinned by their communication systems, which allow the quick dissemination of valuable information among members.<sup>3,4</sup> Among social animals, social insects have evolved a diversity of communication systems, ranging from direct one-to-one interactions<sup>5,6</sup> to indirect mass interactions through chemical signals deposited in the shared environment.<sup>7</sup> The diversification of communication systems has been accompanied by instances of remarkable evolutionary convergence. For example, *Lasius* ants and *Nasutitermes* termites produce similar sponge-like nest structures,<sup>8</sup> and the iconic mass raids of new- and old-world army ants result from independent evolutionary origins.<sup>9</sup> The convergent evolution of behavior is often considered an outcome of adaptation to the same evolutionary pressure.<sup>10</sup> However, convergence can also occur with differentiated functions or through distinct evolutionary contexts.<sup>11</sup> Thus, superficially similar social behaviors may conceal substantial differences in their function and mechanisms for social interactions.

Tandem recruitment by ants is a unique communication technique. This behavior is used by an informed ant to share the location of a valuable resource by directly leading a naive follower to it.<sup>12</sup> It has evolved independently multiple times<sup>13,14</sup> but is especially well documented in the genus *Temnothorax*.<sup>15</sup> In these ants, a successful tandem run is achieved through bidirectional feedback between partners.<sup>16</sup> This feedback allows the follower to regulate the movement speed of the pair so that the follower can learn the route demonstrated by the leader.<sup>17,18</sup> In other ant lineages where tandem running has arisen (Figure 1), it is also used to guide colony members to a goal but in a variety of contexts, including recruitment to food, colony emigration, or brood raids by social parasites.<sup>19–21</sup> However, as tandem recruitment has been studied in each genus independently, integration of knowledge through comparative behavioral analysis is required to determine whether tandem communication is functionally and mechanistically equivalent across different evolutionary origins.

A comparison of tandem runs by *Temnothorax* and *Diacamma* is a potentially revealing example of convergent evolution. These two represent distantly related ant groups, but both use tandem runs during colony

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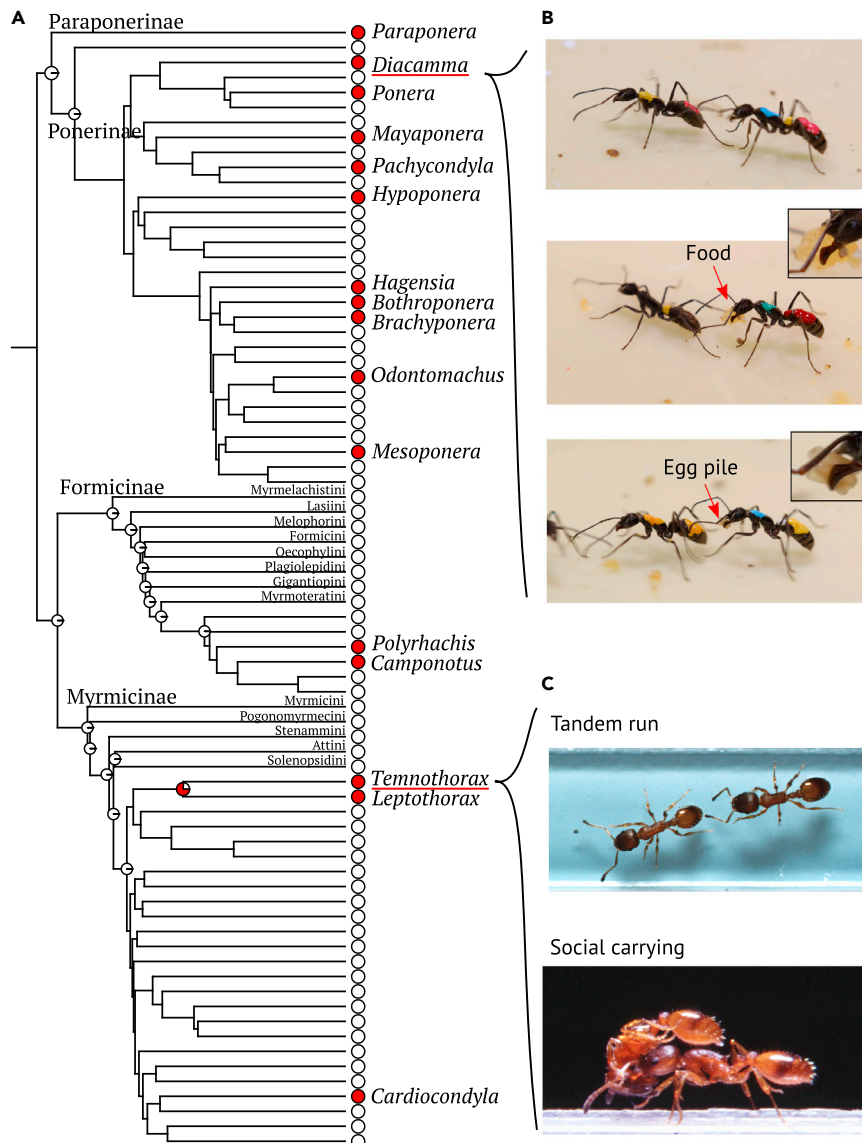
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**Figure 1. Evolution of ant tandem runs and studied species**

(A) Ant phylogeny focusing on the evolution of tandem runs, simplified from Nelsen et al.<sup>22</sup> Red circles at tips indicate the presence of the tandem run. Node pie charts represent the probability of the estimated ancestral states of tandem run, inferred with maximum likelihood estimation methods (see supplemental information). Estimated ancestral states are shown for roots of each tribe or where the tandem run was present with a probability larger than 70%. The full genus-level phylogeny used for ancestral state reconstruction is shown in Figure S1. Genera containing studied species are underlined in red. Tandem running evolved at least 15 times independently in ants.

(B) Tandem running of *Diacamma cf indicum*. Followers can carry food or brood while traveling.

(C) Tandem running and social carrying in *Temnothorax albigennis*. The photo is reused from the author's previous published paper,<sup>23</sup> following Royal Society's permission policy.

emigration. Tandem runs of these two genera have many common points and appear indistinguishable in some respects. For example, leaders release a short-range pheromone to help guide followers,<sup>12,24</sup> and leaders pause to wait for the follower upon accidental interruption.<sup>20,25</sup> However, previous observations imply they use tandem recruitment in different contexts (Figure 1). In *Temnothorax*, tandem runs occur at the initial stage of the emigration, and leaders recruit followers to assess potential new nest sites. Once these ants decide on a site, they switch from tandem runs to social carrying, which they use to transport the rest of their colony members, one at a time, to the new nest.<sup>15</sup> Social carrying is not thought to

allow the transportees to learn the emigration route, but its greater speed makes it more suitable for moving large numbers of ants that will not need to retrace the route.<sup>26</sup> On the other hand, *Diacamma* uses only tandem runs throughout the colony emigration.<sup>27</sup> All colony members move between the old and new nest sites either as leaders or followers. This implies that the tandem run of *Diacamma* may be functionally more similar to *Temnothorax* social carrying than to *Temnothorax* tandem runs: a means of moving the bulk of the colony rather than a means of recruiting additional recruiters.

If tandem running fulfills different functions in different species, this should be reflected in the information flows between leaders and followers. For example, a previous study distinguished similar tandem running behaviors between *Temnothorax* ants and termites.<sup>17</sup> In *Temnothorax* ants, leaders and followers alternately influence their partner's behavior while teaching the follower route information. The leader determines the course of tandem movements, while the follower determines the leader's speed to effectively gather spatial information.<sup>16,17</sup> On the other hand, in termites, tandem running is used by a mated pair and functions to keep them together as they perform a random search for nest sites: there is no need for route learning. Thus, their interactions lack bidirectional feedback, i.e., termite followers just follow their leaders.<sup>17</sup> *Diacamma* tandem runs may similarly lack route-learning protocols used by *Temnothorax* if they primarily play a simple nestmate-moving function like social carrying. Then one can expect interindividual interactions in a *Diacamma* tandem pair to resemble those of termites.

