The adaptive significance of social information in tandem running ants

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SUMMARY

Eusocial insects, especially ants, are widespread and play an important role in many ecosystems all over the world. They have developed communication strategies to collaborate with nestmates to exploit resources and raise their brood. In this dissertation I mainly focused on two ant species that perform tandem runs to recruit nestmates: *Temnothorax nylanderi* and *Pachycondyla harpax*. In **chapter 1** I focused on the question which recruitment strategy was used by ancestral ant species. We found that mass or group recruitment are the most probable ancestral strategies. Over time, ant species with smaller colony size developed and species started recruiting via tandem running, which evolved several times independently. Also, several ant lineages lost recruitment of nestmates. Environmental factors like food source distribution play important roles and can influence the behaviour of workers. In empirical tests (**chapter 2**) and with an agent-based simulation model (**chapter 3**) we analysed the influence of food quality and quantity on the information-use strategy of foragers. The results suggest that recruitment by tandem running is beneficial when food sources are hard to find and long lasting. Additionally, we found that scouts find food sources of average quality, but recruit nestmates to high quality food sources more often, when resources are abundant. Remarkably, scouts can improve their success over time by switching food sources more often. In **chapter 4** we tested if foragers adapt their recruitment behaviour in response to increasing foraging distances. We found that different foraging distances did not influence the success rate of tandem runs, but tandem success increased when leaders gained experience and lead several tandem runs. Also walking speed increased with distance, probably to reduce the exposure to risks. Furthermore, tandem followers waited longer after a break-up or contact loss, when the tandem run lasted longer. In **chapter 5** we analysed the influence of body size difference on the success of tandem runs. We found that with increasing size variation, the success rate of tandem pairs decreased. When the leader-follower size differed more than 10% in body length, tandem runs were unsuccessful, whereas ~80% of tandem runs were successful, when body length differed less than 5 %. In **chapter 6** we performed recruitment experiments in the field with *P. harpax* and tested how competition affects the recruitment behaviour in tandem running ants. When foragers were able to recruit nestmates, they had a higher probability to maintain access to food sources and could exploit them successfully. Even when competition was intense, a slow recruitment method like tandem running is likely to be beneficial for the colony when they reach a threshold of foragers at the resource.

GENERAL INTRODUCTION

The value of social information in tandem running ant species

Simone M. Glaser

Ants are so much like human beings as to be an embarrassment. They farm fungi, raise aphids as livestock, launch armies into war, use chemical sprays to alarm and confuse enemies, capture slaves, engage in child labour, exchange information ceaselessly. They do everything but watch television.

Lewis Thomas

Communication and recruitment strategies

Living in a society is a daily challenge for animals. They constantly communicate to exchange up-to-date information about environmental changes. Individuals exchange information between two (or more) individuals to actively distribute the information (Boyd & Richerson, 1995; Brown & Laland, 2003; Horner *et al.*, 2010; Dawson & Chittka, 2014; Lanan, 2014). Social insects in particular have evolved a large number of varied communication forms and routes of information flows (von Frisch, 1967; Wilson, 1971; Beckers *et al.*, 1989; Hölldobler & Wilson, 1990; Leadbeater & Chittka, 2007; Lanan, 2014). We can find a variety of different strategies like using tactile interactions (Möglich *et al.*, 1974; Grooters, 1987; Laland, 2004; Franklin, 2014), different chemosensory signals for finding a mate or as a defence or alarm signal (Jaffe, 1984; Hölldobler & Wilson, 1990, 2009; Czaczkes *et al.*, 2015b). Probably the most important and valuable information are about the condition of the brood, nest quality and food supply. To relocate the colony or to exploit food sources successfully, several species recruit nestmates to increase the number of individuals at a special location.

In ants, the most common strategies to recruit nestmates are tandem running, group-recruitment and mass-recruitment, which are strongly connected to colony size (Jaffe, 1984; Beckers *et al.*, 1989; Czaczkes & Ratnieks, 2012; Czaczkes *et al.*, 2015b). During a tandem run a scout leads a naïve follower to a location in a tandem pair (Wilson, 1959). The leader and follower constantly interact with each other to ensure body contact (Franks & Richardson, 2006; Richardson *et al.*, 2007). In group recruitment or group raids, a single individual provides social information for a few to dozens of individuals. After finding a valuable resource, the scout usually lays a chemical trail back to the nest, and then leads a group of nestmates to the location (Hölldobler, 1971). Thereby, the leader can have body contact with several followers. Ant species that use chemical mass recruitment may lay short- or long-term trails by depositing pheromones on a trail from a food source back to the nest. Nestmates can follow the trail and often add more pheromones on the trail to reinforce it (Wilson, 1962).

It was hypothesized that ancestral ant species that appeared around 100 mya did not use recruitment strategies (Moreau *et al.*, 2006). With time, more complex recruitment strategies may have evolved (Hingston, 1929; Hölldobler *et al.*, 1974; Ward, 2014; Schultheiss *et al.*, 2015). These authors speculated that tandem running evolved first as a primitive and simple recruitment strategy. According to this view, this further developed via group recruitment to pheromone trails (Fig. Intro. 1). Reeves & Moreau (2019) analysed the ancestral state of foraging in ants and support the hypothesis that the ancestral state was solitary foraging. An alternate evolutionary scenario could be that the ancestral ant species used mass recruitment and evolving species lost the ability and developed other recruitment strategies. As far as is known, early ants had medium sized colonies, containing up to several thousand ants (Burchill & Moreau, 2016) which is generally connected to the use of group or mass recruitment (Beckers *et al.*, 1989). In ant species with smaller colonies, often not enough workers are available to reinforce and maintain the pheromone trails.

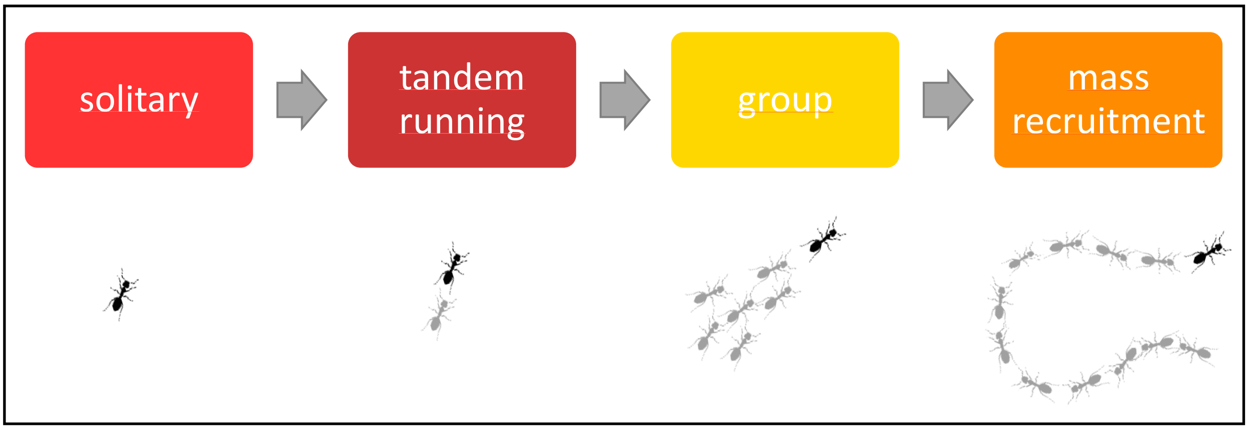

Figure Introduction 1: Possible evolutionary steps of recruitment strategies in ants. No recruitment developed via tandem running and group recruitment to chemical mass-recruitment.

Another intriguing question in the early evolution of recruitment is the original context where recruitment was first used. Recruitment communication could have evolved to support nest relocations for colonies to enable fast and precise emigrations to new nest sites, e.g. to improve nest quality or because of an emergency, like the destruction of the old nest. Alternatively, ants might have first used recruitment communication during foraging. When individual ants find a scarce or high quality food source it can be advantageous to guide nestmates to the resource (Dornhaus *et al.*, 2004; Langridge *et al.*, 2004; Grüter *et al.*, 2018). Foragers can exploit the resource faster and increase the energy intake for the colony. Depending on the recruitment strategy and environmental conditions, ants can choose between different strategies for foraging.

Information-use strategies

Recruitment communication is not the only option that foragers have to collect food. Another possibility to forage successfully is to use different kinds of information. Insect species and especially ants utilize different information-use strategies to collect information about valuable resources (Leadbeater & Chittka, 2007; Czaczkes *et al.*, 2011a; Grüter *et al.*, 2013; Sasaki & Pratt, 2013; Franklin, 2014; Smolla *et al.*, 2015; Dunlap *et al.*, 2016). If individuals need or want to gather information about their environment, like high quality nest sites or good food sources to provide their colony with necessary nutrients, they can use individual, private or social information. Individual learning means that they explore the environment by themselves, also called scouting, collecting and learning new information through trial-and-error (Laland, 2004; Galef & Laland, 2005; Rendell *et al.*, 2010; Heyes, 2012). If individuals already possess knowledge about their environment, they can rely on their memory and use information they already collected before, e.g. returning to the location of a food source and exploit it, that is private (or personal) information use (Kendal *et al.*, 2005; Czaczkes *et al.*, 2011a; Grüter & Leadbeater, 2014; Smolla *et al.*, 2016). Finally, and as discussed earlier, an important strategy in insect societies is the use of social information/learning. Social learning is learning that is influenced by observing or interacting with other individuals or their products, like pheromones (Laland, 2004; Galef & Laland, 2005; Kendal *et al.*, 2005; Heyes, 2012; Grüter *et al.*, 2013). Social learning involves an informed individual providing social information during recruitment, chemical cues, and transmitting the information to an observer (recruit) that learns the information. Using social information is thought to provide a higher pay-off than individual learning because individuals recruit their nestmate to above average and highly rewarding food sources (Shaffer *et al.*, 2013; I'Anson Price *et al.*, 2019). This means the recruiters filter information by removing unrewarding or redundant information about low quality resources (Rendell *et al.*, 2010). It is also expected that recruits benefit from social information because they do not have to do costly trial-and-error-learning (Pratt, 2008; Grüter *et al.*, 2010; Rendell *et al.*, 2010). The probability of finding poor food sources, meeting competitors and predators is lower when receiving and using social information.

Generally, social insects use their memory, *i.e.* they rely on private information, visiting the same location again, as long as the food source is stable and rewarding (Al Toufailia *et al.*, 2013b; Dawson *et al.*, 2013). If the environment changes and food sources are not valuable or rewarding anymore individuals can adapt to the situation and switch their information-use strategy, trying to collect new information or receive social information from a nestmate (Grüter & Ratnieks, 2011; Shaffer *et al.*, 2013). Often individuals use a combination of different strategies. The question of when and why individuals use different information-use strategies received a lot of attention during the last decades (Laland, 2004; Rendell *et al.*, 2010).

Despite this research, however, it is still not well understood in which situations social learning is more beneficial than individual exploration or private information use (Franks *et al.*, 2010; Schürch & Grüter, 2014; I'Anson Price & Grüter, 2015). There can be different reasons why and when individuals copy others, *i.e.* use social information. "When"-strategies include situations where individuals copy others when their own established behaviour is unproductive, like when a food source that they visited in the past is not rewarding anymore. Then individuals might switch from using private information to social information (Laland, 2004). A further possibility is to copy others when individual learning is costly. When exploring the environment by themselves, individuals do not have knowledge about predators, competitors or other risks, so individuals can copy others that have the information about safe options (Laland, 2004; Rendell *et al.*, 2010). Another possibility is that individuals copy others when they are uncertain or inexperienced, because they might possess no relevant knowledge about the environment (Laland, 2004; Smolla *et al.*, 2016). Copying particularly successful individuals could also be beneficial, e.g. after a food transfer via trophallaxis an individual can compare the sample with its memory and estimate the quality of the food source (Laland, 2004; Leadbeater & Chittka, 2007).

Social insects as models to study social learning

Social insects are easy to trance and manipulate individually when they use variable forms of social learning. Probably the most famous recruitment strategies in insects are the waggle dance in honey bees (*Apis mellifera*) (von Frisch, 1946, 1967). During the dance, a forager communicates the location of a valuable food source to her nestmates. The dance followers thereby can learn and decode the distance and direction of the location and fly to the indicated food patch. In bumble bees (*Bombus terrestris*) and many stingless bees (Meliponini) another process called local enhancement is often used. Thereby, individuals use the presence of nestmates and other bees on flowers to learn flowers that might be rewarding food sources (Leadbeater & Chittka, 2005; Dawson *et al.*, 2013; Grüter, 2020). In different ant species we find a variety of different recruitment strategies where scouts provide social information to their nestmates (Lanan, 2014). Mass-recruiting ants lay pheromone trails to provide information for nestmates about the resource location (Beckers *et al.*, 1989; Hölldobler & Wilson, 1990). In ant species that have smaller colonies another form of recruitment called "tandem running" is used. Currently, tandem running is the only known example of "teaching" in non-mammals (Franks & Richardson, 2006; Richardson *et al.*, 2007). This high variability of strategies, mainly during foraging, suggest that social information is certainly advantageous. Independent of the recruitment strategy, the provision of social information leads to a faster sharing of information among the nestmates and thus, they can exploit resources faster.

Costs and benefits of social information

Several factors can influence the value and thus the benefit of social information like the current state of a colony or ongoing environmental changes. Generally, individuals just transmit information about high valuable resources like rewarding food sources or suitable new nest sites (Dornhaus *et al.*, 2004; Grüter & Ratnieks, 2011). When the environment is changing or food sources are overexploited, new and more valuable food source can appear. Then it can be advantageous when foragers use individual learning and are searching for new food patches (Shaffer *et al.*, 2013; Grüter &

Leadbeater, 2014). By sharing social information, the colony can adapt and switch to more rewarding food sources (Shaffer *et al.*, 2013). The receivers of social information then do not have to invest energy and time in costly trial-and-error learning but can receive low-cost and filtered information about valuable resources (Grüter *et al.*, 2010; Rendell *et al.*, 2010). Furthermore, it is expected, that individuals can save time when they receive social information (Franks *et al.*, 2003). Colonies can then emigrate faster to a nest site or reach a food source quicker (but see Seeley, 1983; Seeley & Visscher, 1988) and can share the information in the nest. Another important factor during recruitment is the distance to the resource because it can influence the foraging success and recruitment probability. So far there were just a few studies testing the effects of resource distance on tandem running ants (e.g. O`Shea-Wheller et al., 2016). Individuals have to spend more energy for a longer trip, so the net energy gain decreases with an increasing foraging distance (Fewell *et al.*, 1992). For mass recruiting ants, honeybees and stingless bees it has been found that foragers decrease their recruitment intensity with increasing food distance (von Frisch, 1967; Seeley, 1986; Devigne & Detrain, 2006). Contrary, in tandem running ants the opposite has been found. With an increasing travel duration the number of tandem runs increased in *Temnothorax albipennis* (O'Shea-Wheller *et al.*, 2016). A negative side effect of an increasing distance could be that the tandem pair likely loses contact which leads to a break-up. When leader and follower do not find each other again the follower often is lost and returns to the nest without collecting food (Basari *et al.*, 2013).

The food source distribution plays an important role for the costs and benefits of recruitment. When food sources are scarce and hard to find, recruitment of nestmates can safe time (Seeley, 1986; Schürch & Grüter, 2014). Often individuals have to invest time and wait in the nest for their nestmates to return and share information with them (Seeley, 1989; Dechaume-Moncharmont *et al.*, 2005). The use of social information is likely to be beneficial if the waiting time combined with the travelling time after the information exchange is shorter than the time spent for individual trial-and-error learning. When food sources are abundant, individuals can easily find them by themselves and do not need to wait for a nestmate to be recruited (Seeley, 1986; I'Anson Price *et al.*, 2019). Research with honeybees suggest that using social learning and following waggle dances does not always increase colony foraging success (Schürch & Grüter, 2014; I'Anson Price *et al.*, 2019) and strongly depends on the environment. Furthermore, social information can be outdated and the resource not rewarding anymore. The food source could already be overexploited by nestmates or competitors (Laland, 2004; Rendell *et al.*, 2010). In mass recruiting ant species the use of social information can also be costly (Grüter *et al.*, 2011; Czaczkes *et al.*, 2016). On pheromone trails, foragers provide positive feedback and strengthen the trail, thus even more nestmates follow the trail. This makes it difficult to relocate foragers to a better food source that appears later. Then foragers often do not follow a new emerging foraging trail, because the pheromone signal is weaker than on the old trail and the group is trapped in following the foraging trails with more pheromones on it (Czaczkes *et al.*, 2016).

Social information could also be important when competition is intense and many colonies forage for the same food sources. When resources are scarce, the species that discovers the source first or can exploit it most rapidly will be the most successful colony. Often species adapt to this by occupying special dietary niches, e.g. by exploiting selected types of food (Torres, 1984; Houadria *et al.*, 2015) or they show temporal activity patterns to avoid competition (Stuble *et al.*, 2013; Rosumek, 2017). Despite these adaptations of niche differentiation, ant species cannot avoid competition entirely. In this case the recruitment of nestmates can help colonies to monopolize and exploit food sources successfully (Traniello, 1987; Lach *et al.*, 2010; Drescher *et al.*, 2011).

Study system of this thesis and tandem running as rare recruitment strategy

In this thesis, I studied two ant species, one of which allowed me to perform controlled experiments in the laboratory (*Temnothorax nylanderi*), whereas the second species was particularly suited for field experiments (*Pachycondyla harpax*). The ant species *Temnothorax nylanderi* is widespread in Western and Central Europe and is often found in small rotten sticks or acorns on the forest floor (Foitzik & Heinze, 1998). Competition for nests is harsh as nests are easily destroyed, which forces colonies to migrate to new nest sites. The colonies are rather small and generally contain between a few dozen to a couple of hundred workers and a single queen in a nest (Foitzik & Heinze, 1998). They forage for small insect prey or collect honey dew on the forest ground (Stroeymeyt *et al.*, 2017). The Neotropical species *Pachycondyla harpax* ranges from the Southern USA to Northern Argentina. Colonies range from 15-100 individuals and are most active during the night (Wheeler, 1900). Nests are usually found in the shade and they can have several entrances (Grüter *et al.*, 2018). They are diverse in their food selection and forage for carbohydrate and protein-rich food sources. When the resource or prey is too large or heavy to be transported by a single worker, both species perform tandem runs to recruit nestmates to exploit the food source.

After returning to the nest, a successful ant tries to attract a naïve nestmate by releasing pheromones, so called "tandem calling" (Möglich *et al.*, 1974). When a nestmate reacts to the signal and as soon as she touches the body of the recruiter, the tandem run starts. From now on the pair constantly maintain body contact. The tandem follower touches the gaster and hind legs of the tandem leader to ensure contact. If the pair lose contact, the leader waits and the follower searches around the position of contact loss. During the tandem run, the follower learns the position of the resource by memorizing landmarks that help to orient herself (Basari & Laird-Hopkins, 2014). Thereafter she can herself become a tandem leader. Thereby more and more nestmates can learn the location (Fig. Intro. 2). In *Temnothorax albipennis* it has been shown that tandem followers learn the specific routes from their tandem leaders (Sasaki *et al.*, 2020). Trajectories back from the food source to the nest were significantly more similar compared to routes taken by other tandem runs.

Figure Introduction 2: Marked foragers of the species *Temnothorax nylanderi* forage on a droplet of sugar solution in the lab (left, photo by S. Glaser). A dead insect is exploited by nestmates of a *Pachycondyla harpax* colony (from Grüter et al. 2018).

T. nylanderi and *P. harpax* are very promising model organisms for studying social learning during foraging. In honeybees, the most studied social insect, it is often impossible to see the information-use strategy dance followers use after following a waggle dance because it is difficult to follow the flight of bees (Grüter & Farina, 2009). As a result, it is often unclear if dance followers use private or social information. Also for mass recruiting ant species it is difficult to know if foragers walking on a patch follow the pheromone trail, use their memory or a combination of the two strategies (Czaczkes *et al.*, 2016). While observing tandem running ant species in the laboratory or in nature it is easy to observe which information-use strategy individuals use. Furthermore, individual ants can easily be tracked and manipulated individually. Also the environmental conditions like food source distribution, food quality and quantity can be easily manipulated.

Aims of this Thesis

A vast number of social insect species recruit nestmates to valuable resources. It has been intensely studied whether they use scents or pheromones and how the environment influences colony decisions. Many studies focused on the influence of colony size (Beckers *et al.*, 1989; Bengston & Dornhaus, 2013) and foraging behaviour of the individual, measuring trail laying (Czaczkes *et al.*, 2011a; Basari & Laird-Hopkins, 2014), but very few studies have quantified individual or colony foraging success.

The overall aim was to expand our knowledge about how different information-use strategies influence foraging success in tandem running ants with the focus to better understand the importance of social learning. In tandem running ants we can easily identify the information-use strategy that foragers are using, which makes this recruitment strategy a uniquely suited behaviour to explore some of the major open questions regarding social learning and the value of recruitment communication. As mentioned earlier, it has been hypothesized that early ants did not recruit nestmates to food sources and that different recruitment strategies evolved over time (Reeves & Moreau, 2019). In chapter 1, I investigated the ancestral recruitment strategy in the Formicidae, focusing on the question if recruitment strategies in general became more complex during ant evolution. Furthermore, in chapter 2 and 3 I studied when and how tandem running ants adapt their information-use strategy depending on the environment. This topic has mainly been studied in honeybees (Dornhaus *et al.*, 2006; I'Anson Price *et al.*, 2019) and ant species that use pheromone trails (Czaczkes *et al.*, 2011a; Grüter *et al.*, 2011; Czaczkes *et al.*, 2016). Thus, I concentrated on the question how social learning affects the success of individuals and the colony of *T. nylanderi* depending on the abundance and variability of food sources in the environment. In chapter 4, I focused on the question how food source distance influences the behaviour of tandem leader and follower of *T. nylanderi* and how they adjust their behaviour to increasing distances. In many ant species there is a natural variation of worker body size, which is related to specific tasks (Dejean *et al.*, 2005; Westling *et al.*, 2014). Also, in colonies that are apparently of similar size, small differences in their development can lead to phenotypic variation in body size (Okada *et al.*, 2013). In chapter 5, I studied the question if there are intra-colonial differences in information-use linked to variation in worker morphology of *T. nylanderi*. In chapter 6, I more closely investigated the importance of tandem recruitment in a highly competitive habitat. Under natural conditions I observed individuals of *P. harpax* and how they reacted and adapted their recruitment strategy in the presence of competitors at food sources.

CHAPTER 1

Ancestral state reconstruction suggests repeated losses of recruitment communication during ant evolution (Hymenoptera: Formicidae)

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Abstract

Eusocial insects have evolved different strategies to share information about their environment and can recruit nestmates to food sources or new nest sites. Ants are the most species-rich social insect group and are known to use pheromones, visual and tactile signals to communicate and inform nestmates about resources. However, how these different strategies evolved and whether there was a predominant evolutionary sequence that led to present day recruitment strategies is not well understood. In our study we explored two competing hypotheses about the ancestral recruitment communication: (1) ant ancestors did not recruit nestmates and species evolved more complex recruitment strategies over time vs. (2) early ants used mass-recruitment, which was lost repeatedly in some lineages. We combined an extensive search of scientific literature and ancestral state reconstruction to estimate the ancestral recruitment strategy, focusing on (i) no recruitment, (ii) tandem running, (iii) group-recruitment and (iv) chemical mass-recruitment. Stochastic character mapping suggests that mass-recruitment was ancestral in ants (59-61%), whereas "no recruitment" was unlikely to be the ancestral condition (21%). Similarly, marginal ancestral state reconstruction suggests that mass-recruitment (44-81%) or group-recruitment (48-50%) represented the original state. Our results are consistent with the finding that early ants lived in colonies containing up to several thousand individuals, which are typically associated with mass-recruiting in ants. However, our ability to robustly identify patterns in the evolution of communication in ants remains hampered by a lack of natural history information for most ant species.

Introduction

Communication about resources is widespread in social insects and different species have evolved a variety of strategies to communicate with other individuals (von Frisch, 1967; Wilson, 1971; Hölldobler & Wilson, 1990; Leadbeater & Chittka, 2007; Franklin, 2014; Grüter & Leadbeater, 2014; Czaczkes *et al.*, 2015b; Grüter, 2020). Senders can use visual, tactile (body contact in honeybees, Grooters, 1987; antennation, Hölldobler & Wilson, 1990; von Frisch, 1967) or chemical (e.g. pheromones in ants, Czaczkes et al., 2015; Hölldobler & Wilson, 1990) signals to share information about food locations, nesting locations, dangers or the needs of individuals and the colony.

Ants (Family: Formicidae) are an extraordinarily diverse, widespread and ecologically important group of social insects, containing over 13,000 extant species (Reeves & Moreau, 2019). Ants first appeared in the early Cretaceous, more than 100 million years ago, most likely from a lineage of solitary wasps (Moreau *et al.*, 2006; Ward, 2014). Ants have evolved a variety of different communication strategies to bring nestmates to places where work is needed. Recruitment communication can be behaviourally complex (Hölldobler, 1999) and some species combine several strategies during recruitment.

Several classification systems for recruitment behaviours have been proposed (Jaffe, 1984; Beckers *et al.*, 1989; Lanan, 2014). The most common strategies are the use of pheromone trails (Hölldobler & Wilson, 1990, 2009; Czaczkes *et al.*, 2015b), group-recruitment (Hölldobler, 1971; Liefke *et al.*, 2001), tandem recruitment (Franklin, 2014; Glaser & Grüter, 2018; Grüter *et al.*, 2018) or no recruitment at all (solitary/individual) (Jaffe, 1984; Beckers *et al.*, 1989). In tandem recruitment, a scout with knowledge about a valuable resource recruits a single nestmate to a resource. The follower maintains contact with the leader by antennating the gaster and hind legs of the latter. In group-recruitment, a scout leads a group of up to thirty nestmates to a resource. A short-lived, volatile pheromone emitted by the leader helps recruited ants stay close to the leader (Möglich *et al.*, 1974). In mass-recruitment, successful scouts lay pheromone trails between the nest and the resource, which is followed and strengthened by nestmates. Some species use a particular strategy in one context but not in another. For example, *Neoponera* or *Diacamma* species perform tandem runs when they relocate to a new nest site, but when they are foraging for prey they do so solitarily (Fresneau, 1985; Kaur *et al.*, 2017). Furthermore, recruitment communication can depend on the type of food source that is collected. When collecting small prey, ant species often do not recruit nestmates as they can carry home the food item by themselves, but they can use recruitment communication when finding larger resources, like honey dew-secreting aphid colonies or large prey items (Lach, 2005; Detrain & Deneubourg, 2008; Czaczkes *et al.*, 2011a; Czaczkes & Ratnieks, 2012). These examples highlight that the use of recruitment communication often depends on the ecological context.

Another important determinant of recruitment strategy is colony size. Species with small colonies (<1000 individuals) often do not use recruitment or they perform tandem runs, whereas medium size colonies (up to a few thousand individuals) tend to recruit nestmates by group-recruitment or by pheromone trails and large colonies use mainly pheromone trails (Beckers *et al.*, 1989). Recruitment via pheromone trails requires colonies to have a certain number of foragers to continuously deposit pheromones on the path and, thereby, keep the trail attractive for recruits (Beekman *et al.*, 2001). The link between colony size and recruitment strategy is not rigid, however, and species with similar colony sizes can differ in the strategies they employ (Beckers *et al.*, 1989).

This diversity in recruitment strategies raises the question how these strategies evolved and whether certain forms of recruitment tend to precede other methods in the Formicidae. One hypothesis is that recruitment communication increased in complexity over evolutionary time. According to this scenario, early ants would have foraged solitarily, like many present-day ponerine ants (Maschwitz & Schönegge, 1983; Beckers *et al.*, 1989; Villet, 1990). Subsequently, small-scale communication mechanisms evolved, like tandem-running and group-recruitment. From these forms of communication, mass-recruitment with longer-lasting chemical trails may have evolved (Beckers *et al.*, 1989; Traniello, 1989b; Hölldobler & Wilson, 1990). Hingston (1929) and Wilson (1959), for instance, suggested that tandem running may have been used by early ants. Short-lived pheromones released by the tandem leader play a potentially important role for the cohesion between the leader and follower in a tandem run (Basari & Laird-Hopkins, 2014). It would then have been a small evolutionary step to produce longer lasting trail pheromones that allowed mass-recruitment in species with larger colony sizes. The hypothesis of an increase in scale – from small-scale to large-scale recruitment – has recently been supported by Reeves & Moreau (2019) who suggest solitary foraging as the ancestral recruitment strategy.

A phylogenetic analysis by Burchill & Moreau (2016), on the other hand, suggested that early ant species had medium colony sizes, with colonies containing up to several thousand individuals, which is typically associated with mass-recruitment in extant ants (Beckers *et al.*, 1989). This suggests that mass-recruitment may have been a more suitable strategy for early ants. Following this argument, recruitment ability would have been lost over time in some lineages as ant species with small colony sizes evolved (Burchill & Moreau, 2016). Likewise, the antiquity of tandem running has been questioned given that this behaviour is also found in species that are considered more derived, such as in *Temnothorax* and *Leptothorax* (Planqué *et al.*, 2010). This suggests that tandem running may be a derived behaviour, at least in some groups, that evolved multiple times in different ant lineages.

The main aim of this study was to estimate the ancestral state of recruitment communication of the Formicidae. Unlike Reeves & Moreau (2019) who focused on recruitment communication during foraging only, we also considered whether species use recruitment during emigrations. This was done because there are numerous ant species that do not communicate during foraging even though they use recruitment communication in other situations (Fresneau, 1985; Kaur *et al.*, 2017; Grüter *et al.*, 2018). The value of communication in foraging depends on the foraging ecology of a species, such as the kind of food that is exploited and the food source distribution (Sherman & Visscher, 2002; Dornhaus *et al.*, 2006; I'Anson Price *et al.*, 2019). Thus, the strategies used during foraging reflect the foraging ecology of a species and provide only a partial picture of the recruitment strategies in the behavioural repertoire of a species. To better understand the evolution of recruitment communication mechanisms, we deem it important to consider communication to different types of resources, including nest sites. In addition, we aimed to answer the question whether tandem running was indeed an early recruitment strategy that preceded group- and mass-recruitment (Hingston, 1929; Wilson, 1959).

Material & Methods

Literature research for recruitment strategies

Data were collected on the recruitment strategies used by extant ant species via an extensive search of the published scientific literature (from October 2019 to March 2020). For many ant species, information about recruitment was collected from reviews or articles about recruitment strategies (Jaffe, 1984; Beckers *et al.*, 1989; Silvestre *et al.*, 1999). Furthermore, we searched in Google Scholar using the search terms (ant species or genus in combination with "recruit", "forag", "prey", "individual", "solitary", "tandem", "group", "trail", "pheromone"). We included species-level information when the recruitment strategy was described based on observations or collected in controlled experiments. Data were coded as discrete character traits. Each species was allocated to one of four different recruitment strategies, similar to Jaffé (1984), Beckers et al. (1989) and Lanan (2014): no recruitment, tandem running, group-recruitment and mass-recruitment (Table 1.1). Reeves & Moreau (2019) found that the different classification systems led to very similar outcomes in their ancestral state reconstruction.

Table 1.1: Recruitment classifications and definitions, largely based on Jaffe (1984).

Recruitment strategy	Definition
Solitary/individual	No recruitment, no information transfer between nestmates
Tandem running	A single ant (scout) attracts a single nestmate using antennal contact and then physically leads a nestmate to the goal. Physical contact is often maintained between scout and nestmate, chemical signals may be used.
Group-recruitment	A scout recruits "up to thirty nestmates" and leads them to the goal. Chemical signals are often used for short-distance attraction but physical contact between scout and the group is also used.
Mass-recruitment	Groups are guided via chemical trails alone. Large numbers of ants can be recruited by a small number of recruiters. Chemical trails are laid on the substrate.

The literature search highlighted that there is a lack of detailed information about foraging strategies in the ant literature (see also Reeves & Moreau, 2019). Authors often mention that ants recruit their nestmates without providing details of the strategy and the context that was observed. Studies were excluded from our analysis if they only mentioned recruitment of nestmates without providing sufficient information about the method. If possible, we also collected and compared the recruitment data to Reeves and Moreau (2019), who collected data on the foraging strategies of ants. In some cases, we were unable to recover the information provided by these authors (e.g. *Buniapone amblyops*, *Mayaponera constricta* or *Megaponera analis*). Their study focused on recruitment during foraging only and analysed data at genus level. Thus, our analysis differs from theirs in the following ways: (1) we were interested in whether a species uses recruitment communication independent of context, *i.e.* we included recruitment during nest emigrations and during foraging. (2) We analysed our data at species level. *Tetramorium caespitum* uses both group and mass-recruitment (Collignon & Detrain, 2010). We considered this to be a mass-recruiting species for our analysis. Additionally, we used a subset that included only ant species that perform tandem runs and analysed if recruitment to nest sites or food sources was more likely to be the ancestral state.

Phylogenetic comparative methods

We modified the phylogenetic tree of Branstetter et al. (Branstetter *et al.*, 2017), which contains ~1000 ant species. In our literature research we found information for 161 species, 82 genera and 11 sub-families (Table S1.1) that were also present in the phylogenetic tree. Overall, the species included in our study represent 25% of genera and 65% of sub-families. Species with no recruitment data available were removed from the dataset with the *drop.tip* function in the R package "ape" (Paradis & Schliep, 2019; R Core Team, 2020).

We performed marginal ancestral state reconstructions (ASR) for the dataset, using the functions *fitMk* and *ace* from the R package "phytools" (Revell, 2012) and "ape" (Paradis & Schliep, 2019) to estimate the transition rates and the ancestral states for our tested character using a maximum likelihood (ML) approach. The *fitMk* function assumes that the probability to change from one state to another depends only on the current state and not on the state that has come before. Furthermore, every character state is equally likely to change to one of the other states. The *ace* function utilizes marginal reconstruction and returns the marginal ancestral state likelihood of all nodes within a phylogeny.

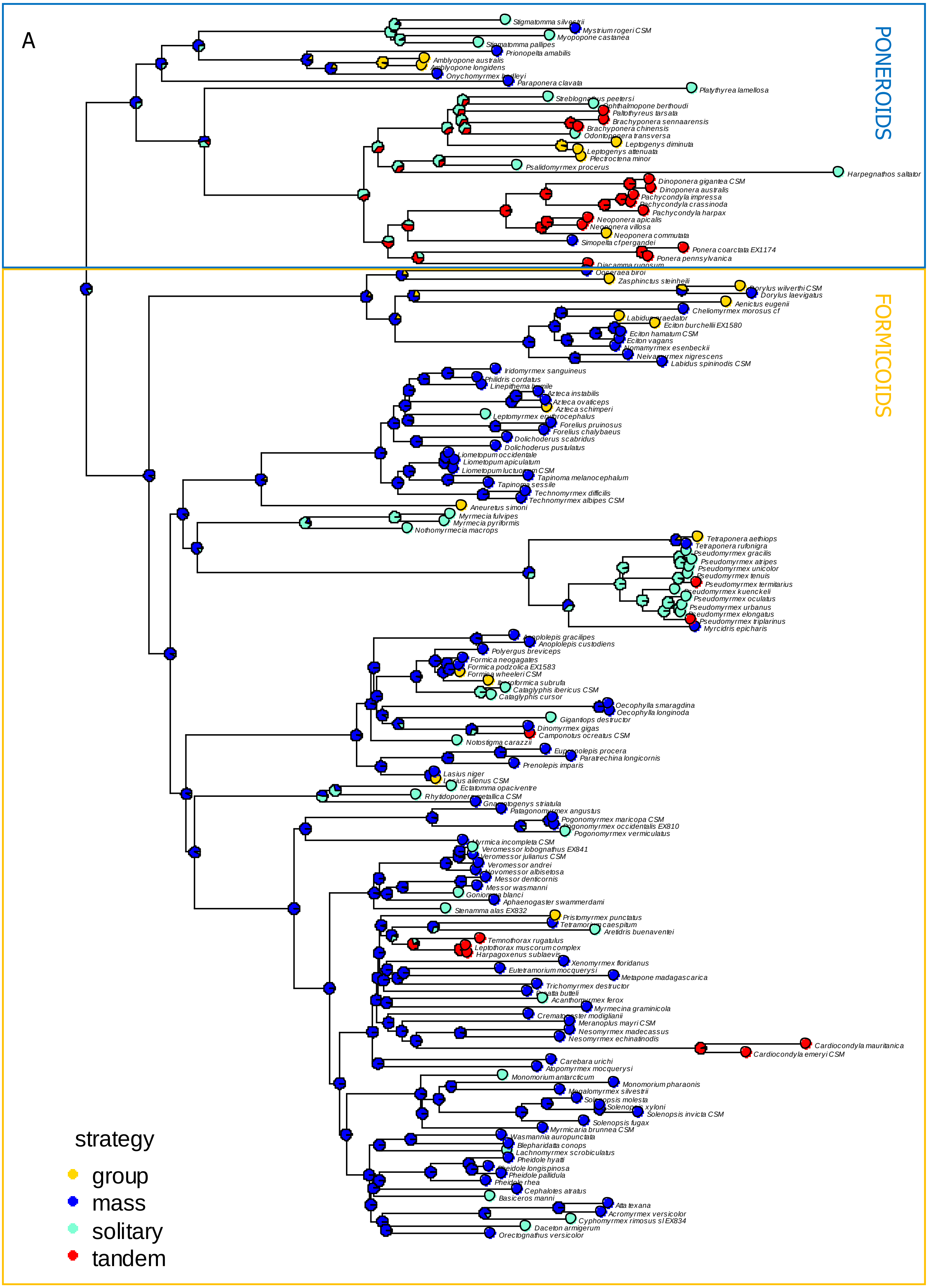
Additionally, we performed a stochastic character mapping (SCM) by using *make.simmap* from the R package "phytools". For the stochastic reconstructions of character states we used an MCMC approach, to explore the posterior probabilities of all nodes and provided the number of changes between the character states (1000 simulations performed).

Three commonly used transition rate models were analysed for the ancestral state reconstructions: "equal rates" (ER), "symmetrical rates" (SYM) and "all rates different" (ARD) with names referring to transition rates between each state. We used the Akaike information criterion (AIC) values corrected for small sample sizes (AICc values) for the three transition rates. We calculated the AIC-weights which standardize the AIC scores of the fitted models and measured the relative weight of evidence for the three models used in our data (Harmon, 2019). We visualized the results by mapping the ancestral state on the phylogeny with the function *plotTree*.

Results

Evolution of recruitment strategy

The ancestral state reconstruction results were mapped to our phylogeny (Fig. 1.1). The log-likelihood values, AIC values, AICc values and the number of free parameters per model are presented in Table 1.2. We compared the AIC and AICc values, which revealed that simple ER models were inferior and, thus, were rejected. A "symmetric model" and "all-rates-different model" best explained our transition between recruitment states. Thus, both transition models were used to analyse the ancestral state of recruitment strategies in ants.


A
PONEROIDS
Stigmatomma silvestrii
Mystrium rogeri CSM
Myopopone castanea
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Prionopelta amabilis
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Amblyopone longidens
Onychomyrmex hoedleyi
Paraponera clavata
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Streblognathus peetersi
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Paltothyreus tarsata
Brachyponera sennaarensis
Brachyponera chinensis
Odontoponera transversa
Leptogenys diminuta
Leptogenys attenuata
Plectroctena minor
Psalidomyrmex procerus
Harpegnathos saltator
Dinoponera gigantea CSM
Dinoponera australis
Pachycondyla impressa
Pachycondyla crassinoda
Pachycondyla harpax
Neoponera apicalis
Neoponera villosa
Neoponera commutata
Simopelta cf pergandei
Ponera coarctata EX1174
Ponera pennsylvanica
Diacamma rugosum
FORMICOIDS
Ooceraea biroi
Zasphinctus steinheili
Dorylus wilverthi CSM
Dorylus laevigatus
Aenictus eugenii
Cheliomyrmex morosus cf
Labidus praedator
Eciton burchellii EX1580
Eciton hamatum CSM
Eciton vagans
Nomamyrmex esenbeckii
Neivamyrmex nigrescens
Labidus spininodis CSM
Iridomyrmex sanguineus
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Tapinoma sessile
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Technomyrmex albipes CSM
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Myrmecia fulvipes
Myrmecia pyriformis
Nothomyrmecia macrops
Tetraponera aethiops
Tetraponera rufonigra
Pseudomyrmex gracilis
Pseudomyrmex atripes
Pseudomyrmex unicolor
Pseudomyrmex tenuis
Pseudomyrmex termitarius
Pseudomyrmex kuenckeli
Pseudomyrmex oculatus
Pseudomyrmex urbanus
Pseudomyrmex elongatus
Pseudomyrmex triplarinus
Myrcidris epicharis
Anoplolepis gracilipes
Anoplolepis custodiens
Polyergus breviceps
Formica neogagates
Formica podzolica EX1583
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Iberoformica subrufa
Cataglyphis ibericus CSM
Cataglyphis cursor
Oecophylla smaragdina
Oecophylla longinoda
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Dinomyrmex gigas
Camponotus ocreatus CSM
Notostigma carazzii
Euprenolepis procera
Paratrechina longicornis
Prenolepis imparis
Lasius niger
Lasius alienus CSM
Ectatomma opaciventre
Rhytidoponera metallica CSM
Gnamptogenys striatula
Patagonomyrmex angustus
Pogonomyrmex maricopa CSM
Pogonomyrmex occidentalis EX810
Pogonomyrmex vermiculatus
Myrmica incompleta CSM
Veromessor lobognathus EX841
Veromessor julianus CSM
Veromessor andrei
Novomessor albisetosa
Messor denticornis
Messor wasmanni
Goniomma blanci
Aphaenogaster swammerdami
Stenamma alas EX832
Pristomyrmex punctatus
Tetramorium caespitum
Aretidris buenaventei
Temnothorax rugatulus
Leptothorax muscorum complex
Harpagoxenus sublaevis
Xenomyrmex floridanus
Eutetramorium mocquerysi
Metapone madagascarica
Trichomyrmex destructor
Proatta butteli
Acanthomyrmex ferox
Myrmecina graminicola
Crematogaster modiglianii
Meranoplus mayri CSM
Nesomyrmex madecassus
Nesomyrmex echinatinodis
Cardiocondyla mauritanica
Cardiocondyla emeryi CSM
Carebara urichi
Atopomyrmex mocquerysi
Monomorium antarcticum
Monomorium pharaonis
Megalomyrmex silvestrii
Solenopsis molesta
Solenopsis xyloni
Solenopsis invicta CSM
Solenopsis fugax
Myrmicaria brunnea CSM
Wasmannia auropunctata
Blepharidatta conops
Lachnomyrmex scrobiculatus
Pheidole hyatti
Pheidole longispinosa
Pheidole pallidula
Pheidole rhea
Cephalotes atratus
Basiceros manni
Atta texana
Acromyrmex versicolor
Cyphomyrmex rimosus sl EX834
Daceton armigerum
Orectognathus versicolor
strategy
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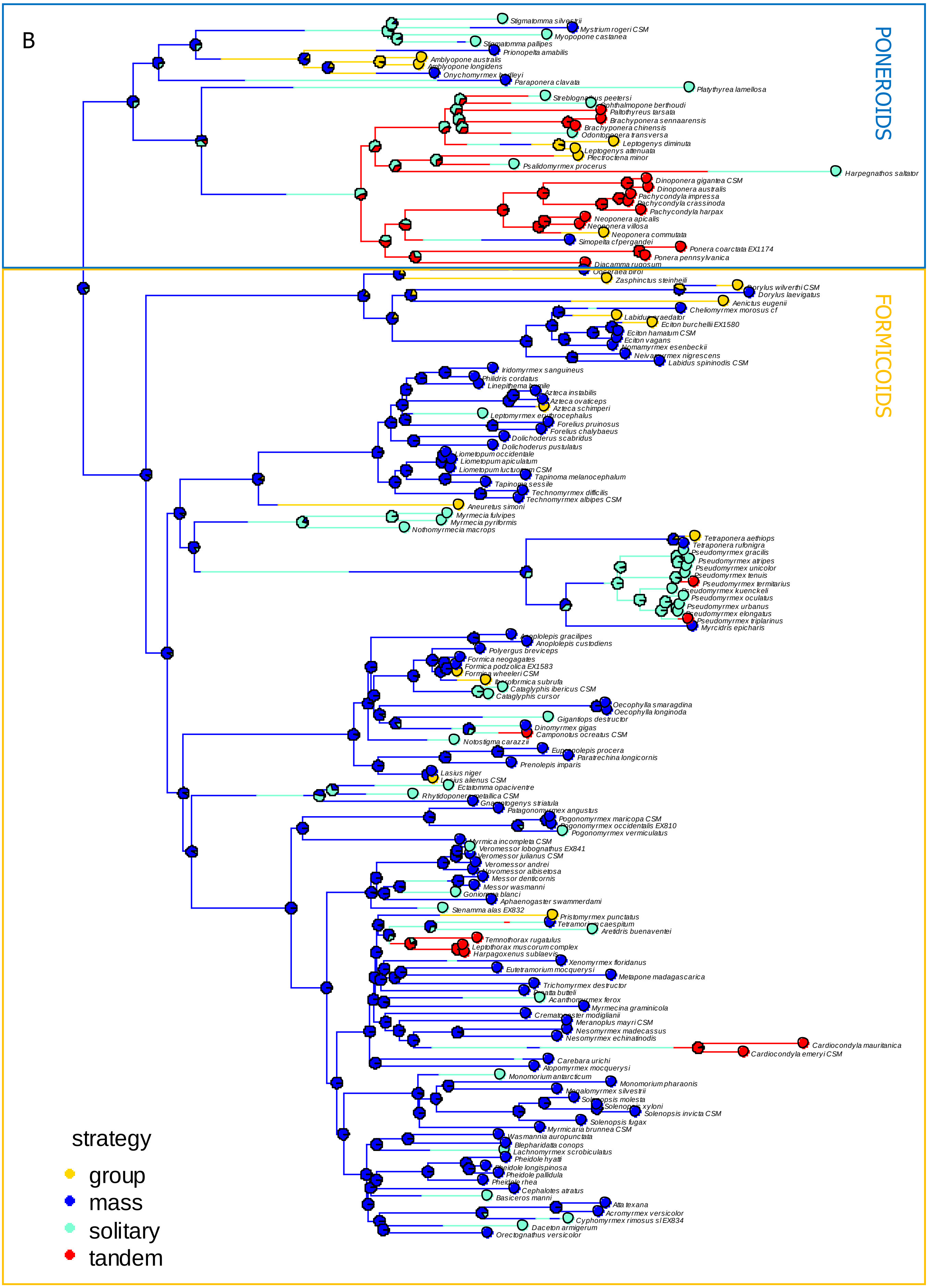
solitary
tandem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Pheidole rhea
Cephalotes atratus
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Acromyrmex versicolor
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Daceton armigerum
Orectognathus versicolor
strategy
group
mass
solitary
tandem