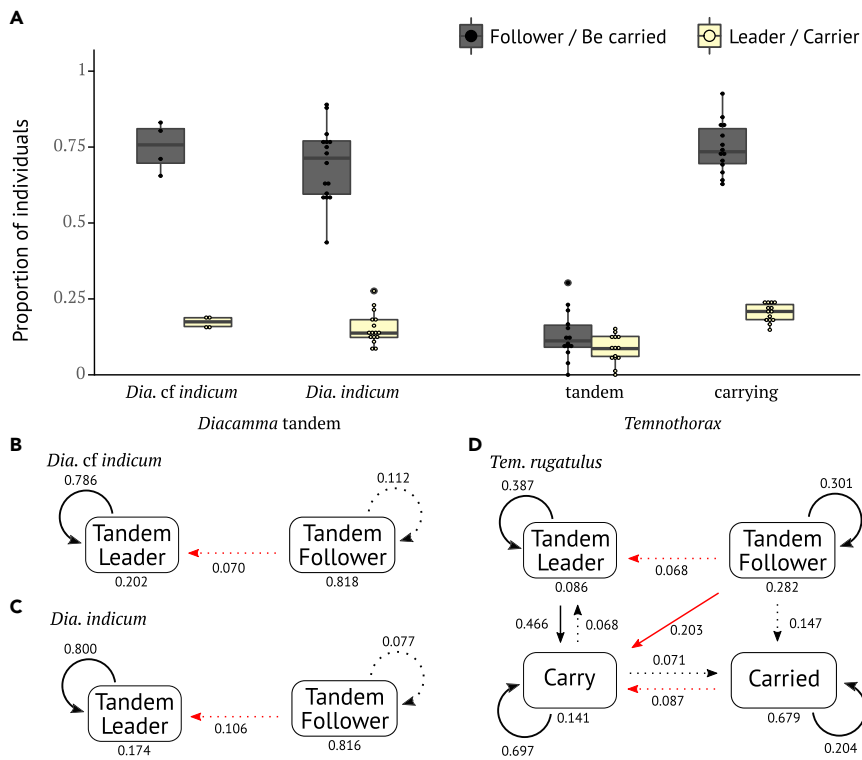
Here we compare tandem runs of both *Temnothorax* and *Diacamma* in a quantitative manner, to reveal how these two are distinguishable in their recruitment function and coordination mechanisms. For comparative analysis, we employed data on colony emigration and tandem runs in five different ant (sub)species: *Diacamma indicum*, *D. cf. indicum* (from Japan), *Temnothorax albipennis*, *Temnothorax nylanderii*, and *Temnothorax rugatulus*. We additionally used data on tandem runs in two termite species as an outgroup contrast. Using these datasets, we explore whether *Diacamma* tandem running is functionally more similar to tandem running or social carrying in *Temnothorax*. First, we tested for between-species differences in the probabilities of engaging or switching between leading, following, and carrying roles. Then, we compared the recruitment network structures among species to test if recruitment dynamics in *Diacamma* tandem runs are similar to those of *Temnothorax* tandem runs or *Temnothorax* social carrying. Finally, we measured the direction of the information flow between leaders and followers within tandem runs.

## RESULTS

### Species differences in the functions of tandem runs

During colony emigration in *Diacamma*, most individuals participated in tandem runs (Mean  $\pm$  SD; *D. cf. indicum*: 86.2  $\pm$  8.5%; *D. indicum*: 76.0  $\pm$  12.0%). In contrast, during emigration in *Temnothorax*, only a minority of workers engaged in tandem runs (*T. rugatulus*: 18.8  $\pm$  9.9%). Participation in tandem running was significantly lower in *Temnothorax* than in *Diacamma*, both for leaders and followers (generalized linear mixed model [GLMM]:  $p < 0.001$ , Figure 2A). *Temnothorax* instead showed a high prevalence of social carrying (*T. rugatulus*: 83.5  $\pm$  6.0%). Indeed, the proportion of *Temnothorax* workers that carried nestmates was similar to the proportion of *Diacamma* workers that led tandem runs in *D. cf. indicum* (GLMM; vs. *D. cf. indicum*:  $\chi^2_1 = 1.77$ ,  $p = 0.18$ ; vs. *D. indicum*:  $\chi^2_1 = 8.27$ ,  $p = 0.004$ , Figure 2A). Similarly, the proportion of the *Diacamma* followers was similar to the proportion of *Temnothorax* workers that were carried (GLMM; vs. *D. cf. indicum*:  $\chi^2_1 = 0.008$ ,  $p = 0.93$ ; vs. *D. indicum*:  $\chi^2_1 = 1.54$ ,  $p = 0.22$ , Figure 2A). Thus, in terms of the proportion of individuals involved, tandem running in *Diacamma* is more similar to social carrying in *Temnothorax* rather than *Temnothorax* tandem running.

In *Temnothorax*, tandem runs function to recruit new recruiters as tandem leaders or carriers.<sup>26</sup> We examined this evidence by measuring the transition probabilities between different recruitment roles. In *Diacamma*, we found that tandem followers rarely went on to become recruiters (*D. cf. indicum*: 7%; *D. indicum*: 10.6%; Figures 2B and 2C). This contrasted with *T. rugatulus*, where 27.1% of tandem followers went on to become recruiters (Figure 2D), a significantly higher proportion than in *D. cf. indicum* (Fisher's exact test:  $p < 0.001$ ) or *D. indicum* (Fisher's exact test:  $p < 0.001$ ). Note that tandem runs of *T. nylanderii*, but not *T. albipennis*, showed a similar pattern to those of *T. rugatulus*, although information on social carrying was unavailable in these species (Figure S2). On the other hand, *T. rugatulus* ants that were transported by social carrying showed a similar pattern to *Diacamma* tandem followers. Only 8.7% of carried workers became recruiters (Figure 2D), which was not significantly different from tandem followers in *D. cf. indicum* (Fisher's exact test:  $p = 0.199$ ) or *D. indicum* (Fisher's exact test:  $p = 0.295$ ).