Figure 1.1: Ant phylogeny including recruitment strategy and (**A**) marginal ancestral state reconstruction or (**B**) stochastic character mapping. Nodes provide estimates based on Markov chain models. The phylogeny is based on Branstetter et al. (2017).

Table 1.2: Results of the transition rate models. Log likelihoods, Akaike information criterion values, number of free parameters and Akaike-weights are shown.

Model	LogL	AIC	AICc	free parameters	AICcW
ER	-170.0	336.9	342.0	1	0.03
SYM	-161.4	331.6	335.4	6	0.45
ARD	-155.7	331.4	337.6	12	0.52

Marginal ancestral state reconstruction analyses and stochastic character mapping both suggest that mass-recruitment is the most probable strategy at the root of the phylogeny (71.2% and 60.2%, respectively) (Table 1.3). Also, internal nodes (lineage splitting events) were dominated by high probabilities for the mass-recruitment category. Mass-recruitment was the most likely ancestral state in both the Poneroids and the Formicoids (Figure 1.1). The stochastic character mapping revealed that there were an estimated 81.3 changes between recruitment strategies (Table 1.4). The most common transitions were from mass-recruitment to solitary/individual behaviour (33.7%) or to group-recruitment (18.5%) (Figure 1.2). Furthermore, there were transitions from no recruitment to tandem running (14.8%) or mass-recruitment (12.7%). Tandem running evolved several times independently in the subfamilies Ponerinae, Pseudomyrmecinae, Formicinae and Myrmecinae. Furthermore, it seems that recruitment was lost at least once in all subfamilies, except in the Dorylinae (army ants). Similarly, we found group-recruitment in nearly all included sub-families, except in the Paraponerinae. The Myrmeciinae were the only group without species that perform mass-recruitment.

Table 1.3: Ancestral character estimation using marginal ancestral state reconstruction and stochastic character mapping. Values represent likelihoods of recruitment strategies at the root.

	Character states	Scaled root likelihood (fitMk)	Scaled root likelihood (ace)	Stochastic charact mapping (make.simmap)
SYM	No recruitment	0.15	0.16	0.21
	Tandem running	0.01	0.01	0.10
	Group-recruitment	0.02	0.02	0.07
	Mass-recruitment	0.81	0.81	0.61
ARD	No recruitment	0.05	0.06	0.21
	Tandem running	0.01	0.01	0.09
	Group-recruitment	0.50	0.48	0.12
	Mass-recruitment	0.44	0.45	0.59

Table 1.4: Changes from stochastic character mapping. GR = group-recruitment, MS = mass-recruitment, NR = no recruitment, TR = tandem running

	SYM		**ARD**	
Total changes	75.783		104.472	
Type	Number	Percentage	Number	Percentage
GR → MS	3.44	4.5%	32.013	30.6%
GR → NR	0.595	0.7%	0	0%
GR → TR	1.224	1.6%	0	0%
MS → GR	14.902	19.7%	23.572	22.6%
MS → NR	26.57	35.1%	27.514	26.3%
MS → TR	0.489	0.6%	0.952	0.9%
NR → GR	1.412	1.9%	7.409	7.1%
NR → MS	10.061	13.3%	0	0%

NR → TR	9.8	12.9%	9.572	9.2%
TR → GR	2.626	3.4%	3.44	3.3%
TR → MS	0.117	0.2%	0	0%
TR → NR	4.547	6.0%	0	0%

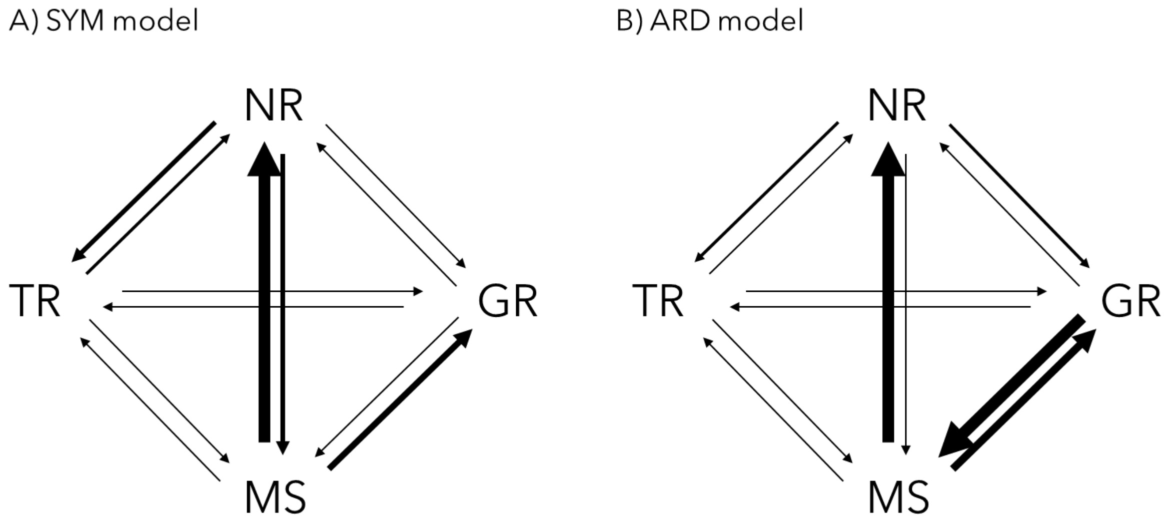

Figure 1.2: Transitions between states from stochastic character mapping for the methods (**A**) SYM and (**B**) ARD. The thickness of the arrow reflects the relative commonness of a transition. GR = group-recruitment, MS = mass-recruitment, NR = no recruitment, TR = tandem running

Evolution of tandem running

Twenty-one species included in our study perform tandem runs. For some species it is not known if they recruit to food sources and during nest relocations. For other species it is known that they perform tandem runs to new nest sites, but forage solitarily for food sources (*Neoponera, Diacamma* or *Paltothyreus*) (Table S1.1).

After mapping the recruitment strategy onto the phylogenetic tree (Figure 1.3), we found that all species that can perform tandem runs do so during colony emigrations to new nest sites. Several species (43%) that use recruitment via tandem running do not do so during foraging.

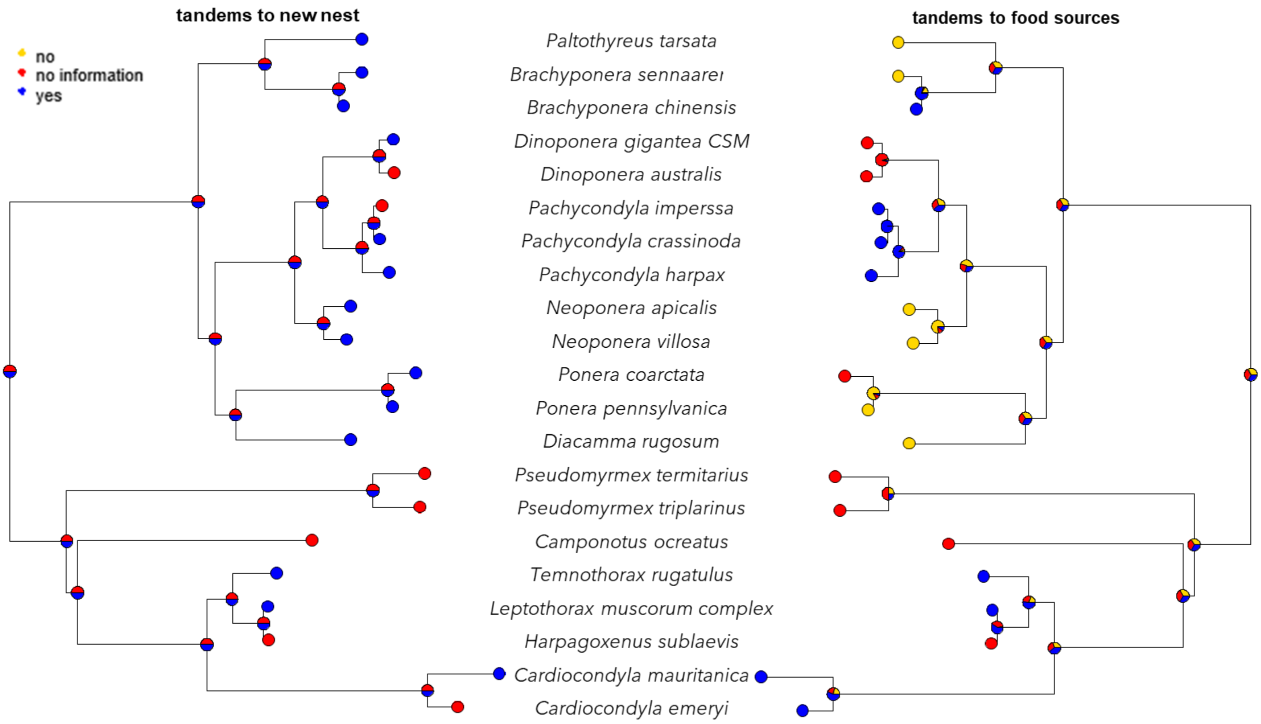

Figure 1.3: Ancestral state reconstruction for ant species that perform tandem running to either new nest sites, food sources or both. Nodes provide estimates based on Markov chain models.

Discussion

Our ancestral state analyses indicate that mass-recruitment or group-recruitment were likely used for recruitment by the common ancestor of present-day ants. During the course of their evolutionary history, all included subfamilies show switches from mass-recruitment to other recruitment strategies. Strikingly, most transitions occurred from mass recruitment to group or no recruitment. The repeated loss of communication seems puzzling, but probably coincided with the emergence of lineages with small colony sizes (e.g. in Amblyoponinae, Myrmeciinae, Ponerinae, Pseudomyrmecinae), where communication is less beneficial (Beckers *et al.*, 1989). Transitions between mass- and group-recruitment were also more frequent than between tandem running and no recruitment. Tandem running evolved independently in 4 of 11 subfamilies. Furthermore, there were also several transitions from no recruitment to tandem running or mass-recruitment. These findings highlight that recruitment strategies are an evolutionary flexible and reversible trait.

Many lineages appear to have lost the ability to recruit nestmates. The putative loss of chemical mass-recruitment could be explained by the constraints of living in a small colony (Beckers *et al.*, 1989; Dornhaus *et al.*, 2012) and/or a switch to a diet or foraging strategy that does not require pheromone trails. Smaller colonies tend to exploit resources solitarily or they use tandem running. One reason could be that smaller colonies do not have a sufficient number of ants to maintain pheromone trails (Beckers *et al.*, 1989; Beekman *et al.*, 2001; Planqué *et al.*, 2010). A recent study has found that the ancestral Formicidae probably had medium colony sizes containing up to several thousand individuals (Burchill & Moreau, 2016). These findings in combination with the findings that medium sized colonies often use group or mass-recruitment (Beckers *et al.*, 1989) are consistent with our results that the best-supported ancestral recruitment strategy was mass- or group-recruitment.

Another reason for the loss of mass-recruitment could be that recruitment by pheromone trails can have disadvantages. When a pheromone trail is continuously reinforced during foraging it can be difficult for the colony to re-allocated workers to a newly available higher-quality resource due to the strong positive feedback created by a pheromone trail. This makes recruitment less flexible and colonies are more likely to miss out on new food sources when the environment changes. During group-recruitment or tandem running, individual scouts can independently search for new food sources. Thereby, the foragers can more easily adapt to a changing environment and recruit their nestmates to the high-quality resources.

Reeves & Moreau (2019) found evidence that solitary foraging, rather than mass recruitment, represented the original state in terms of recruitment strategies. Our and their results do not necessarily contradict each other given that there are several differences between the studies that could account for the different results. Most importantly, we considered whether a species uses recruitment communication during colony emigrations and/or during foraging. As a result, we often allocated species to different recruitment categories than Reeves & Moreau (2019). It is well known that foraging methods in ants strongly depend on the foraging ecology (Davidson, 1977;

Hölldobler & Wilson, 1990; Dornhaus *et al.*, 2006; Dejean *et al.*, 2012; Lanan, 2014) and focusing only on foraging is likely to obscure the true taxonomic distribution of recruitment communication. For example, in numerous ant species, foragers follow a solitary foraging strategy when collecting insects prey, but they use recruitment communication when the colony emigrates to a new nest-site (Lanan, 2014). Another difference between the two studies is that we analysed ancestral states at species level to include differences among the species in a genus whereas Reeves & Moreau (2019) analysed the ancestral states at genus level. Therefore, in their study, the foraging strategy of a genus depended on the majority of the species in that genus.

The observation that numerous species recruit to new nest sites, but do not use recruitment communication during foraging (e.g. *Neoponera* or *Diacamma* species, Fresneau, 1985; Grüter et al., 2018) raises the question if recruitment communication evolved first to help colonies during emigrations rather than to communicate the location of food sources, as has also been suggested in the case of the honeybee waggle dance (Beekman *et al.*, 2008; I'Anson Price & Grüter, 2015). This seems plausible given that during nest relocations of cavity nesting species, nest locations have to be communicated very precisely. If the old nest is damaged or destroyed, a fast and precise relocation is critical (Franks *et al.*, 2003; Dornhaus *et al.*, 2004). During foraging, on the other hand, communication might often be less important because foragers can discover new food sources by themselves. Especially when food sources are abundant and evenly distributed, communication might not provide benefits or even be disadvantageous due to time costs (Dechaume-Moncharmont *et al.*, 2005; Dornhaus *et al.*, 2006; I'Anson Price *et al.*, 2019; Goy *et al.*, 2021). The hypothesis that recruitment evolved first in colony emigrations and was co-opted by some species in a foraging context is also supported by our results suggesting group- or mass-recruitment as the ancestral state and those of Reeves & Moreau (2019) who suggested solitary foraging as the ancestral condition.

Some authors have suggested that tandem running is a "primitive" recruitment strategy (*i.e.* more ancestral) (Hingston, 1929; Wilson, 1959; Hölldobler *et al.*, 1974; Schultheiss *et al.*, 2015). Our results do not support this view. We found that tandem running evolved several times and independently in the subfamilies Ponerinae, Pseudomyrmecinae, Formicinae and Myrmecinae. Transitions to tandem running occurred most often from no recruitment and, more rarely, from mass or group-recruitment. The benefits of tandem running compared to no recruitment could have been particularly high in species experiencing strong competition for nest sites or food sources, where tandem recruitment allows small colonies to build up a critical mass of workers to defend resources against competitors (Glaser *et al.*, 2021).

In summary, our results suggest that mass or group-recruitment were the most likely recruitment strategies used by the common ancestor of present-day ants. There were repeated, independent transitions to smaller-scale strategies, such as tandem running or no recruitment, but also transitions back to group or mass-recruitment. It should be noted that our analysis is restricted to a small proportion of ant species and we currently lack information about recruitment behaviours for the majority of species (see also Reeves & Moreau, 2019). We echo their call to collect more information about ant behaviour and ecology. This will allow us to better understand the connections between different traits of ant behaviour, ecology and natural history.

Supplement

Table S1.1: Species in the phylogenetic tree with the recruitment strategy and source of information.

species	subfamily	strategy	nest	food	raids	reference
Amblyopone australis	Amblyoponinae	group	-	-	-	Hölldobler & Palmer 1989
Amblyopone longidens	Amblyoponinae	group	-	-	-	Hölldobler & Palmer 1989
Myopopone castanea	Amblyoponinae	solitary	-	-	-	Ito 2010
Mystrium rogeri	Amblyoponinae	mass	-	-	-	Hölldobler et al. 1998
Onychomyrmex hedleyi	Amblyoponinae	mass	-	-	-	Miyata et al. 2009
Prionopelta amabilis	Amblyoponinae	mass	-	-	-	Hölldobler et al. 1992

Stigmatomma pallipes	Amblyoponinae	solitary	-	-	-	Traniello 1978
Stigmatomma silvestrii	Amblyoponinae	solitary	-	-	-	Masuko 1993
Aneuretus simoni	Aneuretinae	group	-	-	-	Traniello & Jayasuriya 1981
Azteca instabilis	Dolichoderinae	mass	-	-	-	Philpott et al. 2004
Azteca ovaticeps	Dolichoderinae	mass	-	-	-	Dejean et al. 2009
Azteca schimperi	Dolichoderinae	group	-	-	-	Dejean et al. 2008
Dolichoderus pustulatus	Dolichoderinae	mass	-	-	-	Johnson 1989
Dolichoderus scabridus	Dolichoderinae	mass	-	-	-	Shattuck & Marsden 2013
Forelius chalybaeus	Dolichoderinae	mass	-	-	-	Aranda-Rickert & Fracchia 2012
Forelius pruinosus	Dolichoderinae	mass	-	-	-	Hölldobler 1995
Iridomyrmex sanguineus	Dolichoderinae	mass	-	-	-	Andersen & Patel 1994
Leptomyrmex erythrocephalus	Dolichoderinae	solitary	-	-	-	McClehan et al. 2016
Linepithema humile	Dolichoderinae	mass	-	-	-	Roulston & Silverman 2002
Liometopum apiculatum	Dolichoderinae	mass	-	-	-	Hoey-Chamberlain et al. 2013
Liometopum luctuosum	Dolichoderinae	mass	-	-	-	Hoey-Chamberlain et al. 2013
Liometopum occidentale	Dolichoderinae	mass	-	-	-	Hoey-Chamberlain et al. 2013
Philidris cordatus	Dolichoderinae	mass	-	-	-	Benzing & Clements 1991
Tapinoma melanocephalum	Dolichoderinae	mass	-	-	-	Tomalski et al. 1987
Tapinoma sessile	Dolichoderinae	mass	-	-	-	Buczkowski & Bennett 2008
Technomyrmex albipes	Dolichoderinae	mass	-	-	-	Warner 2003

Technomyrmex difficilis	Dolichoderinae	mass	-	-	-	Warner et al. 2014
Aenictus eugenii	Dorylinae	group	-	-	-	Gotwald & Cunningham 1976
Cheliomyrmex morosus	Dorylinae	mass	-	-	-	O'Donnel et al. 2005
Dorylus laevigatus	Dorylinae	mass	-	-	-	Berghoff et al. 2003
Dorylus wilverthi	Dorylinae	group	-	-	-	Franks et al. 2001
Eciton burchellii	Dorylinae	group	-	-	-	Beckers et al. 1989
Eciton hamatum	Dorylinae	mass	-	-	-	Jaffe 1984
Eciton vagans	Dorylinae	mass	-	-	-	Silvestre et al. 2005
Labidus praedator	Dorylinae	group	-	-	-	Beckers et al. 1989
Labidus spininodis	Dorylinae	mass	-	-	-	Kaspari & O'Donnel 2003
Neivamyrmex nigrescens	Dorylinae	mass	-	-	-	Beckers et al. 1989
Nomamyrmex esenbeckii	Dorylinae	mass	-	-	-	Swartz 1998
Ooceraea biroi	Dorylinae	mass	-	-	-	Goudie & Oldroyd 2018
Zasphinctus steinheili	Dorylinae	group	-	-	-	Wilson 1958
Ectatomma opaciventre	Ectatomminae	solitary	-	-	-	Silvestre et al. 2002
Gmptogenys striatula	Ectatomminae	mass	-	-	-	Blatrix et al. 2002
Rhytidoponera metallica	Ectatomminae	solitary	-	-	-	Thomas & Frameu 2005
Anoplolepis custodiens	Formicinae	mass	-	-	-	Mpuru & Brand 1993
Anoplolepis gracilipes	Formicinae	mass	-	-	-	Lester & Tavite 2004
Camponotus ocreatus	Formicinae	tandem	no info	no info	no info	Hölldobler & Wilson 1990

Cataglyphis cursor	Formicinae	solitary	-	-	-	Beckers et al. 1989
Cataglyphis ibericus	Formicinae	solitary	-	-	-	Ruano et al. 2002
Dinomyrmex gigas	Formicinae	mass	-	-	-	Pfeiffer & Linsenmaier 2000
Euprenolepis procera	Formicinae	mass	-	-	-	Von Beeren et al. 2014
Formica neogagates	Formicinae	mass	-	-	-	Weseloh 2001
Formica podzolica	Formicinae	mass	-	-	-	Dias & Breed 2008
Formica wheeleri	Formicinae	group	-	-	-	Topoff & Zimmerli 1991
Gigantiops destructor	Formicinae	solitary	-	-	-	Silvestre et al. 2001
Iberoformica subrufa	Formicinae	group	-	-	-	Castro-Cobo et al. 2019
Lasius alienus	Formicinae	group	-	-	-	Feener 1987
Lasius niger	Formicinae	mass	-	-	-	Beckers et al. 1989
Notostigma carazzii	Formicinae	solitary	-	-	-	Taylor 1992
Oecophylla longinoda	Formicinae	mass	-	-	-	Beckers et al. 1989
Oecophylla smaragdi	Formicinae	mass	-	-	-	Hölldobler 1983
Paratrechi longicornis	Formicinae	mass	-	-	-	Silvestre et al. 1999
Polyergus breviceps	Formicinae	mass	-	-	-	Topoff et al. 1989
Prenolepis imparis	Formicinae	mass	-	-	-	Talbot 1943
Myrmecia fulvipes	Myrmeciinae	solitary	-	-	-	Merrill & Elgar 2000
Myrmecia pyriformis	Myrmeciinae	solitary	-	-	-	Jayatilaka et al. 2011
Nothomyrmecia macrops	Myrmeciinae	solitary	-	-	-	Hölldobler & Taylor 1983

Acanthomyrmex ferox	Myrmicinae	solitary	-	-	-	Moffett 1985
Acromyrmex versicolor	Myrmicinae	mass	-	-	-	Jaffe 1984
Aphaenogaster swammerdami	Myrmicinae	mass	-	-	-	Voigt et al. 2002
Aretidris bueventei	Myrmicinae	solitary	-	-	-	General 2015
Atopomyrmex mocquerysi	Myrmicinae	mass	-	-	-	Kenne et al. 2009
Atta texa	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Basiceros manni	Myrmicinae	solitary	-	-	-	Wilson & Hölldobler 1986
Blepharidatta conops	Myrmicinae	mass	-	-	-	Diniz et al. 1998
Cardiocondyla emeryi	Myrmicinae	tandem	no info	yes	no info	Wilson 1959
Cardiocondyla mauritanica	Myrmicinae	tandem	yes	yes	no info	Heinze et al. 2006, Seifert 2002
Carebara urichi	Myrmicinae	mass	-	-	-	Wilson 1986
Cephalotes atratus	Myrmicinae	mass	-	-	-	Silvestre et al. 1999
Crematogaster modiglianii	Myrmicinae	mass	-	-	-	Menzel et al. 2010
Cyphomyrmex rimosus	Myrmicinae	solitary	-	-	-	Blum et al. 1964
Daceton armigerum	Myrmicinae	solitary	-	-	-	Beckers et al. 1989
Eutetramorium mocquerysi	Myrmicinae	mass	-	-	-	Morgan 2009
Goniomma blanci	Myrmicinae	solitary	-	-	-	Hensen 2002
Harpagoxenus sublaevis	Myrmicinae	tandem	no info	no info	yes	Buschinger & Winter 1977
Lachnomyrmex scrobiculatus	Myrmicinae	solitary	-	-	-	Feitosa & Brandao 2008
Leptothorax muscorum complex	Myrmicinae	tandem	yes	yes	no info	Möglich 1978

Megalomyrmex silvestrii	Myrmicinae	mass	-	-	-	Silvestre et al. 2004
Meranoplus mayri	Myrmicinae	mass	-	-	-	Voigt et al. 2003
Messor denticornis	Myrmicinae	mass	-	-	-	Ruano et al. 2000
Messor wasmanni	Myrmicinae	mass	-	-	-	Solida et al. 2009
Metapone madagascarica	Myrmicinae	mass	-	-	-	Hölldobler et al. 2002
Monomorium antarcticum	Myrmicinae	solitary	-	-	-	Jaffe 1984
Monomorium pharaonis	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Myrmeci graminicola	Myrmicinae	mass	-	-	-	Buschinger 2010
Myrmica incompleta	Myrmicinae	mass	-	-	-	Lenoir et al. 1992
Myrmicaria brunnea	Myrmicinae	mass	-	-	-	Sardar & Ghorai 2017
Nesomyrmex echitinodis	Myrmicinae	mass	-	-	-	Stuart & Moffett 1994
Nesomyrmex madecassus	Myrmicinae	mass	-	-	-	Csosz & Fisher 2016
Novomessor albisetosa	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Orectogthus versicolor	Myrmicinae	mass	-	-	-	Hölldobler 1981
Patagonomyrmex angustus	Myrmicinae	mass	-	-	-	Johnson & Moreau 2016
Pheidole hyatti	Myrmicinae	mass	-	-	-	Bestelmeyer 2005
Pheidole longispinosa	Myrmicinae	mass	-	-	-	Lampaso 2015
Pheidole pallidula	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Pheidole rhea	Myrmicinae	mass	-	-	-	Gordon et al. 2018
Pogonomyrmex maricopa	Myrmicinae	mass	-	-	-	Hölldobler 1976

Pogonomyrmex occidentalis	Myrmicinae	mass	-	-	-	Fewell 1988
Pogonomyrmex vermiculatus	Myrmicinae	solitary	-	-	-	Torres-Contreras et al. 2007
Pristomyrmex punctatus	Myrmicinae	group	-	-	-	Suwabe et al. 2007
Proatta butteli	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Solenopsis fugax	Myrmicinae	mass	-	-	-	Hölldobler 1973
Solenopsis invicta	Myrmicinae	mass	-	-	-	Beckers et al. 1989
Solenopsis molesta	Myrmicinae	mass	-	-	-	Wilson 1975
Solenopsis xyloni	Myrmicinae	mass	-	-	-	Jaffe 1984
Stemma alas	Myrmicinae	solitary	-	-	-	Branstetter 2013
Temnothorax rugatulus	Myrmicinae	tandem	yes	yes	no info	Pratt 2008
Tetramorium caespitum	Myrmicinae	mass	-	-	-	Ruano et al. 2001
Trichomyrmex destructor	Myrmicinae	mass	-	-	-	Srisakrapikoop et al. 2016
Veromessor andrei	Myrmicinae	mass	-	-	-	Plowes et al. 2014
Veromessor julianus	Myrmicinae	mass	-	-	-	Plowes et al. 2015
Veromessor lobogthus	Myrmicinae	solitary	-	-	-	Johnson 2000
Wasmannia auropunctata	Myrmicinae	mass	-	-	-	Silvestre et al. 2003
Xenomyrmex floridanus	Myrmicinae	mass	-	-	-	Hölldobler 1970
Paraponera clavata	Paraponerinae	mass	-	-	-	Breed & Bennett 1985
Brachyponera chinensis	Ponerinae	tandem	yes	yes	no info	Guérd & Silverman 2011
Brachyponera senarensis	Ponerinae	tandem	yes	no	no info	Billen & Al-Khalifa 2016

Diacamma rugosum	Ponerinae	tandem	yes	no	no info	Maschwitz et al. 1974
Dinoponera australis	Ponerinae	tandem	-	-	-	Billen et al. 1995
Dinoponera gigantea	Ponerinae	tandem	-	-	-	Paiva & Brandao 2010
Harpegthos saltator	Ponerinae	solitary	-	-	-	Maschwitz & Schönegge 1983
Leptogenys attenuata	Ponerinae	group	-	-	-	Maschwitz & Schönegge 1983
Leptogenys diminuta	Ponerinae	group	-	-	-	Attygalle et al. 1988
Neoponera apicalis	Ponerinae	tandem	yes	no	no info	Fresneu 1985
Neoponera commutata	Ponerinae	group	-	-	-	Mill 1984
Neoponera villosa	Ponerinae	tandem	yes	no	no info	Grüter,p.o.
Odontoponera transversa	Ponerinae	solitary	-	-	-	Berghoff et al. 2003
Ophthalmopone berthoudi	Ponerinae	solitary	-	-	-	Beckers et al. 1989
Pachycondyla crassinoda	Ponerinae	tandem	yes	yes	no info	Nicolas Chaline,p.c.
Pachycondyla harpax	Ponerinae	tandem	yes	yes	no info	Hölldobler & Engel 1978
Pachycondyla impressa	Ponerinae	tandem	no info	yes	no info	Hölldobler & Wilson 1990
Paltothyreus tarsata	Ponerinae	tandem	yes	no	no info	Hölldobler 1984
Platythyrea lamellosa	Ponerinae	solitary	-	-	-	Villet 1990
Plectrocte minor	Ponerinae	group	-	-	-	Satz et al. 2001
Ponera coarctata	Ponerinae	tandem	yes	no info	no info	Liebig et al. 1997
Ponera pennsylvanica	Ponerinae	tandem	yes	no	no info	Pratt et al. 1994

Psalidomyrmex procerus	Ponerinae	solitary	-	-	-	Dejean et al. 1999
Simopelta cf pergandei	Ponerinae	mass	-	-	-	Krouer 2009
Streblogthus peetersi	Ponerinae	solitary	-	-	-	Tanner & Keller 2012
Myrcidris epicharis	Pseudomyrmecinae	mass	-	-	-	Vicente et al. 2012
Pseudomyrmex atripes	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 2000
Pseudomyrmex elongatus	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 1999
Pseudomyrmex gracilis	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 2000
Pseudomyrmex kuenckeli	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 1999
Pseudomyrmex oculatus	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 1999
Pseudomyrmex tenuis	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 2001
Pseudomyrmex termitarius	Pseudomyrmecinae	tandem	no info	no info	no info	Beckers et al. 1989
Pseudomyrmex triplarinus	Pseudomyrmecinae	tandem	no info	no info	no info	Beckers et al. 1989
Pseudomyrmex unicolor	Pseudomyrmecinae	solitary	-	-	-	Silvestre et al. 2000
Pseudomyrmex urbanus	Pseudomyrmecinae	solitary	-	-	-	Silvestreetal.1999
Tetraponera aethiops	Pseudomyrmecinae	group	-	-	-	Dejean et al. 2008
Tetraponera rufonigra	Pseudomyrmecinae	mass	-	-	-	Norashmah et al. 2012

CHAPTER 2

Social and individual learners use different pathways to success in an insect mini-society

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Abstract

Animals can acquire information through individual learning or by copying others. Simulations suggest that social learning often leads to better rewards, but experimental studies confirming this remain scarce. Social insect colonies vary greatly in their use of social learning. We tested how a well-known form of social learning in ants, tandem running, affects the foraging success of individuals and colonies. We manipulated the number and the variability of food sources and found that social learners indeed discovered better food sources, but only in rich environments. However, social learners collected less food because they paid additional time costs. Strikingly, individual learners improved their success over time by switching food sources frequently. A simulation model found that colony size and composition critically affect the value of social learning at colony level. Higher food discoverability, favoured colonies consisting only of individual learners. These findings help explain why many social insects, especially those with small colonies, do not use communication in foraging.

Introduction

Animals constantly acquire and update information about their environment to make adaptive decisions (Boyd & Richerson, 1995; Brown & Laland, 2003; Horner *et al.*, 2010; Slagsvold & Wiebe, 2011; Dawson & Chittka, 2014; Lanan, 2014). Individuals can collect new information via individual (trial-and-error) learning or they can use social learning, *i.e.* learning by observing or interacting with other individuals or their products (Laland, 2004; Galef & Laland, 2005; Rendell *et al.*, 2010; Shettleworth, 2010; Heyes, 2012). If an individual already has acquired information, it can rely on its memory and use this "private information" (Kendal *et al.*, 2005).

Social information is often considered to be a "cheap" option, whereas individual learning might be more costly but also more accurate (Laland, 2004; Rieucau & Giraldeau, 2011). Private information, on the other hand, is immediately available, but is prone to being outdated (e.g. Grüter & Leadbeater, 2014; Kendal et al., 2005; Rendell et al., 2010; Smolla et al., 2016). Despite an increasing interest in the circumstances that favour different information-use strategies, still little is known about the relative payoffs of the different strategies, and how these depend on the environment. Rendell et al. (2010) used a simulated computer tournament to test the success of different information-use strategies and found that social learning tended to be more successful than individual learning because "demonstrators" show the most productive behaviour in their repertoire, thereby "filtering" information for observers (Rendell *et al.*, 2010). However, experimental evidence showing that social learning leads to better rewards than individual learning remains scarce (Rendell et al., 2010 but see e.g. Thornton & McAuliffe, 2006). One challenge is that it is often difficult to demonstrate that a behaviour is acquired socially, rather than through individual learning and innovation (Shettleworth, 2010).

Eusocial insects are well-known to rely on social information during foraging or colony migration. Honeybees, for example, use the waggle dance communication (von Frisch, 1967; Seeley, 1995; Al Toufailia *et al.*, 2013a) and many ants, termites and stingless bees rely on trail pheromones (Hölldobler & Wilson, 2009; Hrncir & Maia-Silva, 2013; Czaczkes *et al.*, 2015b). Empirical studies confirm that ant or honeybee foragers often only share information with nestmates when the food source is of high quality (von Frisch, 1967; Czaczkes *et al.*, 2015b), which leads to information filtering as described in Rendell et al. (2010). As predicted, there is evidence that honeybees following waggle dances find better food sources than scouts using an individual learning strategy (Seeley, 1983; Seeley & Visscher, 1988), but recruits also required more time to locate a food source, suggesting that social learning has considerable time costs (Seeley, 1983; Seeley & Visscher, 1988; Dechaume-Moncharmont *et al.*, 2005; Franks & Richardson, 2006; Grüter *et al.*, 2010).

In a cooperative society such as an insect colony, an information-use strategy that increases rewards for the individual might not guarantee success for the colony (Grüter & Leadbeater, 2014). For example, colonies that rely strongly on social learning could be worse off if social learning has time costs (Dechaume-Moncharmont *et al.*, 2005; I'Anson Price *et al.*, 2019). Theoretical models of honeybee foraging suggest that both the benefits and the costs of social information depend strongly on food source distribution (Dornhaus *et al.*, 2006; Beekman & Lew, 2007; Schürch & Grüter, 2014; I'Anson Price *et al.*, 2019). When food sources are easy to find, individual learning is often more efficient. This could explain why many social insect species do not use communication about food source locations. Bumblebees, many stingless bees and many ants with small colony sizes, for example, forage largely independently, even though foragers can use social cues at flowers to make foraging decisions (Beckers *et al.*, 1989; Leadbeater & Chittka, 2005; Worden & Papaj, 2005; Dunlap *et al.*, 2016). Thus, learning socially about where to find food might often not improve colony foraging success.

A better understanding of the consequences of social learning in ants and honeybees has been hampered by the difficulty to distinguish between social and private information use (Grüter & Farina, 2009; Czaczkes *et al.*, 2016). For example, bees might follow a waggle dance, but subsequently use private information to fly to a different food source after leaving the nest (Grüter *et al.*, 2008; Menzel *et al.*, 2011) and ants walking on a trail marked by pheromone might rely strongly on memory rather than chemicals when making decisions (Grüter *et al.*, 2011; Czaczkes *et al.*, 2015b).

Tandem running, on the other hand, is an ideal behaviour to study the outcome of social learning because social learners always pair up with another ant (Franks & Richardson, 2006). This form of communication is used in many ant species with small colony sizes and involves an experienced leader (tutor) and a naïve follower (henceforth also called recruit or social learner) (Möglich *et al.*, 1974; Franks & Richardson, 2006; Richardson *et al.*, 2007; Franklin, 2014; Glaser & Grüter, 2018). After being guided to the food source by a leader, the follower can herself become a tandem leader and guide other ants to the food source.

We studied the common European ant *Temnothorax nylanderi* as a model system and quantified their foraging success in controlled laboratory environments that differed in the number and variability of food sources. We tracked individually marked social and individual learners over repeated foraging trips, analysed their foraging decisions and recorded the outcome of each trip. Additionally, we developed an agent-based simulation model to assess the importance of social learning at the colony level in simulated environments. Our main prediction was that social learners (recruits) find better food sources than individual learners (scouts). We also tested if individuals can improve rewards over successive trips by abandoning food sources that are suboptimal. Lastly, we predicted that the foraging success of simulated colonies depends on the composition of colonies, *i.e.* the ratio of social and individual learners and that tandem running is favoured when food sources vary in quality and are hard to find, similar to what has been suggested in honeybees (Dornhaus *et al.*, 2006; Beekman & Lew, 2007; I'Anson Price *et al.*, 2019).

Material & Methods

Study site and study species

Thirty colonies of *Temnothorax nylanderi* were collected in the Lenneberger forest near Mainz, Germany. Colonies were kept in climate chambers at 25°C with a 12:12 h light/dark cycle. The colonies lived in nests that consisted of two microscope slides (50 x 10 x 3 mm) and between the two slides was a plexiglass slide with an oval cavity and an opening as a nest entrance. The nest was placed in a larger three-chamber-box (100 x 100 x 30 mm) and to prevent the ants from escaping, the walls were coated with paraffin oil. Colonies were provided with an *ad libitum* water source and fed twice a week with honey and cricket. All colonies had a reproductive queen, brood and a mean colony size (adult workers) of 77.0 ± 34.8 (± StDev).

Experimental set-up and procedure

All experiments were conducted in the same climate chamber. Colonies were starved for 10 days before each trial to guarantee foraging motivation. After seven days of starvation the nests were placed in a foraging arena (78 x 56 x 18 cm), which allowed ants to get used to the test environment. The floor of the arena was covered with white paper to improve contrast for filming. The paper was changed after each trial to remove potential chemical traces. To prevent ants from escaping, the walls of the arenas were covered with Fluon.

Fifteen colonies were tested in four conditions each: with few (two) and many (ten) food sources of variable (0.1M and 1.0M sucrose solution) or constant (1.0M) quality. The nest was always positioned in the middle of the arena. The testing order was randomized for each colony. On an experiment day, either two (few food sources) or ten (many food sources) droplets of sucrose solution were provided. Ants of the species *T. nylanderi* typically forage less than 50 cm from the nest (Heinze *et al.*, 1996). Therefore, sugar droplets were positioned 20 cm from the nest entrance with equal distances between food sources. In the case of ten variable food sources, they were positioned in alternating order around the nest, *i.e.* a 1M food source had a 0.1M food source on either side and *vice versa*. After the first forager discovered a food source, video recording (SONY HDR-CX200) started for 90 min. When scouts (*i.e.* individual learners) or tandem followers (social learners, recruits) reached the food source, they were individually marked with a colour dot on their abdomen (POSCA, Mitsubishi Pencil Co., UK). This allowed us to follow the foraging success of individually marked foragers over several foraging trips. If possible, four scouts and four followers were marked per trial. After every trial, colonies were fed for four days before being starved again to be tested in a different setup. Thus, the trial period was 14 days and included 10 days without any food sources in their environment.

In additional experiments, we explored if followers require more or less time than scouts to discover their first food source after leaving the nest. Fifteen different colonies were provided with either two or ten sugar droplets of good quality (1.0 M) sucrose solution. The video recording started after a scout left the nest. We filmed until colonies performed two tandem runs per trial to compare the nest-to-food time of scouts and tandem followers.

Data collection

Video recordings were analysed with the VLC media player V5. We recorded which of the following foraging strategies our marked ants used to locate food sources: scouting (individual learning), returning alone (private information user), leading a tandem run (private information user) or following a tandem run (social learning). We also noted if our focal ants switched food sources and whether they visited a good or a bad food source. We calculated the success rate of tandem runs and the probability to perform a tandem run for each visit (see Glaser & Grüter, 2018 for more details). In the second experiment, we recorded the time that scouts and tandem followers needed from the nest to a food source.

Agent based model

We developed an agent-based simulation model (ABM, see supplement for detailed model description and code) to simulate ant colonies with and without tandem running (Fig S2.1). It simulated the foragers of small ant colonies like e.g. *Temnothorax nylanderi* colonies and matched the experimental conditions: the quality and number of food sources and the duration of simulations were chosen to be equivalent to what we used in the empirical experiments. In the situation without social learning, agents could locate food sources either by independent exploration (scouting) or by using memory about previously visited food sources (private information), but no tandem runs were performed. The probability to return to a food source and recruit to it (in the situation with tandem recruitment) depended on the quality of the food source. Through feeding, agents obtained energy (J), which they brought back to the nest and accounted as total nest energy (NE) for the colony foraging success. We tested colony success under varying conditions: colonies were offered either 2 or 10 food sources of either high quality or a mix of high and low quality (0.75 J/trip = high, 0.075 J/trip = low). We also tested different scout-recruit ratios, varying colony sizes (40-100) and tandem success rates. We performed 30 simulations for each tested parameter combination measuring the gained NE. For a more detailed exploration of this model, see Goy et al. (2021).