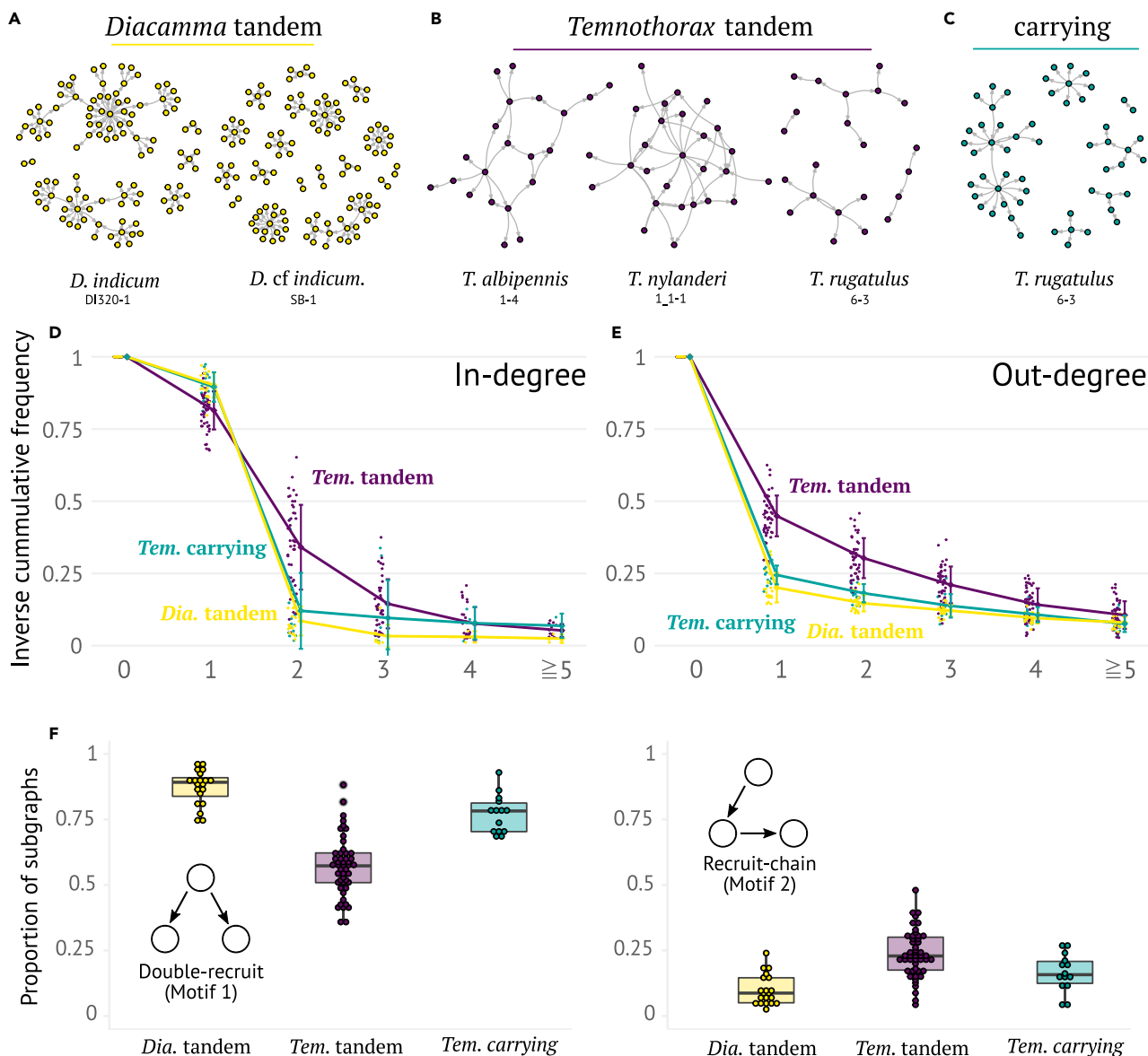
**Figure 2. Worker participation in nestmate recruitment during colony emigration, compared between *Temnothorax* and *Diacamma***

(A) Proportion of workers involved in recruitment (tandem runs and social carrying) either as leaders/carriers or followers/carried.

(B–D) Transition diagram of recruitment tasks during emigration in (B) *D. cf. indicum*, (C) *D. indicum*, and (D) *T. rugatulus*. The results of *T. nylanderi* and *T. albipennis* are in Figure S2. Recursive arrows indicate repetition of the same task. Numbers next to each arrow give the associated transition probability; numbers below each box indicate the probability of concluding the emigration after the task. Dashed lines show transitions with probability <0.2; transitions with probability <0.05 are omitted. Red lines indicate transition from being a recruit (tandem follower or being carried) to recruiting (tandem leader or carrier) roles, suggesting successful recruitment of new recruiters.

These statistical differences between the recruitment dynamics are readily apparent in the structure of the recruitment networks during a given emigration (Figures 3A–3C and S9–S11). In recruitment networks, ants who participated in recruitment were represented as nodes, and tandem runs were represented as directed links pointing from the leader (or carrier) to the follower (or ant being carried). Thus, out-degree and in-degree distributions indicate how many partners the leaders and followers had during emigration events. These two distributions differed between *Diacamma* and *Temnothorax* (Kolmogorov–Smirnov test; in-degree:  $D = 0.26$ ,  $p < 0.001$ ; out-degree:  $D = 0.25$ ,  $p < 0.001$ ; Figures 3D and 3E). In *Diacamma* tandems, the recruitment networks were composed of multiple star-like structures, each with a leader as a central node and its followers as leaves (Figure 3A). Most individuals participated in only one tandem run, as a follower, and had just one incoming edge and no outgoing edges (Figures 3D and 3E). In *Temnothorax* tandems, on the other hand, followers remained involved in recruitment, either by becoming leaders or by being led multiple times by different leaders during emigration (Figure 3B). Thus, followers often had multiple incoming edges and outgoing edges (Figures 3D and 3E). The proportion of passive workers with one incoming edge and no outgoing edge was significantly lower in *Temnothorax* tandems than in *Diacamma* tandems (GLMM; Tukey’s test,  $z = 15.92$ ,  $p < 0.01$ ). The structure of *Diacamma* tandem networks was similar to that of the social-carrying networks of *Temnothorax* due to the presence of many transportees that were carried only once (Figure 3C). There was no significant difference in the proportion of passive workers with one incoming edge and no outgoing edge (GLMM; Tukey’s test;  $z = 2.31$ ,  $p = 0.053$ ; Figures 3D and 3E).

Network motif, a property characterizing the structure of a given network,<sup>28</sup> provided more evidence for differences in the structure of *Diacamma* and *Temnothorax* tandem recruitment networks. We investigated



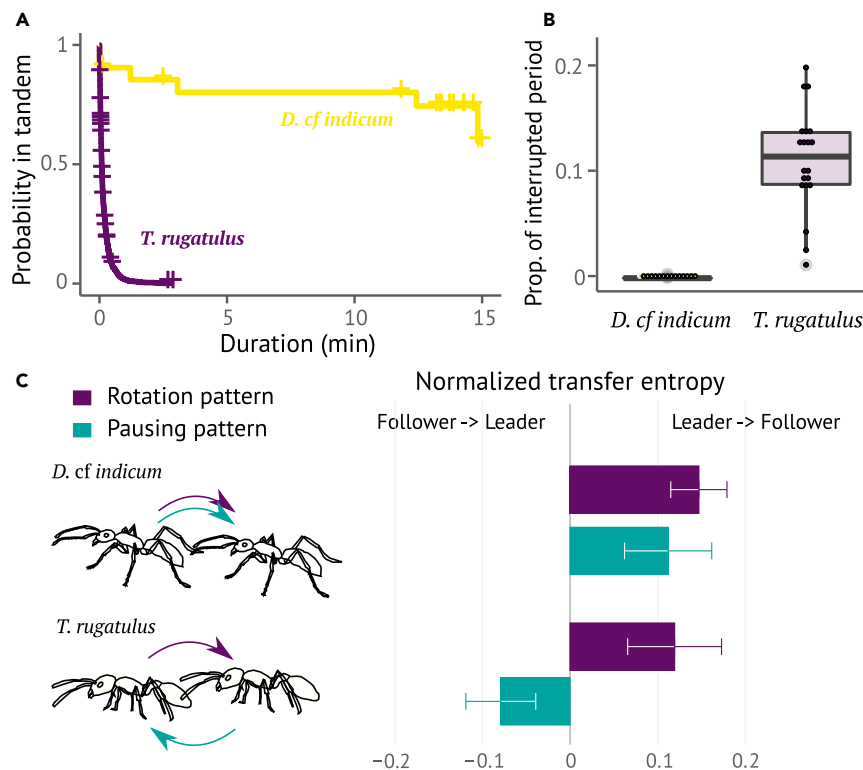
**Figure 3. Comparison of recruitment networks in *Diacamma* and *Temnothorax* ants**

(A–C) Examples of recruitment networks from different species. Nodes represent ants, and edges represent recruitments (tandem run or carrying). Colony id and emigration event are connected by a hyphen following the species name. (A) Tandem recruitment network for *D. cf. indicum* and *D. indicum*. (B) Tandem recruitment network for *T. albipennis*, *T. nylanderi*, and *T. rugatulus*. (C) Carrying network for *T. rugatulus*.