Statistical analysis

All statistical tests were done in R 3.4.2 (R Core Team, 2017). For normally-distributed response variables we used linear mixed-effect models (LME). For response variables with a binomial or Poisson distribution, we used generalized linear mixed-effect models (GLMM) (Zuur *et al.*, 2009). We tested if models with a Poisson distribution were overdispersed (package blmeco; Barry et al., 2003). If necessary, variables were transformed (square root or boxcox transformation) to achieve normality and then analysed with an LME. Colony ID and ant ID were used as random effects to control for the non-independence of data points from the same colony and the same ant (Zuur *et al.*, 2009).

We investigated the role of four fixed effects: foraging setup (few-variable, few-constant, many-variable and many-constant food sources), strategy (scout, leader, follower and private information user), food quality (good or bad) and experience. The effect of foraging experience was analysed as the number of performed tandem runs and as the number of lone visits to food sources. As response variables, we used tandem success rate, the probability of tandem runs, the probability of food switches and the time that scouts and tandem followers needed to reach a food source.

In the agent-based simulation model, we tested the effects of food source distribution, food quality, colony size and the scout-recruit ratio on the total collected energy using standard analysis of variance (Anova) (Crawley, 2007). We performed paired t-tests to analyse the influence of food quality and food quantity on the time until foragers discovered their first food source. For this, we averaged the durations of all scouts and tandem followers for each trial.

Results

Tandem success rate

The success rate of tandem runs (total N = 747) was higher in environments with many food sources (GLMER: variable: few vs. many: z = 4.341, p < 0.001; constant: few vs. many: z = 2.435, p < 0.001). The success rate did not depend on whether food sources were of constant or variable quality (few: 55.5% of 290 vs. many: 75.5% of 457; GLMER: few: variable vs. constant: z = 1.535, p = 0.125; many: variable vs. constant: z = 0.211, p = 0.832), suggesting that tandem runs to bad food sources were not less successful.

Probability of tandem runs & influence of food quality

Unexpectedly, the probability to perform a tandem run was significantly lower when there were more food sources (few: 53.4% vs. many: 31.7%; GLMER: few: variable vs. constant: z = 0.638, p = 0.523; many: variable vs. constant: z = 0.252, p = 0.801; variable: few vs. many: z = -3.377, p < 0.001; constant: few vs. many: z = 3.725, p < 0.001; Fig. S2.3). The probability that ants performed a tandem run increased with their experience (measured as the number of previous visits) (GLMER: few vs. many: z = 0.369, p = 0.712; visit: z = 3.188, p = 0.001; interaction: z = 0.257, p < 0.001). The significant interaction between food source number and visit number indicates that the probability of tandem runs increased more with experience when there were few food sources (based on visual inspection of the results). Furthermore, the probability to start a tandem run was significantly higher after visiting a good food source (Fig. 2.1A) (GLMER: few: z = 3.669, p < 0.001; many: z = 4.989, p < 0.001).

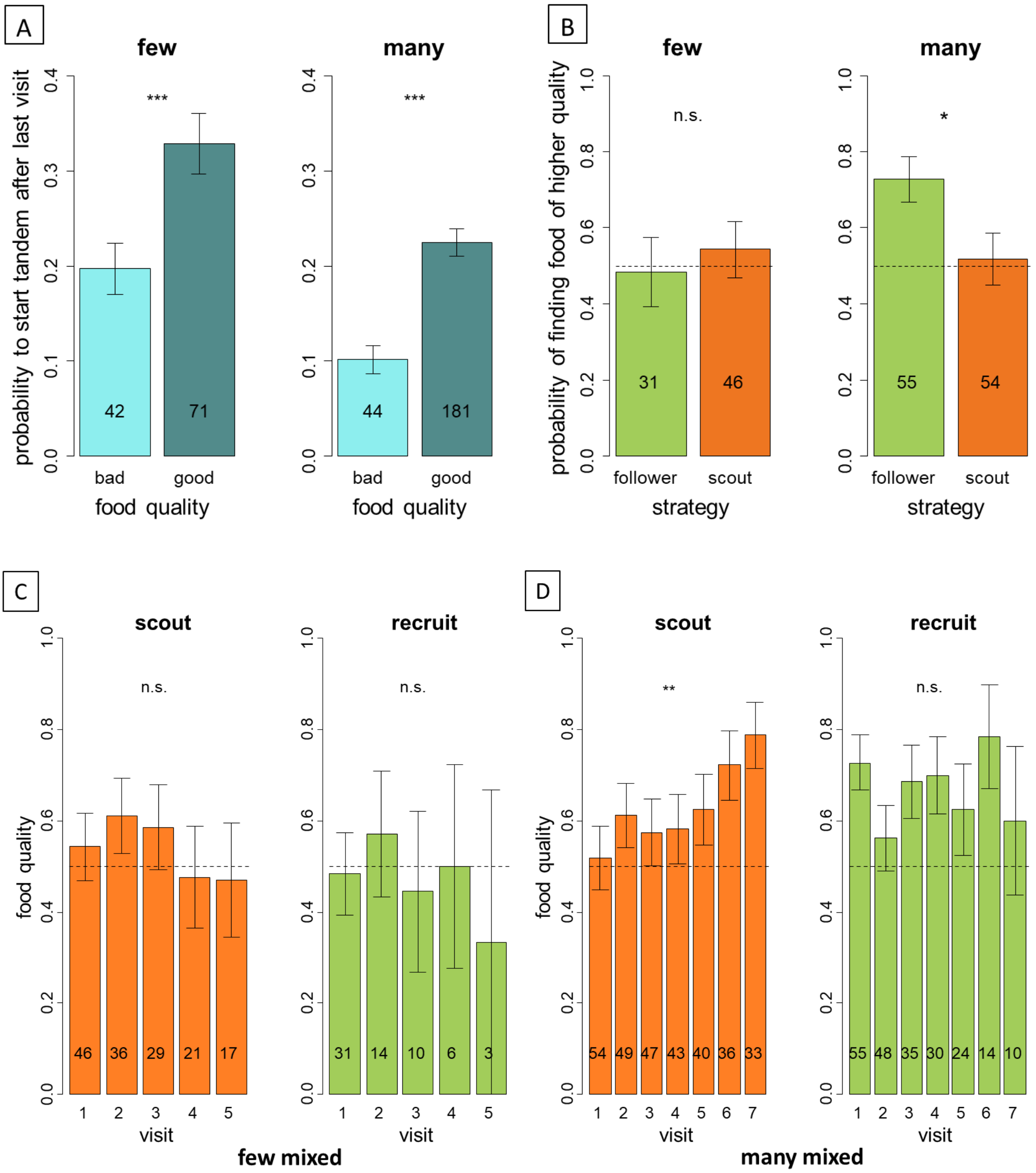

Figure 2.1: (**A**) Probability to start a tandem run (marked ants) depending on the food quality of the last visit. (**B**) Probability of finding food of a better quality on the first food visit depending on the information-use strategy. Mean food quality depending on the food visits of scouts and recruits for (**C**) few mixed and (**D**) many mixed food sources. Bars show mean ± standard error. *p<0.05, **p<0.01, ***p<0.001, ns: not significant

Quality of food sources discovered by individual and social learners

Next, we compared the quality of food sources discovered by scouts (individual learning) and tandem followers (social learning) on their first visit in set-ups that offered both high- and low-quality food sources. In contrast to our prediction, scouts and followers found food sources of similar quality when there were only two food sources that differed in quality: 54.4% (25 of 46) of scouts and 48.4% (15 of 31) of followers first found a high-quality food source (Fig. 2.1B). However, when there were many food sources, followers were significantly more likely to discover a better food source than scouts (scouts: 51.9% vs. followers: 72.7%) (GLMER: few: $z = 0.039$, $p = 0.969$; many: $z = -2.221$, $p = 0.026$). As a result, we found that followers were led to above-average food sources when there were many food sources (GLMER: $z = 2.36$, $p = 0.018$). When there were only two food sources, scouts and original recruits continued to visit food sources of the same, average quality during the course of the experiment (Fig. 2.1C) (GLMER: visits: scouts: $z = 0.837$, $p = 0.403$; recruits: $z = 1.343$, $p = 0.179$). Interestingly, when there were many food sources, scouts switched to better food sources over time (Fig. 2.1D). Recruits continued to visit above average food sources, but without further change in the reward quality over time (GLMER: visits: scouts: $z = 2.663$, $p = 0.008$; recruits: $z = 0.418$, $p = 0.676$).

Probability to switch

The number of different food sources visited by individual ants was significantly lower when there were few food sources compared to many food sources (Fig. 2.2A) (GLMER: few vs. many: variable: $z = 5.727$, $p < 0.001$; constant: $z = 5.719$, $p < 0.001$; variable vs. constant: few: $z = -0.321$, $p = 0.748$; many: $z = -0.249$; $p = 0.804$). When there were few food sources, both scouts and followers visited 1-1.2 food sources during 90 minutes (GLMER: few: variable: $z = 1.145$, $p = 0.252$; constant: $z = 0.039$, $p = 0.969$), meaning that they largely continued to visit the food source they discovered first. However, switching was more common after visiting a low-quality food source (Fig. 2.2B) (GLMER: food quality: $z = 2.258$, $p = 0.024$; strategy: $z = 1.082$, $p = 0.279$). When there was a larger number of food locations, scouts visited on average about 3 different food sources, which was ~50% more than followers did (Fig. 2.2A) (GLMER: many: variable: z = 2.824, p = 0.005; constant: z = 2.835, p = 0.005). Scouts generally switched more often than former followers (Fig. 2.2B) (GLMER: many-variable: strategy: z = 2.333, p = 0.020; food quality: z = 0.71, p = 0.478).

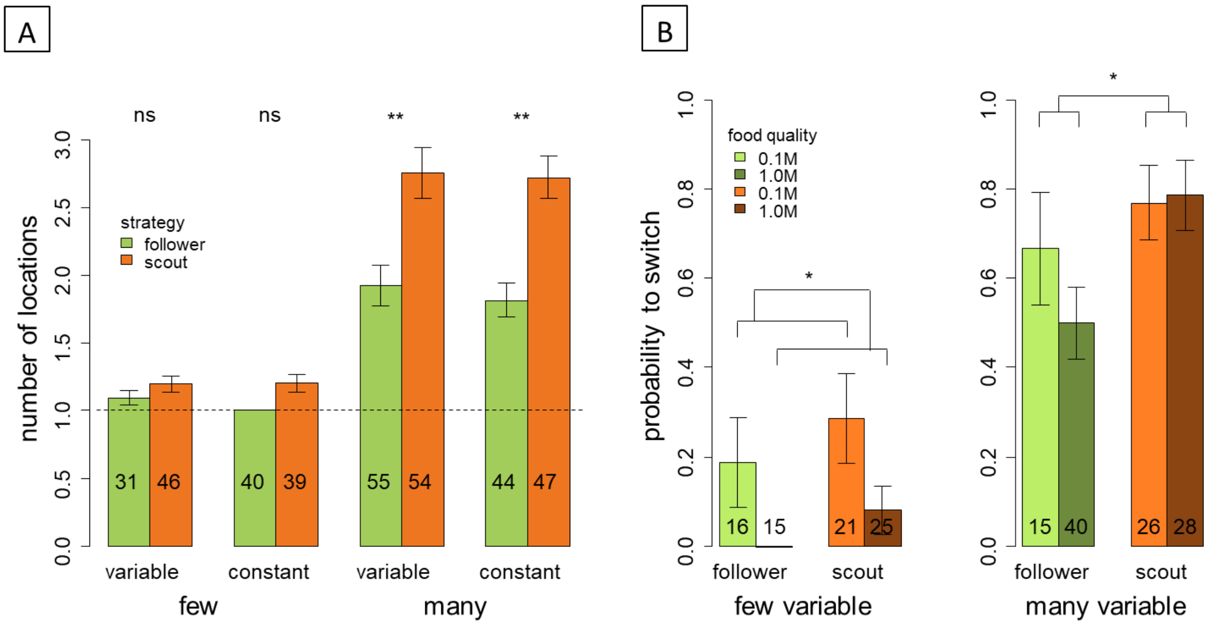

Figure 2.2: (**A**) The number of food locations scouts and tandem followers visited. (**B**) Probability to switch food source depending on the information-use strategy and the food quality. Number in column match the number of tested ants. Bars show mean ± standard error. *p<0.05, **p<0.01, ns: not significant

Total foraging activity

We found that scouts had significantly more food visits than ants that used social learning to discover a food source, irrespective of the number of food sources that were available (Fig. 2.3A) (GLMER: follower vs. scout: few: z = 5.405, p < 0.001, many: few: z = 8.390 , p < 0.001).

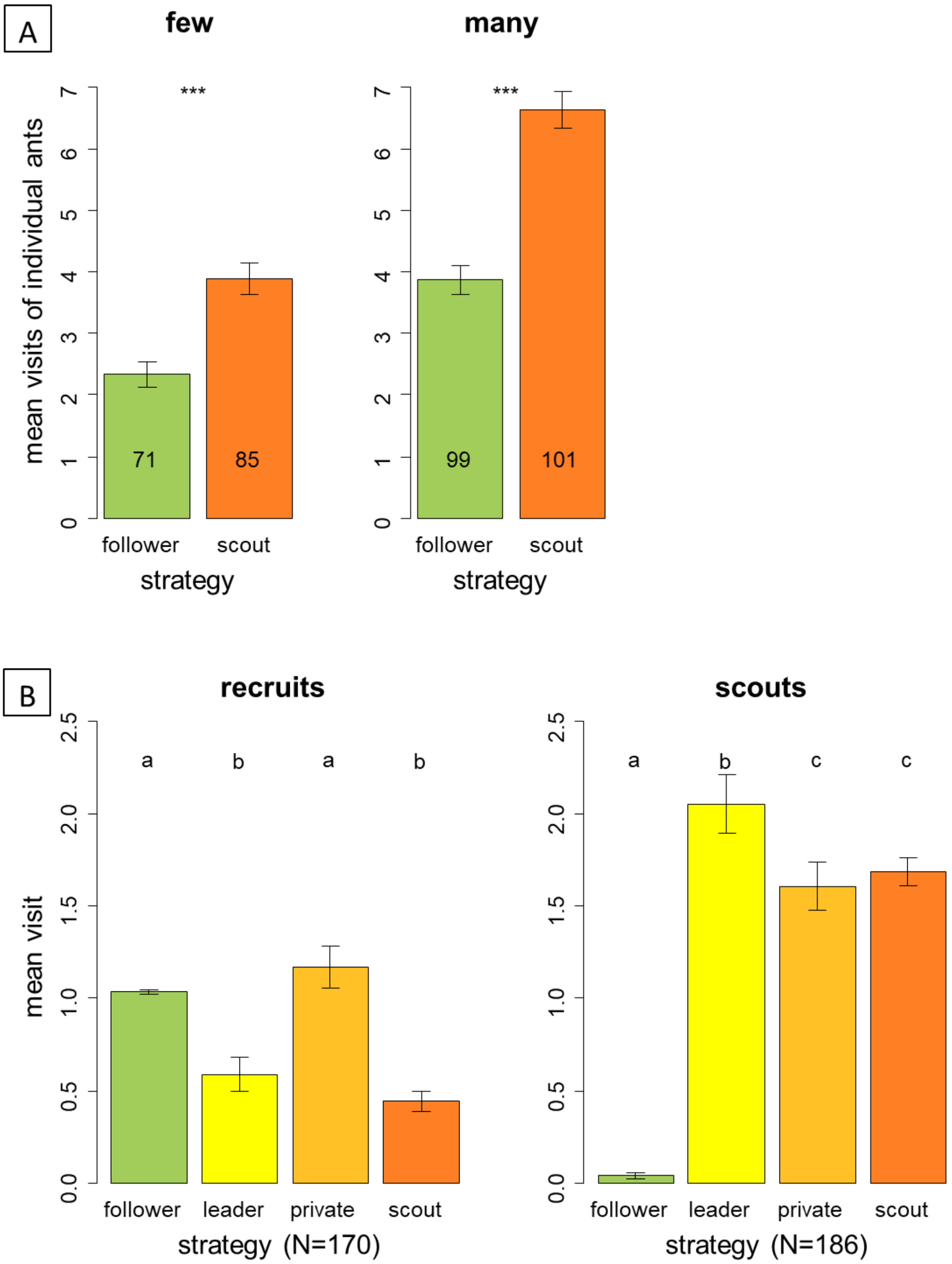

Figure 2.3: Mean visits of individual ants, depending on the information-use strategy they used to reach a food source. (**A**) Mean visits depending on few and many food sources. (**B**) Mean visits for individual ants depending on the information-use strategies for all visits depending on being a scout or a recruit. Number in column match the amount of ants tested. Bars show mean ± standard error. ***p<0.001, different letters indicate significant differences.

When we explored how scouts and followers foraged after the initial food source discovery (Fig. 2.3B), we found that former followers usually used private information (including visits as tandem leaders). They performed fewer individual exploration trips and led fewer tandem runs than former scouts (GLMER: information-use strategies of social learners (recruits): follower vs. leader: z = -4.573, p < 0.001; follower vs. private: z = 1.202, p = 0.229; follower vs. scout: z = -6.266, p < 0.001; leader vs. private: z = 5.687, p < 0.001; leader vs. scout: z = -1.908, p = 0.0564; private vs. scout: z = -7.295, p < 0.001). On the other hand, ants that discovered a food source *via* individual learning almost never followed tandem runs during the experiment (3.8% of 186 cases), but they frequently led them and, therefore, were important providers of social information. Furthermore, they continued to scout and discover new food sources (GLMER: information-use strategies of individual learners (scouts): follower vs. leader: z = 10.595, p < 0.001; follower vs. private: z = 9.922, p < 0.001; follower vs. scout: z = 10.056, p < 0.001; leader vs. private: z = -3.206, p = 0.001; leader vs. scout: z = -2.600, p = 0.009; private vs. scout: z = 0.612, p = 0.540).

Trip duration to the food using social or individual learning

We measured the time tandem followers and scouts needed from leaving the nest until locating their first food source. Overall, scouts needed nearly twice as long as tandem followers to reach the food source (Fig. 2.4A) (LME: scout vs. tandem: few: t = 3.967, p < 0.001; many: t = 4.737, p < 0.001). Furthermore, having more food sources available reduced the time that scouts and followers needed to locate a food source (LME: few vs. many: scout: t = 2.880, p = 0.007; tandem follower: t = 3.848, p < 0.001).

This time to discovery of the first food source in a trial did not include the time that ants have spent waiting inside their nest, which is likely to be different for scouts and recruits. We explored this further using agent-based simulations. We quantified how much time the different forager types needed from the beginning of the experiment until they reached the first food source (100 foragers, 0.2 scout ratio, see detailed model description). In our model, scouts left the nest at the beginning of the simulations, whereas recruits waited until they were recruited by a tandem leader. When there were few food sources, scouts needed more time to find a food source (Fig. 2.4B), independently of food quality (t-test: few food sources: high quality: t = -8.968, df = 29, p < 0.001; variable quality: t = -2.336, df = 29, p = 0.027). With many food sources of high quality, recruits still required less time before discovering their first food source, but the difference was much smaller compared to an environment with few food sources (Fig. 2.4B). When there were many food sources of variable quality, however, scouts needed significantly less time than recruits to discover their first food source (t-test: many food sources: high quality: t = -2.770, df = 29, p = 0.010; variable quality: t = 6.021, df = 29, p < 0.001).

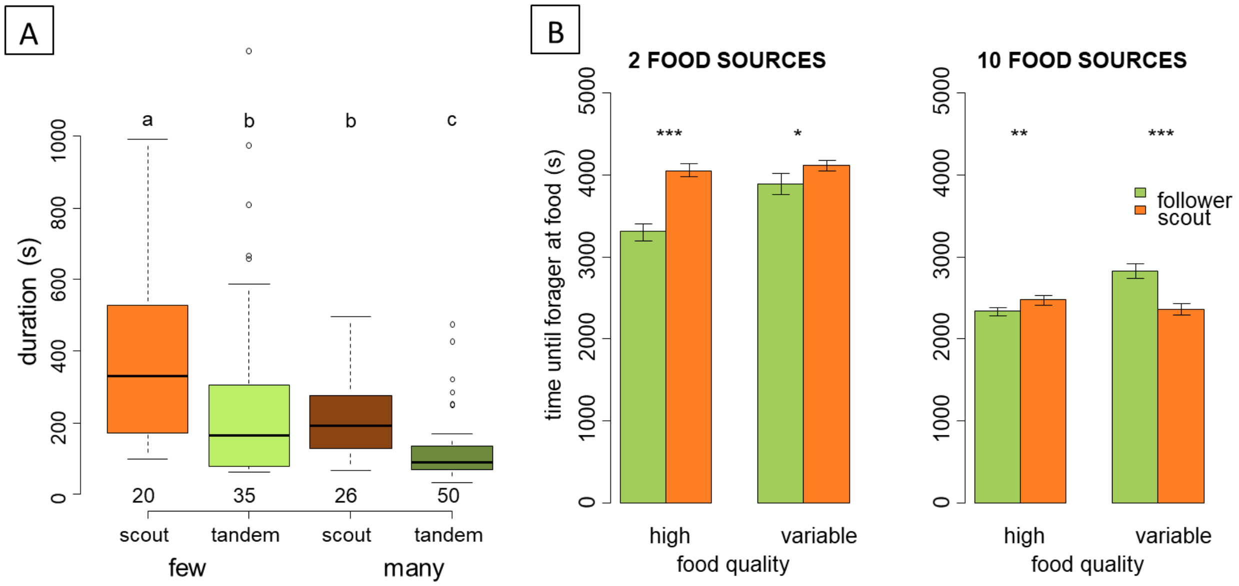

Figure 2.4: Mean time foragers need to find the first food source. (**A**) Duration of tandem runs and scouts from the nest entrance to a food source. (**B**) Few (2) or many (10) food sources of either high or variable quality. Numbers below boxes match tested scouts or tandem runs. Boxplots show medians, quartiles and fifth and 95th percentiles. Different letters indicate significant differences. Bars show mean ± standard error. *p<0.05, **p<0.01, ***p<0.001

Effects of colony size and scout-recruit ratio

Using computer simulations, we analysed the effects of colony size and composition on colony foraging success in an environment with two high quality food sources (other environments with many or variable food sources produced qualitatively similar results and are not shown). When colonies were small, there were no differences in nest energy between colonies consisting only of scouts and colonies containing 60% or 80% social learners (Fig. 2.5) (Anova: 40 foragers: F = 2.117, p = 0.149; 50 foragers: F = 3.095; p = 0.082). However, as colony size increased, colonies containing social learners became more successful than colonies with only individual learners (Anova: 60 foragers: F = 10.47; p = 0.002; 70 foragers: F = 11.46; p = 0.001; 80 foragers: F = 55.33; p < 0.001; 90 foragers: F = 27.69; p < 0.001; 100 foragers: F = 32.57; p < 0.001). Colonies were most successful if they contained around 60% social learners and 40% individual learners (scout-recruit ratio = 0.4) (Fig. 2.5, see Fig. S2.4 for additional ratios).

Figure 2.5: Colony nest energy depending on total number of foragers for different scout-recruit ratios or no recruitment (100% scouts). Few (2) and high quality food sources.

Next, we explored how the food source number and variability affected the value of social learning for colonies (100 foragers). Colonies with social learners (20% and 40% scouts) outperformed colonies consisting only of scouts when there were few food sources, irrespective of whether food sources were of constant or variable quality (Fig. 2.6A,B) (Anova: high quality: $F = 32.57$, $p < 0.001$; 20% vs. 40% scouts: $p = 0.001$; 20% vs. 100% scouts: $p < 0.001$; 40% vs. 100% scouts: $p < 0.001$; variable quality: $F = 16.27$, $p < 0.001$; 20% vs. 40% scouts: $p < 0.001$; 20% vs. 100% scouts: $p = 0.004$; 40% vs. 100% scouts: $p < 0.001$). Interestingly, the pattern changed when there were many high-quality food sources. Now, social learning no longer had a positive effect on colony efficiency. Indeed, nest energy increased with a decreasing number of social learners among the foragers (Fig. 2.6C) (Anova: $F = 19.55$, $p < 0.001$; 20% vs. 40% scouts: $p < 0.001$; 20% vs. 100% scouts: $p < 0.001$; 40% vs. 100% scouts: $p = 0.53$). However, social learners were again beneficial when there were many food sources of variable quality (Fig. 2.6D) (Anova: $F = 17.59$, $p < 0.001$; 20% vs. 40% scouts: $p < 0.001$; 20% vs. 100% scouts: $p = 0.003$; 40% vs. 100% scouts: $p < 0.001$).

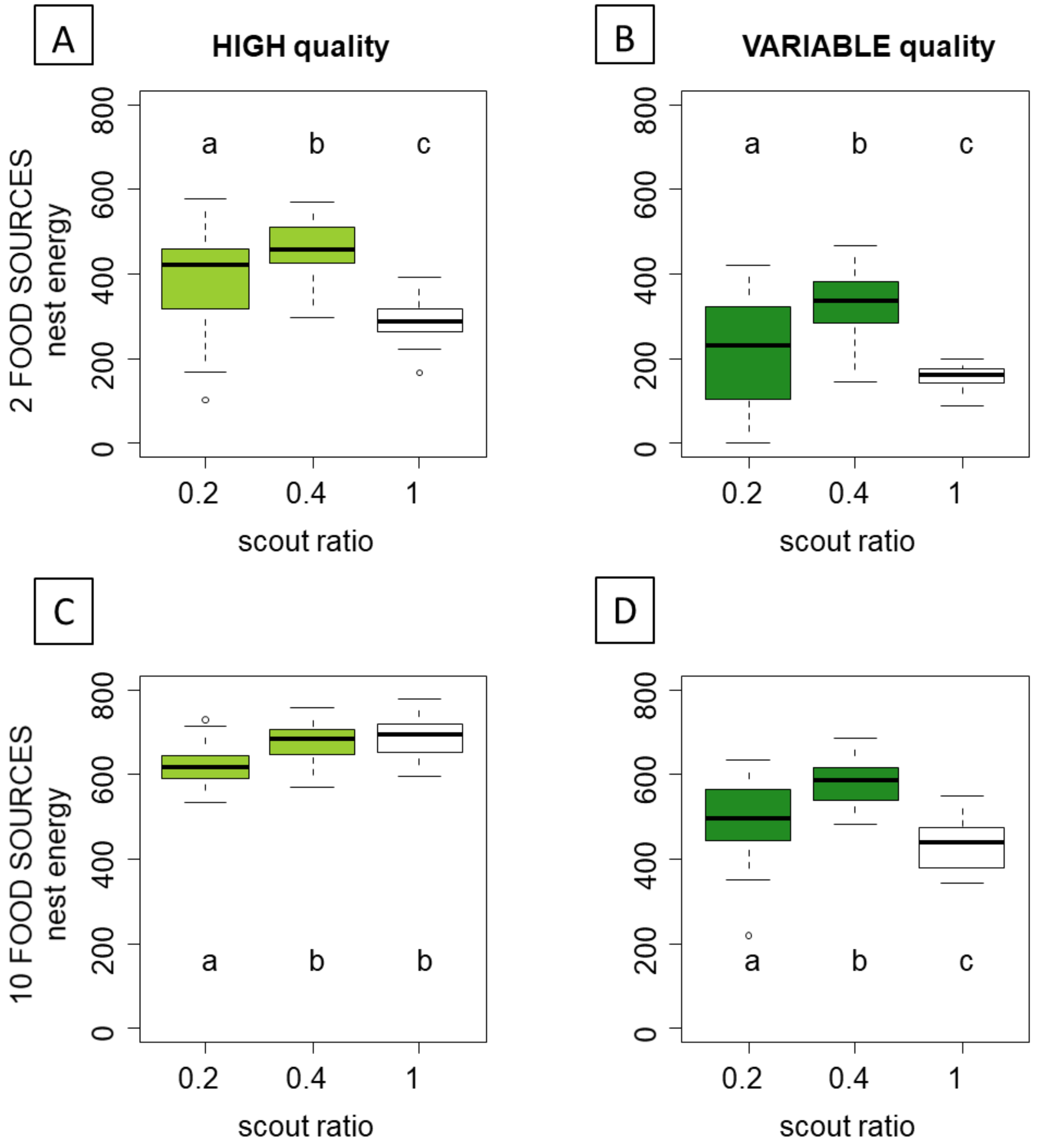

Figure 2.6: Colony nest energy depending on recruitment intensity for 100 foragers. High or variable quality treatments were tested with either few/two (**A** & **B**) or many/ten (**C** & **D**) food sources. Boxplots show medians, quartiles and fifth and 95th percentiles. Different letters indicate significant differences.

We performed the same analyses in smaller colonies, in groups with only 50 foragers. The general patterns were the same as with 100 foragers, but a pure scouting strategy was overall relatively more successful (Fig. S2.5).

In the previous simulations, tandem runs were always successful. However, we found that tandem run success rate was lower when there were only two food sources

(Fig. S2.2). We simulated this situation to explore how it affects colony success. Colonies with tandem runs (0.2 scout-recruit ratio, 100 agents) that had a success rate of 75% still performed better than colonies consisting only of scouts (Anova: high quality: F = 75.01, $p < 0.001$; variable quality: F = 17.42, $p < 0.001$), but they collected significantly less energy than colonies with 100% successful tandem runs (high quality: F = 12.08, p = 0.001; variable quality: F = 5.25, p = 0.026).

Discussion

Our results show that social learners achieved better rewards than individual learners (Rendell *et al.*, 2010) only under certain conditions. Only in a rich environment did social learners (tandem followers) locate a better food source than scouts (individual learners). In such environments, following a tandem run increased the chance of finding a high-quality food source as ants performed more tandem runs to higher quality food sources. Scouts, on the other hand, initially found food sources of average quality but, strikingly, they obtained improved rewards during the course of the experiment by following a strategy of food source switching (Fig. 2.2). In environments where food sources were scarce, scouts and recruits discovered food sources of average quality and overall food quality remained average over several visits. The opportunities to find a better food source are much reduced in a poor environment because there are fewer alternatives. It is surprising, however, that recruits did not discover better food sources given that more tandem runs were initiated after foragers visited a high-quality food source (see also Shaffer et al., 2013). However, the effect of food source quality on tandem runs was less pronounced in a poor environment (Fig. 2.1A) and tandem runs failed more frequently (Fig. S2.2), which is likely to lead to more similar outcomes for social and individual learners. Our finding that ants were overall more likely to perform tandem runs in a poor foraging environment was unexpected (Fig. S2.3), but could be an adaptive response since social information is likely to be more useful to nestmates under these circumstances (Fig. 2.6) (see also Beekman & Lew, 2007; Dornhaus et al., 2006; Schürch & Grüter, 2014). Scouts could assess their environment based on the time they needed to locate a food source.

One way to improve rewards over time is to abandon poor food sources and switch to better ones. As mentioned, switching by either forager type was rare when there were only two food sources. However, switching was more frequent when there were many food sources, particularly after foragers fed on food of low quality. Scouts visited more food sources than tandem followers, suggesting a strategy to actively search for better food sources, whereas tandem followers mostly used private information to return to the location they were guided to (Fig. 2.3B). Frequent food source switching not only allows scouts to improve the quality of exploited food sources over time (Fig. 2.1D), but also highlights the role of scouts as "innovators" that discover new food sources for the colony. This could be particularly important in an unpredictable environment.

Our results indicate that there are two types of foragers in *T. nylanderi* that differ in whether they use social or individual learning to discover a food source. Scouts are the main providers of social information as they lead most of the tandem runs. It is also noteworthy that after the first food source was discovered, the most frequently followed strategy by both scouts and recruits was the use of private information. This seems to be widespread in social insects (e.g. reviewed in Grüter & Czaczkes, 2019; Grüter & Leadbeater, 2014).

Tandem followers needed less time from the nest to the food source than scouts, which is one of the reasons why this behaviour is considered a case of animal teaching (Franks & Richardson, 2006). On the other hand, we found that social learners performed 60% fewer foraging trips. This highlights that there are hidden time and opportunity costs to social learning: social learners need to wait for information inside the nest (Dechaume-Moncharmont *et al.*, 2005; Schürch & Grüter, 2014). We used our simulation model to include these waiting times and compare the time tandem followers and scouts need to locate their first food source. The simulations showed that scouts needed significantly more time than recruits when there were few food sources, but scouts were faster than recruits when there were more, variable food sources (Fig. 2.4B). In the latter situation, it is relatively easy for scouts to find a food source. Scouts discovering a bad food source are unlikely to perform a tandem run and recruit a nestmate, which increases the waiting time of social learners compared to a situation with only high-quality food sources (Fig. 2.4B). These findings are in line with observations that honeybee recruits experienced greater time costs than scouts (Seeley, 1983; Seeley & Visscher, 1988).

The benefits of individual learning depended strongly on the discoverability of high-quality food sources in simulated honeybee colonies (Dornhaus *et al.*, 2006; Beekman & Lew, 2007; Schürch & Grüter, 2014). If there are many good food sources, a scouting strategy was often more successful. On the other hand, when high-quality food sources are rare or unequally distributed, the long-term benefits of finding food of higher quality can outweigh the waiting costs of social learning (Schürch & Grüter, 2014; I'Anson Price *et al.*, 2019). Our simulations corroborate these findings, even though we simulated a very different kind of society that uses a different communication system. When there were few food sources or when food sources were variable, having tandem runs was beneficial for the colony. On the other hand, when food sources were abundant and of high quality, colonies were more efficient without recruits that use social learning.

Our agent-based simulation model showed that colony size plays a critical role in whether social learning is beneficial for colonies. This contrasts with the findings of models simulating honeybee foraging (Dornhaus *et al.*, 2006; Schürch & Grüter, 2014), but is consistent with an empirical study on honeybee colony foraging success (Donaldson-Matasci *et al.*, 2013). If colonies were small in our study, containing only 40-50 foragers, tandem runs did not improve colony success. Under these circumstances, colonies with social learners contained only a handful of scouts, and the probability to find food was small. Occasionally, simulation runs ended with colonies not having discovered any food sources. As colony size increases, this stochasticity decreases and colonies containing both social and individual learners perform better compared to colonies with individual learners only. The contrasting findings of our simulations and previous models of honeybee foraging (Dornhaus *et al.*, 2006; Schürch & Grüter, 2014) could be explained by the large number of simulated food sources in the honeybee models. This, in turn, makes it more likely that even small colonies quickly discover food sources, which could then be advertised by waggle dances.

Our simulations also show that finding the right mix of social and individual learners is a critical challenge for colonies (Fig. 2.5 and S2.4). Especially as colony size increases, small differences in the scout-recruit ratios can have a large effect on foraging success. In our simulations, having about 40% of scouts was usually most successful, but this is likely to depend on the number of food sources.

Communication is often considered to be beneficial, particularly in social insect colonies (but see e.g. Dechaume-Moncharmont et al., 2005; I'Anson Price et al., 2019). However, our empirical and simulation results highlight that there is often a narrow parameter space that favours colonies that use communication, especially when species have small colony sizes. This is consistent with empirical findings showing that ants with small colony sizes often forage solitarily (Maschwitz *et al.*, 1974; Jessen & Maschwitz, 1986; Beckers *et al.*, 1989). For instance, our findings could explain why ants like *Diacamma*, *Neoponera or Paltothyreus*, bumblebees and many stingless bees do not share information about food source locations with nestmates, even though some of these species use communication during colony emigrations. More information about the foraging ecology of these species would help build more accurate models, which might reveal why they do not communicate.

Supplement

Material & Methods

Model description

We developed a spatially explicit agent-based simulation model (ABM) with NetLogo version 6.1.0 (Wilensky, 1999). The purpose of the model was to explore how tandem recruitment affects the foraging success of colonies. We compared colonies that could perform tandem runs and colonies without tandem runs. This latter situation is, for example, found in ant species where tandem runs are performed only during colony migrations, but not during foraging (e.g. in *Diacamma* and *Neoponera*; Kaur *et al.*, 2017; Grüter *et al.*, 2018). We were interested in testing the effects food source distribution, scout-recruit ratios and colony size. The ABM was inspired by

Temnothorax nylanderi to derive some of the basic parameters, but applies to a wider range of social insects with linear recruitment communication in foraging. For a more extensive version of this model that tests a wider range of parameters, see Goy et al. (2021).

Entities, state variables and scale

The agents operated in a two-dimensional square grid of 140 x 140 patches (arena) with a nest in the center and 2 or 10 food sources (FS). The nest was located in the center at coordinates (x=0, y=0), with a radius of 10 patches. The food patches were at a distance of 40 patches from the outer edge of the nest (we also tested a distance of 20 patches; the results were qualitatively nearly identical). For the model we considered 1 tick (time unit used by NetLogo) to be equivalent to 1 second and 1 patch to 1 cm. The experimental duration t_{max} = 5400 ticks (corresponding to 90 minutes real time) was chosen to be similar to the duration of the empirical experiments. Model time and distance were connected via the walking speeds (0.8 patches/tick = $v_{outside}$, 0.4 patches/tick = v_{tandem}), which were chosen to mimic the walking velocity in cm/sec of real *T. nylanderi* ants (Glaser & Grüter, 2018).

Tandem running species tend to have small colonies (Beckers *et al.*, 1989). The colony size in our ABM ranged from 40 to 100 (note that all agents are foragers), covering the natural range of colony sizes of *T. nylanderi* and many other tandem running species. The colonies consisted of varying numbers of two types of agents, scouts and recruits (Table S2.1). The default scout-recruit ratio was 1:4, based on the counts of scouting ants and estimated forager numbers in our empirical experiments. This is similar to honeybees where scouts usually represent 5-25% (Seeley, 1995). However, several other ratios were tested as well (see results). Scouts search for food source locations without following a tandem run, whereas recruits wait in the nest until they find a recruiting ant to follow in a tandem run.

Process overview

We performed 30 simulations per parameter combination. At the beginning of each simulation, the nest and 2 or 10 food sources (NFS) were created. Varying numbers of scouts (N_{scouts}) and recruits ($N_{recruits}$) were setup in the centre of the nest. All agents started with a forager energy (FE) of 0. When leaving the nest, the FE decreased every step by a metabolic cost M_{Cost} (Table S2.1), which was chosen so that the energetic costs that accumulated during a foraging trip corresponded to ~0.1% of the energy obtained during a typical foraging trip (Fewell, 1988) (see Goy et al. 2021 for more details). Agents did not use up energy when they were drinking or inside the nest.

Scouts initiated foraging by performing a random walk with the speed of $v_{outside}$, whereas recruits were patrolling inside the nest with speed v_{nest}, waiting to be recruited. When a scout found a food source, it began feeding for a duration of 120 ticks ($t_{feeding}$). If scouts did not find a food source within 600 ticks (t_{scouts}), they returned to the nest, stayed there for 60 ticks ($t_{nest\text{-}stay}$, simulating food unloading) before leaving the nest again for a new search trip.

Based on the weight difference between full and empty foragers (N = 11), we calculated that a *T. nylanderi* forager feeding at a 1 molar sucrose solution collects ~0.75 Joule per foraging trip, whereas a forager feeding at a 0.1 molar solution would obtain ~0.075 J. Therefore, agents discovering a food source would obtain one of these two amounts, depending on whether the food source was of high or low quality (FS_{high} and FS_{low}). Agents feeding at the high-quality food source would become "satisfied" agents, whereas agents feeding at the low-quality food source would have only a 10% probability to become "satisfied" foragers and a 90% chance of becoming "unsatisfied" foragers. Satisfied agents would become active recruiters with a 50% probability ($p_{recruitment}$) upon return to their nest, leading to tandem run probabilities that are similar to what has been found in *T. nylanderi* and *Pachycondyla harpax* (Glaser & Grüter, 2018; Grüter *et al.*, 2018), whereas unsatisfied agents would not recruit. After $t_{nest\text{-}stay}$, satisfied agents that did not become recruiters (50% of all satisfied agents) would return to the food source they had visited before, i.e. use private information. Unsatisfied agents would have an equal probability to leave the nest as scouts to search for a new food source or become recruits and wait inside the nest. Agents that became active recruiters searched for a potential recruit for 120 ticks ($t_{tandemstarter}$) in order to initiate a tandem run and become tandem leaders. A tandem run started when a recruiter encountered a recruit on the same patch inside the nest. Together they then moved with speed v_{tandem} towards the respective food source. After arrival at the food source, they became feeding ants and again could become either happy or unhappy agents (S2.1). In the default situation, tandem runs did not break up. However, we also ran simulations with a tandem success rate of 75%, similar to what we found in our experiments. In this case, a follower became a "lost tandem follower" with a probability of 0.002 per tick. Lost tandem followers first perform a random walk for 180 ticks ($t_{search\text{-}time}$) and, if they do not find a food source, had an equal probability to become either a scout or return to the nest as an unsatisfied forager. Leaders would continue to the food source.

The simulation finished when t_{max} was reached. The total nest energy (total J of all individual collection trips minus the total J of the metabolic costs) was measured for each simulation run. Additionally, the time until scouts and recruits (as tandem follower) discovered their first food source and the time recruits had to wait inside the nest till recruitment occurred was recorded.

Model variations

A large number of parameter combinations can be simulated with our model. In this study, we only present a sub-set of possible parameter combinations in order to show general patterns of how our variables of interest (e.g. food source number or colony size) affect the value of tandem communication. Different combinations can be run with the NetLogo files we provide as supplementary material (see also Goy et al. 2021 for a more detailed analysis of this model).

Table S2.1: Overview of the model parameters and the used values.

Abbreviation	Explanation	Default Values	Other values tested
N_{scouts}	Number of scouts in agent population	20	8 - 100
$N_{recruits}$	Number of recruits in agent population	80	0 - 80
N_{FS}	Number of food sources	2 or 10	
FS_{size}	Size of food sources	1 patch	
N_{size}	Radius of nest	10 patches	
t_{scouts}	Time a scout searches food before returning to nest	600 ticks	
$t_{nest\text{-}stay}$	Time a returned forager stays at nest	60 ticks	
$t_{tandemstarter}$	Time an active recruiter searches for a recruit in the nest	120 ticks	
$t_{feeding}$	Feeding time of drinking agents	120 ticks	
t_{max}	Duration time of a simulation	5400 ticks	
FS_{High}	Energy gained from the high quality food source	0.75 Joule	
FS_{Low}	Energy gained from the low quality food source	0.075 Joule	
$p_{recruitment}$	Probability to recruit when “satisfied”	50%	
$v_{outside}$	walking velocity of ants outside the nest	0.8 patch/ticks	
v_{nest}	walking velocity of ants inside the nest	0.1 patch/ticks	
v_{tandem}	walking velocity of Tandem leader and Tandem follower towards the respective food source	0.4 patch/ticks	
M_{cost}	Metabolic or energy cost of walking outside	0.000000244699 J/tick	

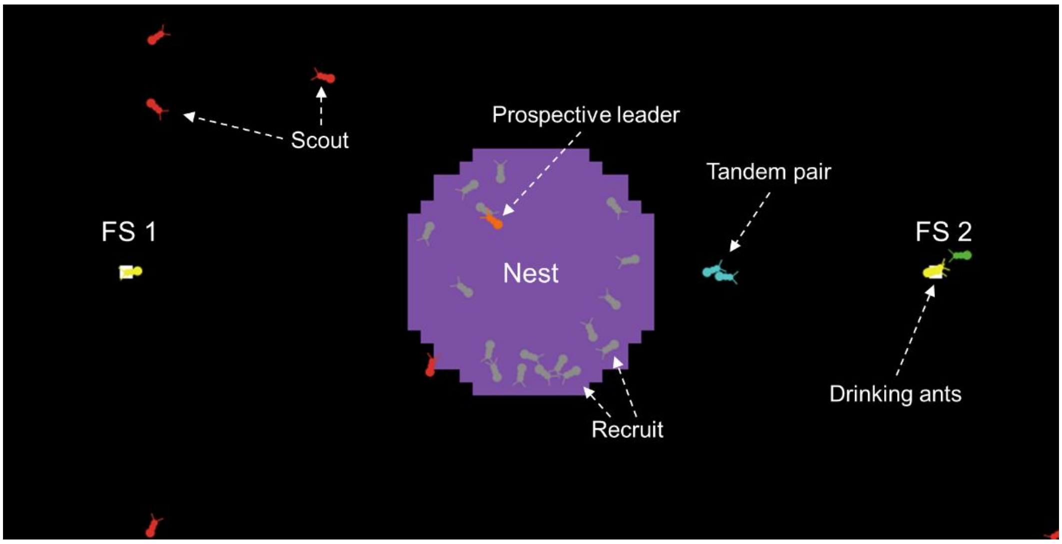

Figure S2.1: Overview of the Agent-based model with a centred nest (violet) and 2 food sources (white) at a distance of 40 patches for the recruitment treatment. Different agents are shown.