(D and E) Comparison of cumulative in-degree and out-degree distributions. Bars indicate mean  $\pm$  s.d.

(F) Comparison of the proportion of network motifs for different types of ant recruitment networks. Networks with  $<20$  nodes were removed from the analysis.

the distribution of triadic motifs to identify representative patterns of information flow in recruitment networks. Among 13 possible subgraphs with three nodes, two motifs predominated, namely the “double-recruit” motif ( $A \rightarrow B, A \rightarrow C$ , motif 1 in<sup>28</sup>) and the “recruit-chain” ( $A \rightarrow B \rightarrow C$ , motif 2 in<sup>28</sup>). The proportion of both motifs was different among recruitment types (GLMM;  $p < 0.001$ ). Double-recruit was more frequent in *Diacamma* tandem recruitment networks than in *Temnothorax* tandem networks (Tukey’s test;  $z = 11.02$ ,  $p < 0.001$ , Figure 3F). On the other hand, recruit-chain was more frequent in *Temnothorax* networks than *Diacamma* networks (Tukey’s test;  $z = 6.82$ ,  $p < 0.001$ , Figure 3F). These differences indicate that the tandem recruitment networks of *Temnothorax* result from individuals switching from following to leading, whereas the networks of *Diacamma* rarely contain these switches and instead mainly represent



**Figure 4. Comparison of movement coordination during tandem runs**

(A) Comparison of the duration of tandem runs until interruptions. Kaplan-Meier survival curves were generated for each species. + indicates censored data due to the end of observations. There was a significant difference in duration until interruption between species (Tukey's test after mixed-effects Cox model,  $p < 0.05$ ).

(B) Comparison of the interrupted period during observation.

(C) The predominant direction of predictive information is given by the proportion of uncertainty reduction explained by the interaction between leader and follower. Bars indicate the mean  $\pm$  s.d.

interactions between a minority of highly active recruiters and a majority of passive followers. In contrast, we found no significant difference between motifs present in *Diacamma* tandem networks and *Temnothorax* social-carrying networks (Tukey's test: double-recruit:  $z = 1.91$ ,  $p = 0.13$ , recruit-chain:  $z = 1.80$ ,  $p = 0.17$ ), and both represent interactions of a minority transporting the majority of other members. Thus, recruitment dynamics of *Diacamma* tandems are rather similar to *Temnothorax* social carrying.

### Species differences in the coordination mechanisms of tandem runs

The two members of a tandem coordinate their movement by adjusting their speed according to that of their partner. Our analysis of the changes in movement speed (i.e., the acceleration) during tandem runs showed that leaders and followers, in both *D. cf. indicum* and *T. rugatulus*, modified their movement acceleration according to the distance to each other (Figure S3). When the distance increased, followers accelerated to catch up with leaders, and leaders decelerated to allow the follower to reconnect. Conversely, when the distance decreased, followers decelerated, and leaders accelerated. However, there were striking differences between *D. cf. indicum* and *T. rugatulus* in the stability and duration of tandem runs. Tandem runs of *T. rugatulus* were frequently interrupted by pauses in which leader and follower were temporarily separated, with all runs experiencing interruptions within 3 min (Figure 4A). In contrast, tandem runs of *D. cf. indicum* were more stable, with more than half of the runs lasting longer than 15 min without interruptions (Figure 4A). As a result, tandem pairs of *T. rugatulus* were interrupted for much longer periods than *D. cf. indicum* over the course of observed tandem runs (Figure 4B).

The frequent interruptions of *Temnothorax* tandems are believed to play a role in information transfer. Followers trigger pauses so that they can learn visual cues that will later help them navigate the route independently. Thus, follower behavior largely drives the temporal pattern of move/pause by leaders. On the other

hand, because only leaders know the route to the new nest, their behavior largely determines the movement direction of followers. This bidirectional feedback was recently formally quantified using an information-theoretic analysis in *T. rugatulus*.<sup>17</sup> The net flow of information between a leader and follower was quantified using transfer entropy<sup>29–31</sup>; that is, information flow is from a leader to a follower if leader behavior predicts the future follower behavior, better than the other direction. They separately analyzed the sequence of pauses and moves, and the sequence of clockwise and counter-clockwise turns, to examine the direction of net flow of information.

We applied the same analysis to *Diacamma* tandem runs, expecting that tandem runs in this species would lack this bidirectionality, given their greater stability and the absence of *Temnothorax*-like route learning. Indeed, for the tandem runs of *D. cf. indicum*, leader behavior was always a better predictor of follower behavior than the other way around, both for motion and for direction (Wilcoxon signed-rank tests,  $p < 0.01$ , Figures 4C and S4). This contrasted with *T. rugatulus* tandem runs, in which leader's turning behavior predicted followers' turning patterns, and follower's pausing behavior predicted leaders' pausing patterns (Wilcoxon signed-rank tests,  $p < 0.01$ , Figure 4C). A previous study showed that similar tandem-running behavior observed in termite mating pairs is distinct from that of *Temnothorax* ants,<sup>17</sup> and we found that the tandem runs of *D. cf. indicum* were qualitatively similar to those of termite mated pairs, where the present behavior of the leader significantly predicted the future behavior of the follower (Wilcoxon signed-rank tests,  $p < 0.01$ , compare Figures 4C and S8). This corresponds to the similar functions inferred for *Diacamma* and termites, but note that *Diacamma* tandems had fewer interruptions than termite tandem runs (Figure S8).

## DISCUSSION

When ants emigrate, they use recruitment communication to share information and reach a consensus on a single nest. Both *Diacamma* and *Temnothorax* ants recruit via tandem runs, a behavior, which at first glance, seems similar in the many ant species where it is found.<sup>32</sup> However, we demonstrated that tandem runs of these two species are functionally and mechanistically distinct. We found that tandem followers of *Diacamma* do not actively regulate the movement of leaders but keep consistent close contact with their leaders. This suggests that *Diacamma* prioritizes avoiding lost followers over providing opportunities for followers to collect spatial information. In *Temnothorax* ants, this mass movement of colony members is conducted by social carrying, an alternative recruitment technique, where an active ant carries a nestmate in her mandibles. *Temnothorax* instead use tandem runs to recruit additional recruiters by sharing route information to the new nest (Figure 2). Unlike in *Diacamma*, *Temnothorax* followers actively regulate their leaders' movements to facilitate route learning. This contrasts with *Diacamma* followers who usually do not need to learn the route as they remain at the goal after arrival.

In addition to the different functions of tandem runs in *Diacamma* and *Temnothorax*, the interaction rules responsible for movement coordination are also distinct. Leaders of *Diacamma* control both the direction and speed of tandem runs, while *Temnothorax* leaders and followers consistently regulate each other's motion (Figure 4). In this sense, *Diacamma* tandem runs are like those used by termite mating pairs, where the female leader decides the course of movement during search for a nest site and the male follower strives not to be separated. Note that *Diacamma* leaders modify their behavior to facilitate coordination even though the interaction is unidirectional. For example, the leader's moving speed during tandem runs is slower than when the leader is alone,<sup>20</sup> contrasting with the termite leaders that show consistent movement speed even with male followers.<sup>33</sup> As a result, *Diacamma* tandem runs are highly stable with few interruptions (Figure 4). We, therefore, suggest that the *Diacamma* tandem run is a recruitment behavior that is specialized for preventing separation during emigration. Conversely, *Temnothorax* tandem runs can be seen as maximizing information sharing at the risk of frequent follower loss.