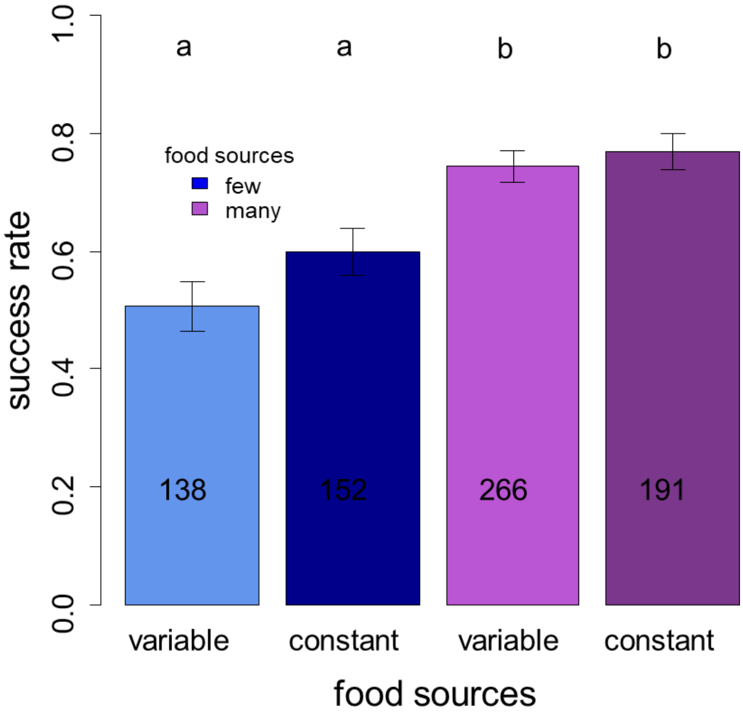

Figure S2.2: Success rate depending on the food quantity, for few and many food sources. Numbers in columns represent tandem runs to a food source. Bars show mean ± standard error. Different letters indicate significant differences.

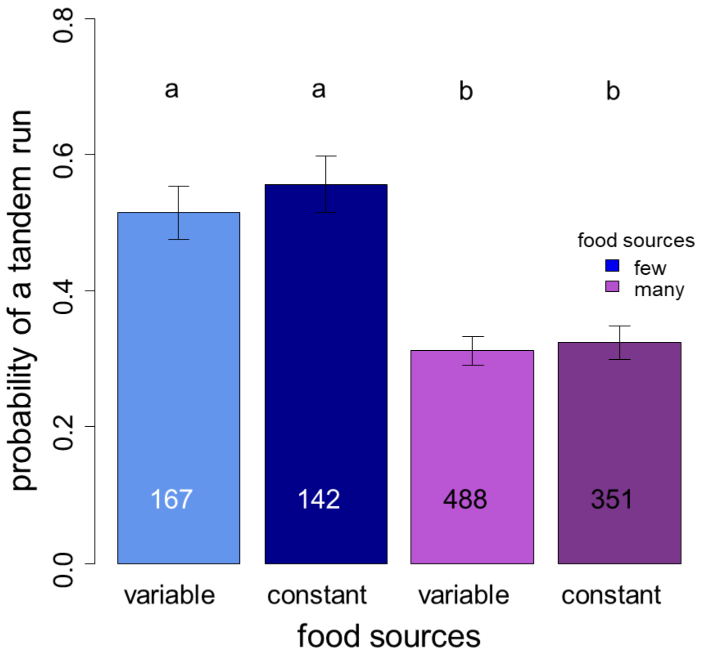

Figure S2.3: Probability of a tandem run depending on the different setups. Numbers in columns represent the number of individual visits (starting with visit 2). Bars show mean ± standard error. Different letters indicate significant differences.

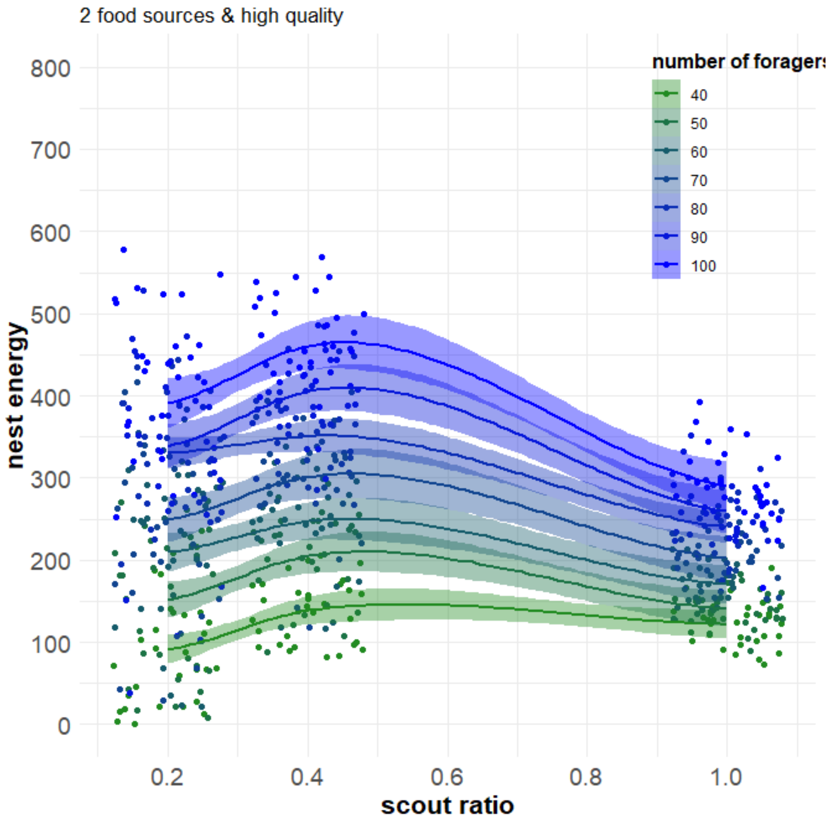

Figure S2.4: Nest energy depending on the scout ratio. Presented are different colony sizes.

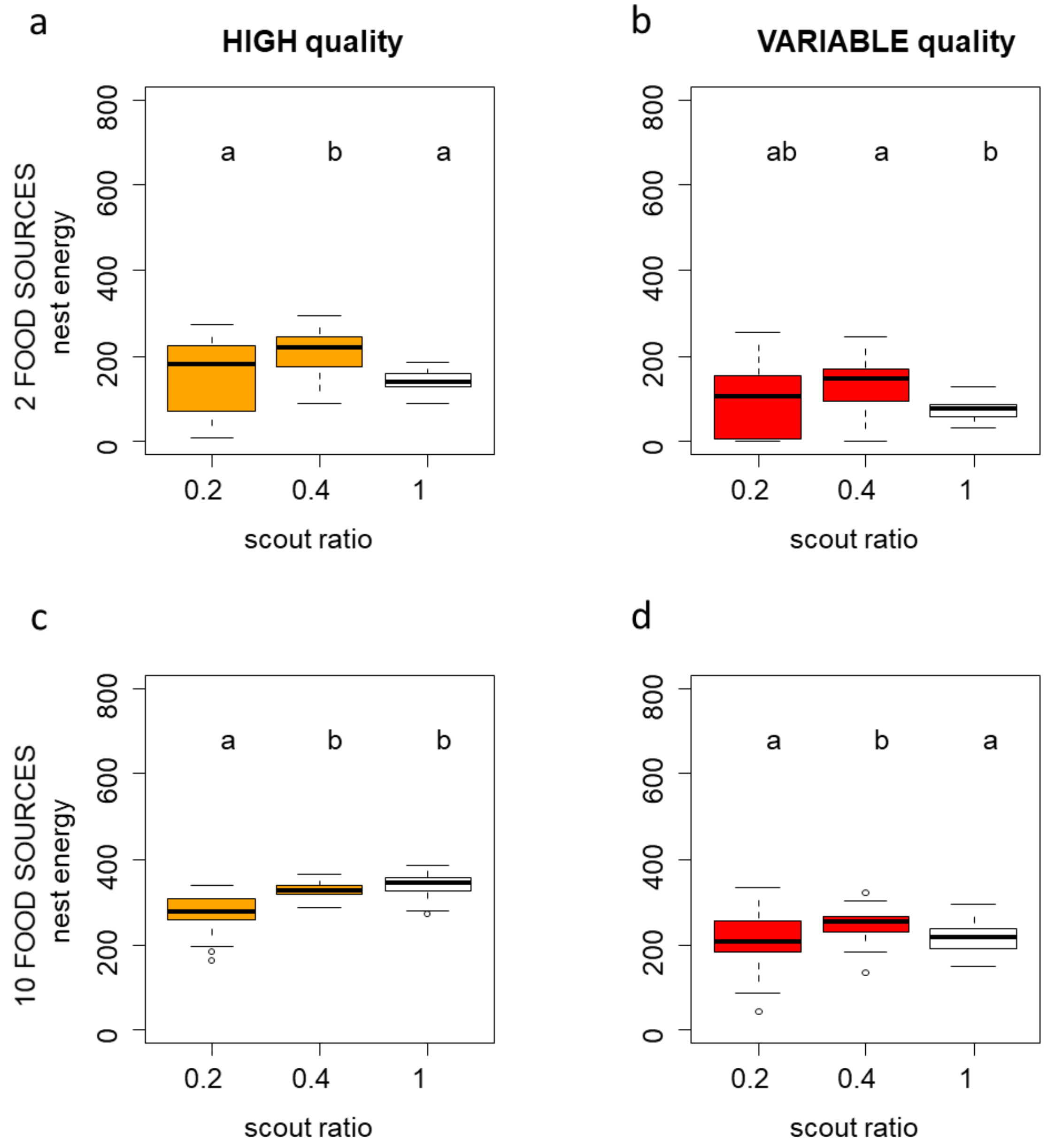

Figure S2.5: Nest energy depending on different scout ratios with a colony size, containing 50 foragers. High or variable quality treatments were tested with either two (**a** & **b**) or ten (**c** & **d**) food sources. Boxplots show medians, quartiles and fifth and 95th percentiles. Different letters indicate significant differences.

CHAPTER 3

The adaptive value of tandem communication in ants: insights from an agent-based model

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Abstract

Social animals often share information about the location of resources, such as a food source or a new nest-site. One well-studied communication strategy in ants is tandem running, whereby a leader guides a recruit to a resource. Tandem running is considered an example of animal teaching because a leader adjusts her behaviour and invests time to help another ant to learn the location of a resource more efficiently. Tandem running also has costs, such as waiting inside the nest for a leader and a reduced walking speed. Whether and when these costs outweigh the benefits of tandem running is not well understood. We developed an agent-based simulation model to investigate the conditions that favour communication by tandem running during foraging. We predicted that the spatio-temporal distribution of food sources, colony size and the ratio of scouts and recruits affect colony foraging success. Our results suggest that tandem running is favoured when food sources are hard to find, differ in energetic value and are long lasting. These results mirror the findings of simulations of honeybee communication. Scouts locate food sources faster than tandem followers in some environments, suggesting that tandem running may fulfil the criteria of teaching only in some situations. Furthermore, tandem running was only beneficial above a critical colony size threshold. Taken together, our model suggests that there is a considerable parameter range that favours colonies that do not use communication by tandem running, which could explain why many ants with small colony sizes forage solitarily.

Introduction

Finding food is critical for survival and reproduction, but also energy- and time-consuming. Foraging for food can be done independently or by using information provided by other organisms (Sumpter, 2010; Hoppitt & Laland, 2013). In social insects, such as ants, social bees or social wasps, new food sources are usually discovered by scouts that explore the environment on their own (Hölldobler & Wilson, 1990; Seeley, 1995). After finding a profitable food source, they return to their nest and often communicate their discovery to nestmates. The communicated information depends on the species, but often includes the location of the resource, e.g. by means of laying a pheromone trail (Hölldobler & Wilson, 1990; Jarau & Hrncir, 2009; Czaczkes *et al.*, 2015b). Recruitment communication allows colonies to exploit profitable feeding sites fast, e.g. before competitors have discovered and consumed the food source. It can also help to build up a critical mass of foragers that can defend the food source against competitors (Glaser *et al.*, 2021). Once the foragers have learned the location of the food source, they can use their route memory to return to the feeding site (e.g. Collett et al., 2013; von Frisch, 1967).

In social insects, foraging strategies should not only take into account short-term individual success, but also how they affect colony foraging success. Thus, the value of communication should ultimately be studied at the colony level. So far, most theoretical and empirical studies that explored the value of communication for colony foraging success have focused on honeybees (but see also e.g. Sumpter & Pratt, 2003; Dechaume-Moncharmont *et al.*, 2005; Czaczkes *et al.*, 2015a). These studies suggest that the value of communicating the location of food sources by means of waggle dances depends on how food sources are distributed (Beekman & Lew, 2007; Donaldson-Matasci et al., 2013; Dornhaus et al., 2006; Dornhaus & Chittka, 2004; I'Anson Price et al., 2019; Schürch & Grüter, 2014; Sherman & Visscher, 2002 reviewed in I'Anson Price & Grüter, 2015). For example, Beekman & Lew (2008) found that the value of the "dance language" (the spatial information provided by the waggle dance) depends on the size and distance of the food patches. When patches were large and close to the hive, colonies that did not use dance communication and instead followed an individual foraging strategy were more successful. Dornhaus et al. (2006) concluded that dance communication does not help colonies collect more energy if there are many food sources that vary little in quality. Their models suggest that communication is beneficial if high-quality food sources are available, but are hard to find and that dance communication could be detrimental if food sources are easy to find (see also (Dechaume-Moncharmont *et al.*, 2005). In the latter case, foragers should search for new food sources through scouting (independent search) and return to known high-quality food sources using route memory (Schürch & Grüter, 2014).

There is a well-known, but not yet fully understood link between colony size and the method of recruitment in ants (Beckers *et al.*, 1989; Planqué *et al.*, 2010; Dornhaus *et al.*, 2012). While large colony size is associated with pheromone-based mass-recruitment, species with smaller colony sizes often forage solitarily or they use a recruitment method called tandem running (Beckers *et al.*, 1989). In tandem running, an experienced ant (tandem leader) guides an inexperienced nestmate (tandem follower) to a new nest-site or a rewarding food source (Franks & Richardson, 2006; Hingston, 1929; Kaur et al., 2017; Möglich et al., 1974; Pratt, 2008; Wilson, 1959; reviewed in Franklin, 2014). It has been argued that tandem followers locate resources quicker than scouts that search for resources by individual exploration and trial-and-error learning (Franks & Richardson, 2006). Additionally, ants that are recruited by a tandem leader might find food sources of higher quality because foragers are more likely to perform tandem runs after finding a better food source (Shaffer *et al.*, 2013). On the other hand, tandem running also has disadvantages. During a tandem run, both ants walk with reduced speed (Franks & Richardson, 2006; Kaur *et al.*, 2017) and a substantial proportion of tandem runs fail (e.g. Glaser & Grüter, 2018; Grüter et al., 2018; Pratt, 2008; Wilson, 1959). Furthermore, recruits experience time and opportunity costs as they wait inside their nest for a leader, rather than search in the environment for food sources by themselves. These disadvantages could explain why some ant species do not seem to use tandem communication when foraging, even though tandem runs are used during colony migrations (Hölldobler, 1984; Traniello & Hölldobler, 1984; Fresneau, 1985; Maschwitz *et al.*, 1986). More generally, a sizeable group of ant species do not seem to use any form of communication during foraging (e.g. Beckers et al., 1989; Lanan, 2014; Reeves &

Moreau, 2019). This raises the question whether, when and how a communication method that is relatively slow and small-scale like tandem running, improves colony foraging success and whether the ecological circumstances that favour tandem running match those that favour honeybee dance communication.

We developed an agent-based simulation model to investigate the importance of recruitment communication in the form of tandem running for the foraging success of virtual ant colonies. We compared colonies that could perform tandem runs with colonies that consisted only of scouts, *i.e.* without tandem running in an environment that varied in the number, quality, distance and longevity of food sources. Additionally, we tested whether colony size affects the importance of tandem communication for colony foraging success. Finally, we explored the role of forager ratio (relative numbers of scouts and recruits) and tested if recruits indeed locate food sources faster than scouts. Based on studies that simulated honeybee foraging, we predicted that tandem running is beneficial when high quality food sources are hard to find (Dornhaus *et al.*, 2006; Beekman & Lew, 2007), but is detrimental to colony success when food sources are short-lived (Schürch & Grüter, 2014). We also predicted that larger colonies benefit more from tandem running.

The agent-based simulation model

An agent-based simulation model (ABM) was developed using the software Netlogo 6.1.1 (Wilensky, 1999; Wilensky & Rand, 2015) (the NetLogo file can be found in the online material). The model simulates the foragers of an artificial ant colony in an environment consisting of their nest and food sources. Some of the basic parameters, like the range of colony sizes, walking speeds of scouts and tandems or energy collected by foragers were derived from the ant species *Temnothorax nylanderi* (Glaser & Grüter, 2018).

Purpose

The aim of our model was to explore the adaptive value of tandem running in ants by measuring the colony foraging success (as gained energy) and the time required by foragers to find a food source. We compared colonies that could perform tandem runs with colonies that consisted only of scouts, *i.e.* without tandem running. This latter situation is found in many ant species with small colony sizes, such as *Diacamma* or *Neoponera* (Fresneau, 1985; Hölldobler, 1984; Traniello & Hölldobler, 1984, Kaur, pers. Communication). In both situations, foragers could also use route memory (or private information) to return to food sources they visited in the past. We assessed the effects of tandem communication depending on food source distribution (number, distance), their quality and stability as well as colony size and the scout-recruit ratio.

Entities, state variables and scale

Netlogo operates with patches that can be used to measure distances and ticks for time steps. In our model, 1 tick is equivalent to 1 second and 1 patch to 1 cm. The agents operate in a two-dimensional square grid of 140×140 patches (arena) with a nest and either 2 or 10 food sources (FS). The border of the square grid represents the boundary of the virtual world at which agents turn around. This simulated environments with few or many food sources. The nest is located in the center (x=0, y=0), with a radius of 10 patches. The food patches were at a distance of either 40 (default) or 20 patches from the outer edge of the nest, simulating natural conditions as *T. nylanderi* mostly forages within 50 cm from their nest (Heinze *et al.*, 1996). Each food source had a size of 1 patch, which could represent a dead insect or a drop of honey dew, and could either be of high or low quality (FS_{high} and FS_{low}), simulating a sugar solution of either 1 molar or 0.1 molar concentration.

Since all agents are foragers, our default colony size of 100 would correspond to a natural colony consisting of ~300-400 workers, assuming that foragers account for about 20-30% of a *Temnothorax* colony (e.g. Shaffer et al., 2013). Simulated colonies consisted of varying ratios of scouts that search for resources independently and recruits that waited in the nest until they are recruited to a food source. The default scout-recruit ratio was 1:4 (*i.e.* 20 scouts + 80 recruits in the default situation), similar to what has been observed in honeybees where scouts represent about 5-35 % of the colony (von Frisch, 1967; Seeley, 1995). In colonies without tandem running, all foragers were scouts. In the default configuration, scouts and recruits can both assume any of the following seven states: (1) idle inside the nest, (2) feeding at food sources, (3) returning to the nest with food, (4) unloading food, (5) searching for a follower inside the nest, (6) leading a tandem run to the food source or (7) returning alone to the food source (*i.e.* use private information). Additionally, scouts search for food sources independently, while recruits wait inside the nest for a tandem leader. Recruits can then follow tandem runs to a food source (Fig. 3.1).