The distinct tandem runs of *Diacamma* and *Temnothorax* presumably reflect their different nest-site selection process. In *Temnothorax* ants, colonies live in preformed cavities such as hollow nuts or rock crevices, rather than building their own nests. This places a premium on their ability to find and select a high-quality nest from the available options.<sup>34</sup> Site quality may be critical for colony defense from competitors,<sup>35</sup> and suitable nest sites are limited resources<sup>36</sup> for which colonies compete.<sup>37</sup> Therefore, *Temnothorax* workers rely on tandem runs for information sharing and collective decision-making,<sup>38</sup> which requires route-learning function at the expense of stability. In contrast, *Diacamma* ants live in rainforests, where heavy rains result in nest flooding and demand rapid evacuation to temporary sites.<sup>39</sup> Thus, colonies usually fragment to



multiple temporary sites and then unite at the final location during nest emigration.<sup>40</sup> In this condition, *Diacamma* workers rely much more on individual decisions than collective decision-making,<sup>41</sup> which enables them to achieve highly stable tandem runs since a route-learning function is not required.

In conclusion, convergent evolution is often considered an outcome of similar evolutionary pressures occurring in different lineages. However, similarity in phenotype does not always guarantee the same functional capacities in natural history.<sup>11</sup> Similar morphology has been associated with different functions among different lineages, as evidenced in the tree growth habit (tall plants with a thickened single trunk<sup>42</sup>), foot webbing in salamanders,<sup>43</sup> and saber-shaped teeth in carnivores.<sup>44</sup> We show that this is even true for animal social behavior. Tandem running behaviors of *Temnothorax* ants and *Diacamma* ants play different functional roles during colony emigration, and similar movement coordination comes from different forms of interactions.

### Limitations of the study

In this study, we engaged in the in-depth functional analysis of two distantly related ant genera, *Temnothorax* and *Diacamma*, to illustrate the clear difference in their behavioral mechanisms. However, this captures only two of the 15 independent evolutionary origins of ant tandem runs (Figure 1). Thus, further comparative studies on other ant genera (e.g., *Camponotus* and *Pachycondyla*) with species-specific ecological contexts will be essential to comprehend the evolutionary causes of tandem runs with distinct functions.

Also, in *Diacamma* tandems, a few followers turn into leaders after tandem runs (Figures 2B, 2C, 3A, and 3F). Thus, followers of *Diacamma* ants might acquire some information without a *Temnothorax*-like route-learning protocol, which remains unknown. For example, tandem followers of *Diacamma* might collect spatial information through a stride integrator<sup>45</sup> or optic flow<sup>46</sup> during travel, as observed in *Cataglyphis* desert ants. Alternatively, some followers might have already experienced the route in a different context (e.g., foraging) and thus could become leaders without learning during tandem runs.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
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- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Comparison of recruitment dynamics
  - Recruitment network analysis
  - Trajectory analysis
  - Information transfer between tandem partners
  - Phylogenetic comparative analysis

### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2023.106418>.

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## AUTHOR CONTRIBUTIONS

N.M., Conceptualization, methodology, software, validation, formal analysis, data curation, writing-original draft, writing-review & editing, visualization, supervision, project administration, and funding acquisition. Y.T., Conceptualization, methodology, formal analysis, investigation, resources, data curation, and writing-review & editing. G.V., Conceptualization, methodology, software, resources, and writing-review & editing. T.O.R., Resources and writing-review & editing. S.A., Resources and writing-review & editing. S.C.P., Conceptualization, methodology, writing-review & editing, and supervision. H.S., Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing-review & editing, supervision, project administration, and funding acquisition.

## DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>Deposited data</i>		
Recruitment networks and movement trajectories of <i>Diacamma cf indicum</i>	This paper	Zenodo: <a href="https://doi.org/10.5281/zenodo.7688262">https://doi.org/10.5281/zenodo.7688262</a> github.com/nobuaki-mzmt/diacamma-tandem
Recruitment networks of <i>Diacamma indicum</i>	Kolay and Annagiri <sup>47</sup>	Dryad: <a href="https://doi.org/10.5061/dryad.3nc0g">https://doi.org/10.5061/dryad.3nc0g</a>
Recruitment networks of <i>Temnothorax albipennis</i>	Richardson et al. <sup>48</sup>	ESM: <a href="https://doi.org/10.1098/rspb.2017.2726">https://doi.org/10.1098/rspb.2017.2726</a>
Recruitment networks of <i>Temnothorax nylanderii</i>	Richardson et al. <sup>49</sup>	Zenodo: <a href="https://doi.org/10.1234/tandem_running">https://doi.org/10.1234/tandem_running</a>
Recruitment networks of <i>Temnothorax rugatulus</i>	Valentini et al. <sup>50</sup>	ESM: <a href="https://doi.org/10.1098/rspb.2019.2950">https://doi.org/10.1098/rspb.2019.2950</a>
Movement trajectories of <i>Temnothorax rugatulus</i>	Valentini et al. <sup>17</sup>	Figshare: <a href="https://doi.org/10.6084/m9.figshare.9786260">https://doi.org/10.6084/m9.figshare.9786260</a>
Movement trajectories of <i>Reticulitermes speratus</i> and <i>Coptotermes formosanus</i>	Mizumoto and Dobata <sup>51</sup>	ESM: <a href="https://doi.org/10.1126/sciadv.aau6108">https://doi.org/10.1126/sciadv.aau6108</a>
Molecular phylogeny and recruitment data of ants	Reeves and Moreau <sup>13</sup>	Senckenberg: <a href="https://doi.org/10.26049/ASP77-2-2019-10">https://doi.org/10.26049/ASP77-2-2019-10</a>
<i>Experimental models: Organisms/strains</i>		
<i>Diacamma cf indicum</i>	Kwansei Gakuin University	N/A
<i>Software and algorithms</i>		
Analysis codes	This paper	Zenodo: <a href="https://doi.org/10.5281/zenodo.7688262">https://doi.org/10.5281/zenodo.7688262</a> github.com/nobuaki-mzmt/diacamma-tandem
FastTrack	Gallois and Candelier <sup>52</sup>	<a href="https://doi.org/10.1371/journal.pcbi.1008697">https://doi.org/10.1371/journal.pcbi.1008697</a>
UMATracker	Yamanaka and Takeuchi <sup>53</sup>	<a href="https://doi.org/10.1242/jeb.182469">https://doi.org/10.1242/jeb.182469</a>

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Nobuaki Mizumoto ([nobuaki.mzmt@gmail.com](mailto:nobuaki.mzmt@gmail.com)).

#### Materials availability

The study did not generate new unique reagents.

#### Data and code availability

- The data and code needed to reproduce the results in the publication are available at Zenodo with <https://doi.org/10.5281/zenodo.7688262>, and Github: [github.com/nobuaki-mzmt/diacamma-tandem](https://github.com/nobuaki-mzmt/diacamma-tandem).
- The datasets associated with previous studies are publicly available. The sources are listed in the [key resources table](#).
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### Network analysis

##### *Diacamma cf indicum*

We used four colonies of *D. cf indicum* collected in Onna and Naha, Okinawa, Japan, between August and October 2021 (colony ID: K4, K8, SA, SB; colony size: 115, 127, 111, 129 workers + one gamergate, respectively). Each colony was maintained in an experimental nest, a plastic container (24.1 cm × 14.1 cm × 3.7 cm) fill with plaster and covered with a translucent red plastic plate. Until the experiment, the nest was connected to a foraging area (41 cm × 26 cm × 7 cm) that was provided with a water tube and food (mealworms and standard artificial diets<sup>54</sup>) under laboratory conditions (25 ± 1 °C with stable light-dark cycle 14: 10 light: dark). We marked all workers by using a unique combination of four colors for individual identification.

### *Diacamma indicum*

Sixteen colonies of *D. indicum*, collected from Mohanpur, West Bengal, India, between September 2013 and May 2014 (colony ID: see Figure S9; colony size:  $133.9 \pm 37$  adults [average  $\pm$  s.d.] + one gamergate), were used in the experiments. All workers had unique colored paint marks. Further details can be found in ref.<sup>47</sup>

### *Temnothorax rugatulus*

Three colonies of *T. rugatulus*, collected in the Pinal Mountains near Globe, Arizona, in 2009 (colony ID: 6, 208, 3004; colony size: 78, 81, and 33 workers + one queen, respectively), were used in the experiments. All workers had unique colored paint marks. Further details can be found in ref.<sup>50</sup>

### *Temnothorax albipennis*

Six colonies of *T. albipennis*, collected on the Dorset coast, UK, during 2011 (colony ID: 1–6; colony size: 72–113), were used in the experiments. All workers were tagged with RFID micro transponders (PharmaSeq, NJ, USA) for individual identification. Further details can be found in ref.<sup>48</sup>

### *Temnothorax nylanderi*

Twelve colonies of *T. nylanderi*, collected in the Forêt de Dorigny, Switzerland, between June and October 2018, were used in the experiments (colony ID: 1\_1-3\_4; colony size 71–116 workers). All workers had unique colored paint marks. Further details can be found in ref.<sup>49</sup>

## Trajectory analysis

### *Diacamma cf indicum*

We used four colonies of *D. cf indicum* (colony size: 136–160) collected in Onna and Nakijin, Okinawa, Japan, in August 2021. Each colony was maintained in a humid artificial nest covered by a red plastic plate (14.8 cm  $\times$  8.4 cm  $\times$  3.2 cm) connected to a foraging area (24 cm  $\times$  18 cm  $\times$  8.5 cm). Other conditions were same as for the network analysis.

### *Temnothorax rugatulus*

Six colonies (colony size: 30–60) collected in the Pinal Mountains near Globe, Arizona, during September 2017 were used in the experiment. Further details can be found in ref.<sup>17</sup>

## Termites

Two colonies with alates of *Coptotermes formosanus* were collected in Wakayama, Japan, in June 2017, and five colonies of *Reticulitermes speratus* were collected in Kyoto, Japan, in May 2017. After a controlled flight in the lab, dealates (individuals that had shed their wings) were used for tandem run experiments. Further details can be found in ref.<sup>51</sup>

## METHOD DETAILS

### Ant recruitment experiment

To examine recruitment dynamics in ant tandem runs, we used datasets for leader-follower combinations of tandem runs during colony emigration. In *Diacamma*, recruitment is only via tandem run, while *Temnothorax* recruitment can be via tandem or social carrying. Only tandem recruitment data were available for *D. cf indicum* from Japan, *D. indicum*, *T. albipennis*, and *T. nylanderi*. Both tandem run and social carrying data were available for *T. rugatulus*. Note that we treated *D. indicum* and *D. cf indicum* separately because of their locational segregation and historical distinction.<sup>55</sup> *D. indicum* is reported from the eastern and southern parts of India and Sri Lanka.<sup>56</sup> *D. cf indicum* refers to an ant population, closely related to *D. indicum* but separated in a distance. This population is from Okinawa Island, Japan, has long been reported as “*Diacamma* sp. from Japan,” and is still awaiting a formal taxonomic description.<sup>57</sup> The context of each emigration experiment is as follows:

### *Diacamma cf indicum*

Colony emigration of *D. cf indicum* was observed in a square arena (100 cm  $\times$  100 cm  $\times$  30 cm) filled with sand collected from the field (Kwansei Gakuin University, Japan). The old and new nests were positioned at 55 cm. To inform the colony about the environment of the experimental arena, we kept the colony in this

arena for three days with sufficient food and water. The new nest was unroofed during this period and thus unavailable for the colony. After three days, we moistened and covered the new nest to make it available. As we did not add water to the old nest while maintenance, the old nest was drier than the newly moistened nest, whereas the new and wet nest was preferable for *Diacamma* colonies. We also removed the red plate on the old nest to induce colony emigration. We recorded nest emigration using three digital video cameras (HC-V480MS, Panasonic, Japan). One recorded the entire arena, and other two were located at the top of the entrance to the new nest and the old nest, so that we can confirm which individuals were coming in or out of the nests. To examine the combination of leaders and followers for each tandem running event, we recorded the time and combination of IDs of each leader and follower at the entrance of the new nest.

#### *Diacamma indicum*

Experiments with *D. indicum* were performed as part of a study on tandem leader removal during multiple emigrations.<sup>47</sup> The colony emigrated from the old and broken nest to the identical new nest. Colony emigrations were observed over a wooden bridge (1520 mm) that connected the old and new nests. Each colony emigrated twice: an initial control emigration and the following leader or random member removal experiment. We used data from the control emigration for this study. We recorded the time and the participating workers of all tandem runs during colony emigration.

#### *Temnothorax rugatulus*

Experiments with *T. rugatulus* were performed as part of a study on the division of labor during multiple emigrations.<sup>50</sup> The colony emigrated from an old and broken nest to either of two new candidate nests with different quality (good and mediocre). The two new nests were 50 cm from the old nest in a rectangular arena (37 cm × 65 cm). Each colony emigrated five times with a rest interval of two to five days between emigrations. The time and involved workers were recorded for all tandem running and social carrying events during colony emigration.

#### *Temnothorax albipennis*

Tandem running interaction in *T. albipennis* was recorded in experiments to study collective decision-making.<sup>48</sup> The colony emigrated from a nest of poor quality to either of two better nests of identical quality. These three nests were placed in a rectangular arena (45–75 cm) with an equilateral-triangle arrangement. The emigration procedure was repeated five times for each colony at seven-day intervals. The time and involved workers were recorded for all tandem running events during colony emigration.

#### *Temnothorax nylanderi*

Tandem running interactions in *T. nylanderi* were recorded in leader or follower removal experiments.<sup>49</sup> The colony emigrated from a low-quality and half-broken nest to either of two new nests with better and identical quality. These three nests were placed in a rectangular arena (46–78 cm) with a triangle arrangement. Each colony was subjected to five emigrations at one-week intervals between successive emigrations. Specialized leaders or followers were removed before the fifth emigration. Thus, we used the data for the 1<sup>st</sup> through 4<sup>th</sup> emigrations in this study. Participating workers were recorded for all tandem runs during colony emigration.

### Obtaining tandem trajectory

We used movement trajectories of leaders and followers in tandem pairs from two ant species, *D. cf indicum* and *T. rugatulus*, and two termite species, *Coptotermes formosanus* and *Reticulitermes speratus*. In ant species, tandem recruitment was used during nest emigration, and all tandem running pairs reached the new nest location. In summary, we obtained trajectories longer than 15 minutes (15, 20, 17, 20 trajectories for *D. cf indicum*, *T. rugatulus*, *C. formosanus*, *R. speratus*, respectively) and used the frames for the first 15 minutes (= 26,972 frames for each trajectory) for the following analysis. We also checked the robustness of our analysis by using different thresholds, such as 10 minutes (29, 20, 17, 20 trajectories for each species) or 5 minutes (46, 20, 17, 20 trajectories for each species), and confirmed that this did not affect our conclusion. The tandem observation experiment for each species is as follows:

#### *Diacamma cf indicum*

To obtain sufficiently long trajectories of tandem runs, we prepared a large experimental arena (100 cm × 100 cm × 30 cm) with a twisting bridge connecting the old nest to a new nest. The bridge consisted of 12

straight paths in parallel with 10 U-turns connecting two straight paths (Figure S5), resulting in a walking distance of ~950 cm between two nests. The design and dimensions of this arena were informed by a previous study on *T. rugatulus*.<sup>17</sup> We introduced the old nest to the experimental arena and then removed the roof to induce colony emigration. After introduction, workers explored the bridges to search for the emigration site (~ a few hours). Then once they found the new nest, they started tandem running from the old nest to the new nest. We recorded the whole this emigration process using a digital video camera (HC-VZX992M, Panasonic, Japan) that was located at the top of the arena at 30 frames per second with FHD resolution.

For each colony, we selected 10 (or 16 for one colony) tandem runs that were longer than 5 minutes. This is because our information transfer analysis requires long time series datasets, and a previous study suggested 5 minutes is sufficiently long to detect information transfer.<sup>17</sup> Note that there were many shorter and successful tandem runs in which ants moved from an old nest to a new nest within 5 minutes. This suggests that our analysis might underestimate the stability and efficiency of tandem runs of *D. cf. indicum*. We subdivided the whole video into multiple clips so that each clip includes one selected tandem running event. In total, we obtained 46 video clips.

Extracting trajectories of a leader and a follower in a tandem running pair is challenging because partners are often in contact with each other. Most tracking software recognizes a tandem pair as a single individual and fails to distinguish partners from each other. The coordinates of tandem partners can be well extracted using UMATracker software,<sup>53</sup> as evidenced by previous studies.<sup>17,33,51,58</sup> One of the prerequisites of the UMATracker is a fixed number of individuals in a video frame. However, this is not the case in ant colony emigration experiments, where ants continuously appear and disappear in the arena by entering or coming out of the nests (Figure S6A). A previous study manually subdivided the video and trimmed the frames so that one video clip included a fixed number of ant individuals.<sup>17</sup> As this process is time-consuming, we needed an alternative solution to efficiently obtain data from multiple species. To overcome this challenge, we established a system to track ant tandem running behavior semi-automatically by combining another tracking software, FastTrack,<sup>52</sup> with UMATracker (Figure S6). Although FastTrack cannot recognize a tandem running pair as two different partners, it can handle a changing number of objects in a region of interest, which is suitable for analyzing ant colony emigration events.

Using FastTrack, we first recognized a tandem running pair as a single object and extracted the rough trajectory of the centroid of the pair (Figure S6B). Because FastTrack skips any frame in which it failed to recognize an object, we gave coordinates of the tandem in skipped frames by using R to calculate the averaged values of the frames before and after the skipped frames. Next, from the tentative coordinates of tandem runs, we created a video that showed only the focal tandem running pair, using the OpenCV library and Python scripts. We did this by making a background frame by averaging every 1,000 frames throughout the clips. Then, we covered the video clips with this background frame except for a 20 × 20 pixel area surrounding the focal tandem coordinates; this masked all individuals other than the focal tandem pair (Figure S6C). Finally, we used UMATracker on this masked video clip to extract the trajectories of the tandem leader and follower (Figure S6D). This process inevitably missed pairs in the masked video clip when they were separated by more than ~20 pixels. To analyze these, we applied UMATracker to the original videos for these frames by manually specifying the region of interest. Finally, our videos sometimes missed tandem running pairs that went behind the bridge (46 events, 25,209 frames in total, ≈2.20% of all frames) or due to the blackout between two successive video clips imposed by our video camera (13 events, 668 frames in total, ≈0.06% of all frames). For these exceptional frames, we estimated the coordinates by assuming that ants were moving linearly and at a constant speed. The Python script for this process is available in the datasets. The tracking results are sufficiently accurate to identify tandem running behavior (Figure S12).

As a result, we obtained 46 trajectories of the centroid of the leaders and followers at 30 frames per second (1,146,126 frames). Among them, 15 are longer than 15 minutes, and 29 are longer than 10 minutes. Trajectories were converted from pixels to millimeters using a scaling factor estimated by measuring known features of the experimental arena with ImageJ.<sup>59</sup> The body length was also measured using ImageJ (mean ± s.d. = 10.54 ± 0.86 mm).



### *Temnothorax rugatulus*

Trajectories of tandem leaders and followers were available from previously published work on *T. rugatulus*.<sup>17</sup> Each colony was induced to move from an old nest to a new nest located at a diagonal position in a large experimental arena (370 × 655 mm). The arena was subdivided by five barriers (10 mm by 310 mm), forming a twisting path to observe sufficiently long tandem runs. The colony emigration was recorded once for each colony at 30 frames per second using a video camera with 1K resolution. One to six tandem runs that lasted more than 15 min were selected for each colony, and the trajectories of the leader and the follower were extracted using the UMATracker software platform. The centroids of each runner's body were tracked at 30 frames per second. Trajectories were converted from pixels to millimeters using a scaling factor estimated by measuring known features of the experimental arena with ImageJ.<sup>59</sup> The body length was also measured using ImageJ (mean ± sd = 2.34 ± 0.3 mm).

### Termites

Trajectories of tandem leaders and followers were available for *Reticulitermes speratus* and *Coptotermes formosanus*.<sup>51</sup> A female and a male termite were introduced in the experimental arena (a Petri dish filled with moistened plaster, diameter = 145 mm), and their behavior was recorded for 60 minutes. In total, 17 pairs were observed for *C. formosanus* and 20 pairs for *R. speratus*. The resolution of the videos was reduced to 640 by 480 pixels for movement tracking. The centroids of each runner's body were tracked at 30 frames per second. Trajectories were converted from pixels to millimeters by using as a scale the diameter of the arena petri dish (145 mm). We used the body length measured in<sup>17</sup> (*C. formosanus*: mean ± sd = 8.89 ± 0.42 mm, *R. speratus*: mean ± sd = 5.5 ± 0.3 mm). In this study, we only present the results of *C. formosanus* because they showed a qualitatively similar pattern compared to ant tandem runs.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Comparison of recruitment dynamics

Information on the whole emigration was available in *D. cf indicum*, *D. indicum*, and *T. rugatulus*, while only tandem run (initial stage) information was available in *T. albipennis* and *T. nylanderii*. We first compared the proportion of individuals engaged in each recruitment activity between *D. cf indicum*, *D. indicum*, and *T. rugatulus*. We used generalized linear mixed models (GLMMs) with binomial errors and a logit link function. The recruitment type was treated as an explanatory variable, and each unique emigration event was included as a random effect (random intercept). The likelihood ratio test was used to assess the statistical significance of the inclusion of each explanatory variable (type II test). The GLMM was used for each of six pair-wise comparisons of four different recruitment types. The analysis was performed for the proportion of leaders and followers separately. GLMM analyses were conducted using the 'car' and 'lme4' packages.<sup>60</sup>

We next investigated the behavioral sequence of individuals during colony emigrations for each species. The behavioral states of *Diacamma* (or *T. albipennis* and *T. nylanderii*) included three states: tandem leader, follower, and end of the colony emigration, while *T. rugatulus* included five states: tandem leader, follower, social carrier, being carried, and end of the colony emigration. We calculated the probability of transitioning from one state to another for each state. Passive individuals (tandem followers or being carried) can become active recruiters (tandem leaders or social carriers) after recruitment. Using Fisher's exact test, we compared the probability of a passive ant being activated across species.

### Recruitment network analysis

In total, we obtained 125 networks (each network corresponds to each emigration event and recruitment tactic: *D. cf indicum*: 4, *D. indicum*: 16, *T. albipennis*: 30, *T. nylanderii*: 48, *T. rugatulus* [tandem]: 13, *T. rugatulus* [social carrying]: 14). The sequence of tandem runs (or social carrying) was represented as a static network. Ants who participated in recruitment were represented as nodes, and tandem runs were represented as directed links pointing from the leader (or carrier) to the follower (or being carried). Note that no tandem was observed in one emigration event in *T. rugatulus*. Networks with <20 nodes were removed from the analysis (*D. indicum*: 0/16, *D. cf indicum*: 0/4, *T. albipennis*: 20/30, *T. nylanderii*: 7/48, *T. rugatulus* tandem: 11/13, *T. rugatulus* carrying: 0/14).

First, we examined the in-degree and out-degree distributions to compare the overall network structures between *Diacamma* and *Temnothorax* tandem recruitment structures. We compared these distributions

using Kolmogorov-Smirnov (KS) test (data were pooled for each genus). Also, we compared the proportion of workers with one incoming edge and no outgoing edge between recruitment types. We fitted a GLMM with binomial error and a logit link function, where the recruitment type was treated as an explanatory variable, and each emigration event was included as a random effect (random intercept). In cases of significant effects, we ran Tukey's post hoc test using the `glht()` function in the 'multcomp' package.<sup>61</sup>

Next, we investigated the motifs of these structures to compare the property of network structures. Network motifs are overrepresented small subgraphs in a given network.<sup>28</sup> The motif property is reported to be independent of colony size in other social insect research (e.g.,<sup>62</sup>) and thus ideal for our comparison between species. There are 13 possible directed subgraphs for three-node patterns. We used the function `graph.motifs()` in the R package 'igraph'<sup>63</sup> to obtain the number of each subgraph for each network. Then, we compared the proportion of two specific patterns (motifs 1 and 2), which were overrepresented in recruitment networks, between lineages and recruitment types (*Diacamma tandem*, *Temnothorax tandem*, and *Temnothorax carrying*).

The proportion of subgraphs was compared between lineages and recruitment types (*Diacamma tandem*, *Temnothorax tandem*, and *Temnothorax carrying*). We used GLMMs, similar to the above comparison of the proportion of passive workers. Note that the size of the network (= number of nodes) was different between recruitment types, and we confirmed that there was no relationship between the number of nodes and the proportion of each motif (Figure S7).

### Trajectory analysis

We down-sampled all videos to a rate of five frames per second (FPS) (= every 0.2002 s) for the analysis in this section. To compare the stability of tandem runs between species, we defined two individuals to be in tandem when the distance between their centroids was less than two body lengths.<sup>17</sup> As a result, we obtained 21, 1523, 43, and 89 tandem run events for *D. cf indicum*, *T. rugatulus*, *C. formosanus*, and *R. speratus*, respectively. We compared the duration of tandem runs using the mixed-effects Cox model (`coxme()` function in the 'coxme' package in R<sup>64</sup>), with species as a fixed effect and pair id as a random effect. The random effect accounted for the inclusion of multiple tandem events for each pair of termites. The likelihood ratio test was used to determine the statistical significance of each explanatory variable (type II test). Observations interrupted by the end of the video were treated as censored data.

### Information transfer between tandem partners

The parallel information flows within the same behavior can be obtained by extending transfer entropy to different symbolic representations of the same trajectory datasets (i.e., patterns embedded in symbols representing the direction of motion or the speed of motion).<sup>17</sup> We applied the same methodology to the tandem running behavior of *D. cf indicum* to compare it with previously published analyses of *T. rugatulus* and termites (refer to<sup>17</sup> for a detailed description of this methodology).

We first discretized trajectories of each runner to obtain time series describing the pausing and rotation patterns.<sup>17</sup> For the pausing pattern, the behavior of each runner was either of two states: pause (P) or motion (M). For the rotation pattern, it was either clockwise (CW) or counter-clockwise rotation (CCW). The P/M states were distinguished from each other using the threshold for step length, the distance traveled by a runner between two successive frames. The threshold was set as the 10th percentile of the probability distribution of step length.<sup>17</sup> A step length shorter than the threshold was represented as P; otherwise was represented as M. The threshold was obtained separately for each species and sampling period. The CW/CCW states were distinguished based on the direction of motion computed as the cross-product of movement vectors between successive frames. If no rotation was detected (i.e., cross-product equal to 0), the rotation direction was copied from the previous time step.

Given that  $L$  and  $F$  are behavioral sequences of the leader and the follower, transfer entropy from  $L$  to  $F$  is defined as

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

where  $l_i$  and  $f_i$  are the values of sequence  $L$  and  $F$  at time  $i$ , respectively, and  $f_i^{(k)}$  is the  $k$ -history of  $F$  at time  $i$  (i.e., the last  $k$  states in the sequence). We computed the transfer entropy in both directions,  $T_{L \rightarrow F}$  and  $T_{F \rightarrow L}$ , and compared them to identify the predominant direction of information flow. The difference in transfer entropy between the two directions,  $T_{L \rightarrow F} - T_{F \rightarrow L}$ , is called net transfer entropy.<sup>17,31</sup> The value is positive when information flow from leader to follower is predominant ( $T_{L \rightarrow F} > T_{F \rightarrow L}$ ) and negative when flow from follower to leader ( $T_{L \rightarrow F} < T_{F \rightarrow L}$ ) predominates.

For comparison purposes, we processed transfer entropy as follows. First, to ensure that the results were not an artifact of finite sample size, we artificially created surrogate datasets by randomly pairing time series of leaders and followers from different tandem pairs.<sup>17,31</sup> We computed transfer entropies for these datasets and discounted their mean from experimental data for data correction. This process enables us to focus on the causal interaction between tandem partners because leaders and followers from different pairs lack causal interactions by definition but are influenced by the same environmental cues of the experimental arena. We generated 50 surrogate datasets for each species and parameter configuration. Additionally, we obtained normalized transfer entropy by dividing it by its maximum value.<sup>17,31</sup> Normalized transfer entropy ranges from 0, when the leader and follower are independent of each other, to 1, when the follower behavior is entirely determined by the leader behavior.

There are two parameters in our information-theoretic analysis, the sampling period of continuous spatial trajectories and the history length of transfer entropy,  $k$ . The parameter combinations that maximize the efficiency of detecting information flow can be variable across species or behaviors.<sup>17</sup> To find optimal parameters, we computed net transfer entropy for 900 different parameter configurations for each species (history length  $k \in \{1, \dots, 20\}$  and sampling period  $\{0.0334s, \dots, 1.5015s\}$ ). The resulting landscapes of net transfer entropy show robustness to different parameter values over most of the tested range (Figure S4). We selected the parameter configurations that maximize the net transfer of information. For the chosen parameter configurations, we performed two statistical tests. First, we tested if the experimental data showed significantly greater values of transfer entropy with respect to the surrogate data. We used one-sided two-sample Wilcoxon rank-sum tests with continuity correction. Second, we tested differences in the flows of information between the two possible directions (from leaders to followers and from followers to leaders) to determine which among the leader and the follower was the predominant source of information. We used one-sided paired Wilcoxon signed-rank tests with continuity correction. All information-theoretic measures were computed using the 'rinform-1.0.1' package in R.<sup>65</sup>

### Phylogenetic comparative analysis

We summarized the information about ant tandem running behavior at the genus level. If at least one species in the genus shows tandem running behavior in any context (recruitment to food, nest, or slave-hunting sites), we counted that genus has tandem running behavior. Almost all of the information comes from,<sup>13</sup> which compiles the information about foraging modes in ant species. As<sup>13</sup> did not consider tandem running behavior during colony emigration, we newly included *Diacamma* (used in this study) and *Hypoponera*<sup>32</sup> as genus that performs tandem running. We considered that tandem running is absent in the genus with information on foraging modes<sup>13</sup> but without tandem running information.

We modified the species-level ant phylogeny reconstructed by.<sup>22</sup> This phylogenetic tree included 1730 ant species from all extant subfamilies and 317 of the 334 extant genera. Because species in this phylogeny did not entirely match with the species with tandem or foraging information, we reduced the tips to build a genus-level phylogenetic tree. Note that this phylogeny randomly selected one representative species for every genus, and thus the polyphyletic genera, such as *Tetramorium*, were represented as monophyletic groups occupying singular phylogenetic positions. We also removed genera without information on foraging mode.<sup>13</sup> As a result, the tree included 161 genera (Figure S1).

We estimated marginal ancestral states of tandem running behavioral ability in ants, using the function `re-rootingMethod()` in R package 'phytools'.<sup>66</sup> We used a maximum likelihood model with an equal rate of transition among states.

All of the data analysis was performed using R v4.0.1.<sup>67</sup> The R scripts and data are available in the datasets.