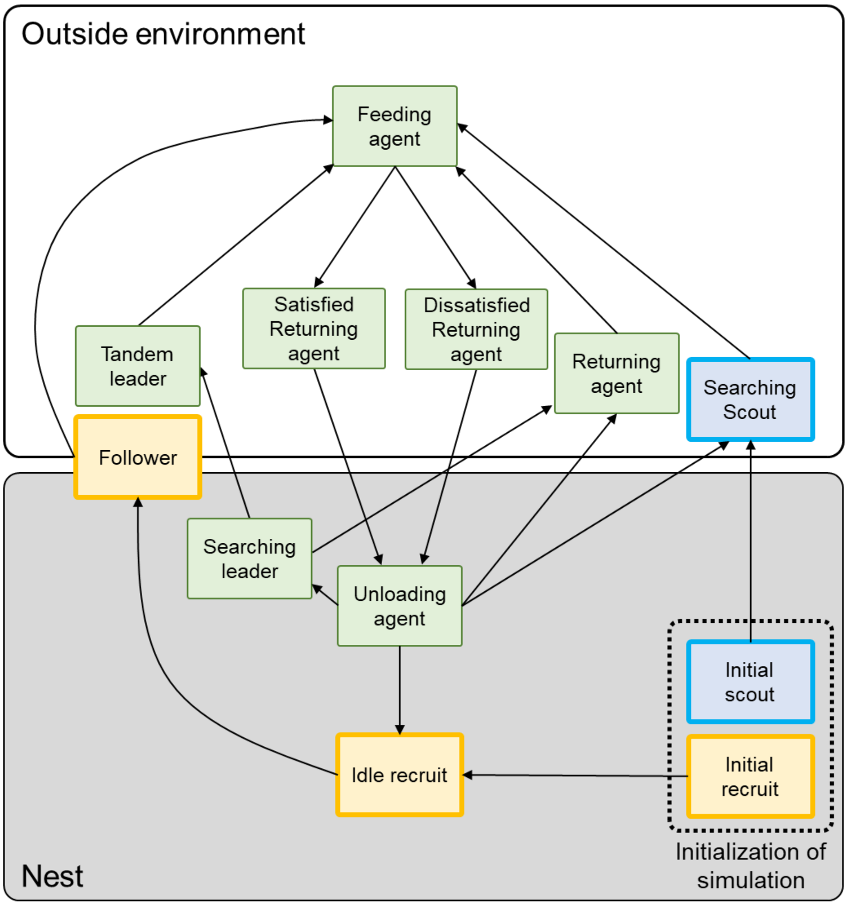

Figure 3.1: State diagram for the agent-based model for colonies with tandem runs. All foragers start inside the nest. Green boxes represent agent states that are possible for both scouts and recruits, blue boxes are states that are only possible for scouts and orange boxes represent states that are only possible for recruits. See Fig. S3.1 for a more detailed state diagram.

Colonies gain nest energy (NE) when agents return to the nest and "unload" the energy gained at food sources. We estimated the energy content of a crop load of a *T. nylanderi* forager the following way: we measured foragers (N = 21, from 3 colonies) and estimated that full foragers carry ~0.15 mg of sugar solution. Given the energetic value of sucrose, we calculated that a forager feeding at a 1 molar sucrose solution collects ~0.75 Joule per foraging trip, whereas a forager feeding at a 0.1 molar solution would obtain ~0.075 J.

Process overview

The default simulation duration t_{max} was 5400 ticks (corresponding to 90 minutes), but we also tested a duration of 21600 ticks (corresponding to 6 h). Time and distance in the model were connected via the walking speeds (0.8 patches/tick outside the nest = $v_{outside}$, 0.4 patches/tick in a tandem run= v_{tandem}), which were chosen to be similar to the walking velocity (in cm/sec) of *T. nylanderi* ants (Glaser & Grüter, 2018, Glaser S., unpublished data).

When the model was initialized (t=0), the nest and either 2 or 10 food sources and the agents were created. The generated food sources always occupied the same locations, *i.e.* the same pre-defined x- and y-coordinates. In the situation without tandem running, only scouts were simulated. All agents started in the centre of the nest. Scouts immediately started to perform a random walk to search for food sources with the speed of $v_{outside}$, whereas recruits patrolled inside the nest with speed v_{nest} (0.1 patches/tick) and waited to be recruited by another agent. All agents started with an energy of zero. When leaving the nest, this energy decreases every tick by a metabolic cost M_{cost} (see Table 3.1). M_{cost} was chosen so that the metabolic costs that accumulate during an average foraging trip correspond to ~0.1% of the value of energy obtained during a typical foraging trip (Fewell 1988). We estimated this by running several simulations and measuring foraging trip duration of our agents. We also ran simulations with metabolic rates that were 10-times higher or 10-times lower than our default value but found that this did not affect the general patterns (Fig. S3.2).

Table 3.1: Overview of the model parameters and the used values.

Variables	Description	Default values	Other values tested	Information source
FS distance	Distance from nest to food source	40	20	Heinze et al. 1996
FS number	Number of food sources	2 or 10		arbitrary
Colony size	Number of agents (foragers) in a colony	100	20-200	Beckers et al. 1996
Scout-recruit ratio	The ratio of scouts and recruits in a colony	1:4 (r = 0.2) or (all scouts)	1:9 to 10:0 (r = 0.1 to 1.0)	arbitrary range
$\mathbf{FS_{High}}$	Energy gained from the high-quality food source	0.75 J		Experimentally determined
$\mathbf{FS_{Low}}$	Energy gained from the low-quality food source	0.075 J		Experimentally determined
$\mathbf{t_{max}}$	Duration of a simulation (1 tick ~ 1 second)	5400 ticks	21600 ticks	arbitrary
$\mathbf{v_{outside}}$	Walking velocity of ants outside the nest	0.8 patch/ticks		Glaser, S., unpublished data for *T. nylanderi*
$\mathbf{v_{nest}}$	Walking velocity of ants inside the nest	0.1 patch/ticks		arbitrary
$\mathbf{v_{tandem}}$	Walking velocity of Tandem leader and Tandem follower towards the respective food source	0.4 patch/ticks		Glaser & Grüter 2018
$\mathbf{M_{cost}}$	Metabolic or energy cost of walking outside	2.446 x 10^{-7} J/tick	2.446 x10^{-6}, 2.446 x10^{-8}	Estimated based on Fewell 1988
$\mathbf{t_{scouts}}$	Time a scout searches food before returning to nest	600-900 ticks		S.M.G., pers. observation in *T. nylanderi*
$\mathbf{t_{nest\text{-}stay}}$	Time a returned forager stays inside the nest	60 ticks		Grüter et al. 2018
$\mathbf{t_{tandemstarter}}$	Time an active recruiter searches for a recruit inside the nest	120 ticks	60, 180 ticks	Grüter et al. 2018, Glaser, S., unpublished data for *T. nylanderi*
$\mathbf{t_{feeding}}$	Feeding time of drinking agents	120 ticks		Glaser, S., unpublished data for *T. nylanderi*
$\mathbf{p_{break\text{-}up}}$	Probability that tandems break up	0/tick	0.002/tick, 0.005/tick	Range observed in different species
$\mathbf{p_{recruitment}}$	Probability to recruit when "satisfied"	50%		Grüter et al. 2018, Glaser & Grüter 2018

When an agent finds a food source, it becomes a feeding agent and feeds for a duration of 120 ticks. It gains either 0.75 J or 0.075 J, depending on whether the food source is of high or low quality. If scouts do not find a food source within a certain time period (t_{scouts}), they return to the nest. If they are at greater distances from the nest, unsuccessful scouts return quicker (600 ticks). Unsuccessful scouts that are closer to the nest (35 patches from the center) return if 900 ticks have passed. This was done to match observations that *T. nylanderi* scouts often return to their nest if they had been searching unsuccessfully for several minutes (S.M.G., personal observation). After their return, unsuccessful scouts wait idle inside the nest for 60 ticks ($t_{nest\text{-}stay}$), before resuming to scout. At the end of the feeding time, agents return to the nest either as "satisfied" or "unsatisfied" foragers. Foragers that found a high-quality food source were always satisfied, whereas agents feeding at a low-quality food source had only a 10% probability to become satisfied. After unloading for the duration of $t_{nest\text{-}stay}$, "satisfied" agents become prospective tandem leaders with a 50% probability ($p_{recruitment}$), whereas unsatisfied agents would not recruit. This leads to a recruitment probability of 5-50% per trip, which is similar to what has been found in both *T. nylanderi* and *Pachycondyla harpax* (Glaser & Grüter, 2018; Grüter *et al.*, 2018). Satisfied agents return to the same food source they had visited before, either in a tandem run or alone. In other words, they use "route memory" to revisit a high-quality food source, but were unlikely to return to a low-quality food source (10% probability). Unsatisfied agents would not recruit and either wait inside the nest for a tandem leader (recruits) or they search for a new food source (scouts). Fig. S3.3 is a screenshot of a simulation showing the arrangement of the nest, food sources and some of the agent states.

Prospective tandem leaders stay inside the nest and search for a potential recruit for the duration of 120 ticks ($t_{tandemstarter}$). In Pachycondyla harpax, most foragers stayed less than 90 seconds inside their nest (Grüter *et al.*, 2018), whereas *Temnothorax nylanderi* foragers stayed on average 160 seconds inside the nest before leading a tandem run to a food source (S.M.G., unpublished data). Therefore, we also tested a $t_{tandemstarter}$ of 60 and 180 ticks, but found no difference with 120 ticks (Fig. S3.4). A tandem run starts when a leader encounters a recruit on the same patch. If a prospective leader does not find a recruit after $t_{tandemstarter}$, they return to the previously visited food source alone.

By default, tandem runs do not break up but we also tested situations with a break-up probability of 0.002 and 0.005 per tick, which corresponds to tandem success rates of ~75 % and ~50% for the default distance (calculated based on an average tandem run duration of 127 ticks for the default food source distance). Lost tandem followers first perform a random walk for 180 ticks ($t_{search\text{-}time}$) and – if they do not find a food source – have an equal probability to become either a scout or to return to the nest as an unsatisfied forager.

In the default settings, food sources were ad libitum, *i.e* they did not disappear during the simulations. Since this may not always be the case, we also simulated food sources that disappeared after they were visited by 10 agents to create a more dynamic foraging environment. If a food source disappears before ants return to it (either alone or in a tandem), agents reaching the old food source location search randomly for 180 ticks ($t_{search\text{-}time}$), then they become unsatisfied foragers and return to the nest. If the food source vanishes during feeding, the agent becomes an unsatisfied forager. Food sources that have disappeared are replaced by an identical food source at the same position after 600 ticks have passed, which means that it has to be discovered again by scouts. For each simulation run, new inexperienced agents were created as described above. Table 3.2 lists the tested factors.

Table 3.2: The different factors tested in our model. See Table 3.1 for values

Tested factor	What was manipulated
Spatio-temporal distribution of food sources	Number of food sources, distance to food sources, simulation duration, longevity of food sources
Variability of food sources	Energetic value of food sources was set to be either identical (only high-quality) or mixed (high- and low-quality food sources). We also simulated conditions with only low-quality food sources. A pure scouting strategy was always better under these circumstances (Fig. S3.5). This is because tandem runs are very rare when all food sources are of low quality and recruits spend most of their time inside the nest.
Colony composition	Different scout-recruit ratios were tested
Colony size	In addition to simulating a colony size of 100 agents, we tested a range of other colony sizes

We measured the total nest energy NE (total J of all individual collection trips minus the total J of the metabolic costs) for each simulation run. Due to the stochasticity of simulations we performed 30 simulation runs for each tested combination of parameters. In addition, we measured the food discovery time of scouts and recruits under default conditions. This refers to the time scouts needed to discover their first food source. In recruits, we measured both their waiting time inside the nest and the duration of the tandem run. These durations were averaged per forager type and per simulation run. Agents that did not discover a food source during an entire simulation were given the maximum value of 5400 ticks. The default condition of $p_{\text{break-up}} = 0$ was simulated.

Statistical analyses

All statistical analyses were performed using the software R 3.6.3 (www.r-project.org). Since different treatments occasionally had unequal variance (heteroscedasticity) or contained zeros and in order to provide a consistent statistical approach we used non-parametric statistical tests throughout. It should be noted, however, that when we compared parametric and non-parametric methods (Anova's), they yielded very similar results. We used Mann-Whitney U tests to compare two independent samples and Wilcoxon signed-rank tests for paired data. In addition to the p-values, the R software provides the test statistic value W, which is a linear transformation of the usual rank sum statistic U. When three groups were compared, we used Kruskal-Wallis tests and Dunn tests with sequential Bonferroni corrections for post-hoc pair-wise comparisons ("FSA" package, Ogle et al., 2020) (Sokal & Rohlf, 1995).

Results

Distribution of food sources

We first tested if the number of food sources and their distance from the nest affect the value of tandem running. When colonies had access to few food sources, they were more successful with tandem recruitment (scout-recruit ratio of 1:4) than colonies consisting only of scouts, irrespective of whether food sources were of high-quality (Fig. 3.2) (Mann-Whitney U Test, $W = 215$, $p = 0.0004$) or of mixed quality ($W = 307$, $p = 0.034$). In a rich environment, with 10 food sources, colonies collected overall more energy (Fig. 3.2). Tandem communication was beneficial when food source quality was mixed ($W = 112$, $p < 0.0001$), whereas colonies consisting only of scouts performed better when all 10 food sources were of high quality ($W = 773$, $p < 0.0001$). This general pattern did not change when food sources were closer to the nest (20 patches instead of 40 patches) (2 food sources, high-quality: $p < 0.0001$; mixed-quality: $W = 210$, $p = 0.0003$; 10 food sources, high-quality: $W = 827$, $p < 0.0001$; mixed-quality: $W = 54$, $p < 0.0001$), but colonies gained overall more energy when all food sources were close to the nest (Fig. 3.2). Fig. 3.2e and 3.2f illustrate the temporal development of nest energy during exemplary simulation runs that correspond to the conditions shown in Fig. 3.2a and 3.2b with high-quality food sources.

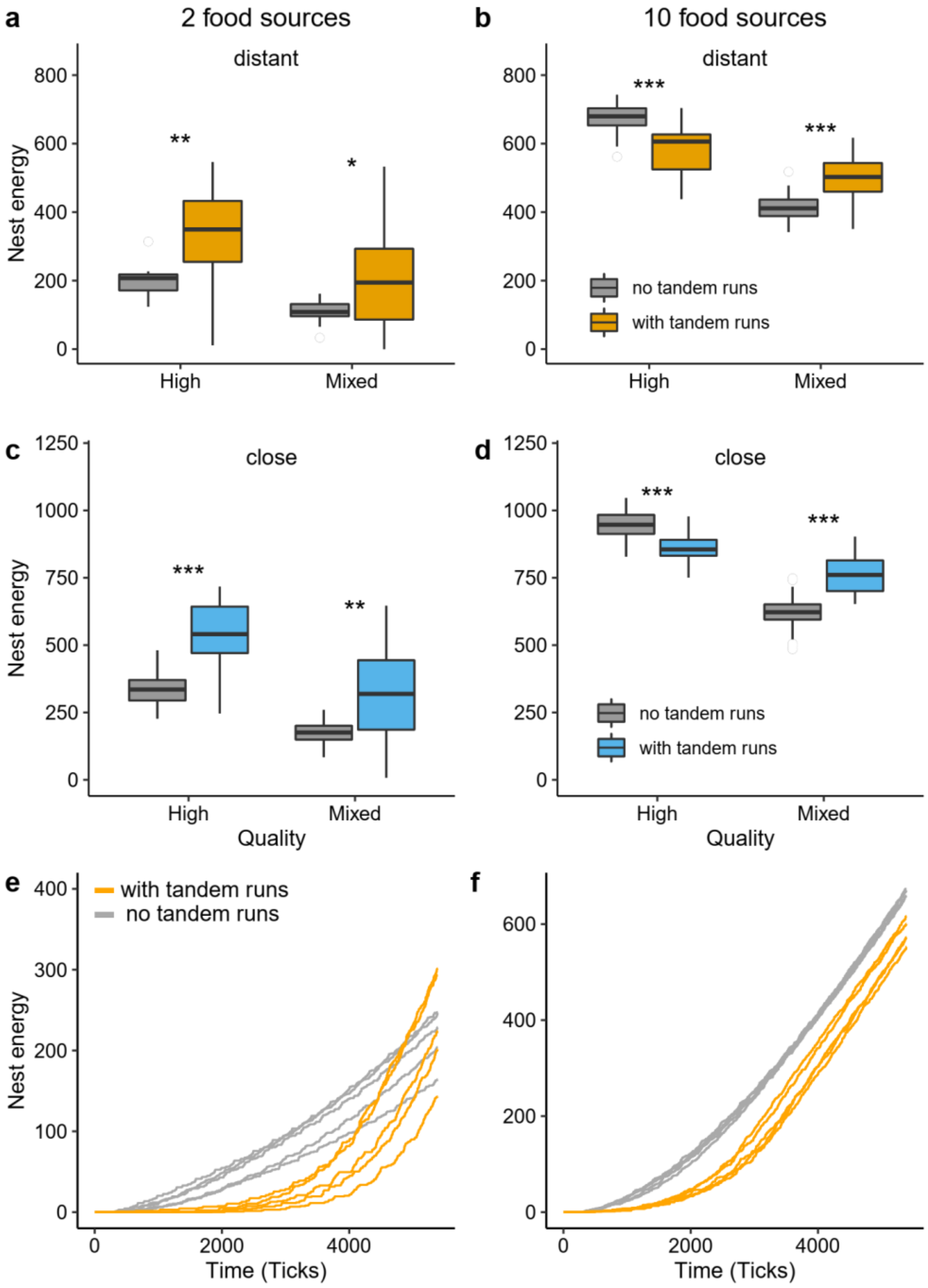

Figure 3.2: Nest energy with 2 or 10 food sources. In (**a**) and (**b**) food sources were at a distance of 40 patches, whereas in (**c**) and (**d**) food sources were at a distance of 20 patches. *p<0.05, **p<0.001, ***p<0.0001. In (**e**) and (**f**), nest energy is plotted over time for conditions as shown in (a) and (b) when all food sources were of high quality (5 simulation runs per treatment for visualisation of the trajectory).

Foraging duration and food source longevity

When we increased the foraging duration (*i.e.* the simulation duration) from 5400 to 21600 ticks, we found a similar pattern. Tandem running was highly beneficial when there were few food sources (high-quality: W = 0, p < 0.0001; mixed-quality: W = 0, p < 0.0001). Tandem runs were also beneficial when there were many food sources of mixed energetic quality (W = 56, p < 0.0001). In the case of many high-quality food sources, pure scout colonies performed better (W = 900, p < 0.0001). It is noteworthy that colonies with tandem communication were almost as successful in an environment with 2 food sources as in an environment with 10 food sources (Fig. 3.3a, b).

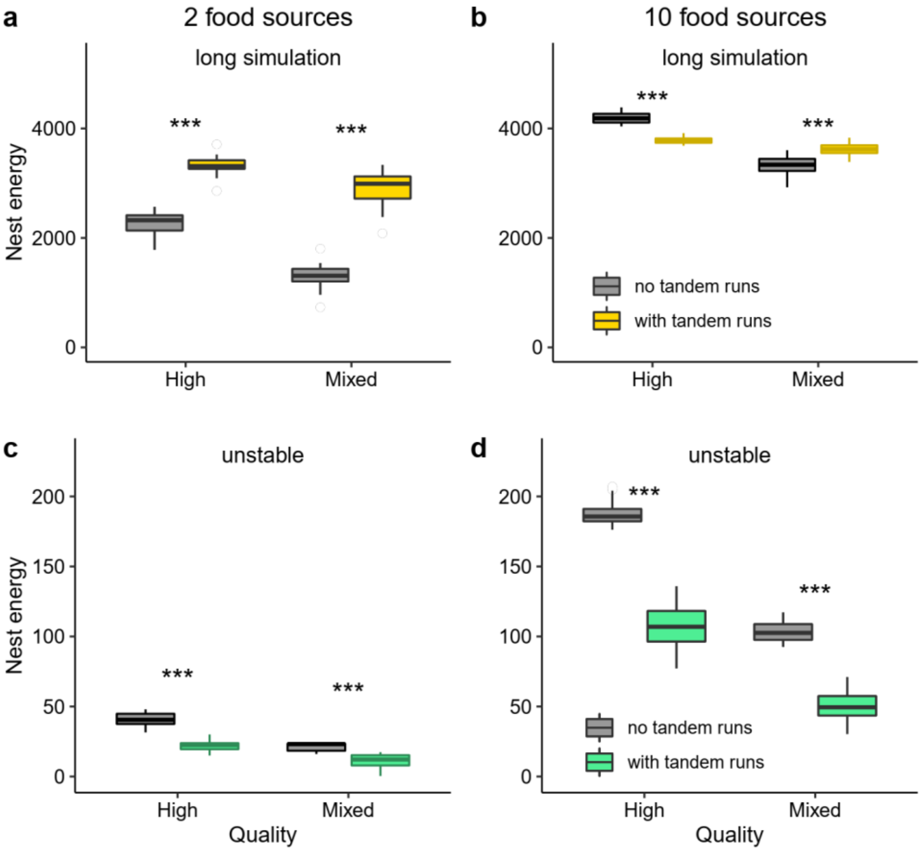

Figure 3.3: Nest energy with 2 or 10 food sources. In (**a**) and (**b**), food sources simulations were 4-times longer (21600 ticks instead of 5400). In (**c**) and (**d**), simulations lasted 5400 ticks and food sources disappeared if they were visited by 10 ants. A new food source appeared after a delay.

So far, we assumed that food sources offered food during the entire simulation. Next, we tested the effects of short-lived food sources. If food sources were unstable (sometimes called short-lived), a scouting strategy was more successful, irrespective of the number of food sources and their variability (Fig. 3.3c,d) (2 food sources, high-quality: W = 897, p-value < 0.0001; mixed-quality: W = 896, p-value < 0.0001; 10 food sources, high-quality: W = 900, p-value < 0.0001; mixed-quality: W = 900, p-value < 0.0001). Differences were particularly pronounced when colonies were offered many food sources. Scouting remained the better strategy when we increased the foraging duration to 21600 ticks (e.g. 2 food sources, high-quality: W = 900, p-value < 0.0001; mixed-quality: W = 900, p-value < 0.0001).

Tandem success rate

Tandems do occasionally break up and we tested how this affects the energy collected by colonies. We compared colonies with 100% (default), ~75% and ~50% successful tandem runs and colonies with only scouts in an environment with few food sources, *i.e.* under conditions where tandem runs are beneficial (Fig. 3.2a). Our simulations show that a reduction in tandem success rate has a negative impact on the energy intake that is collected by colonies (Fig. 3.4). If only about 50% of the tandem runs are successful, colonies without any tandem running collect more energy in an environment with few, stable food sources (Fig. 3.4) (high-quality, 50% success rate vs. no tandems: W = 246, p = 0.002, mixed-quality, 50% success rate vs. no tandems: W = 257, p = 0.004).

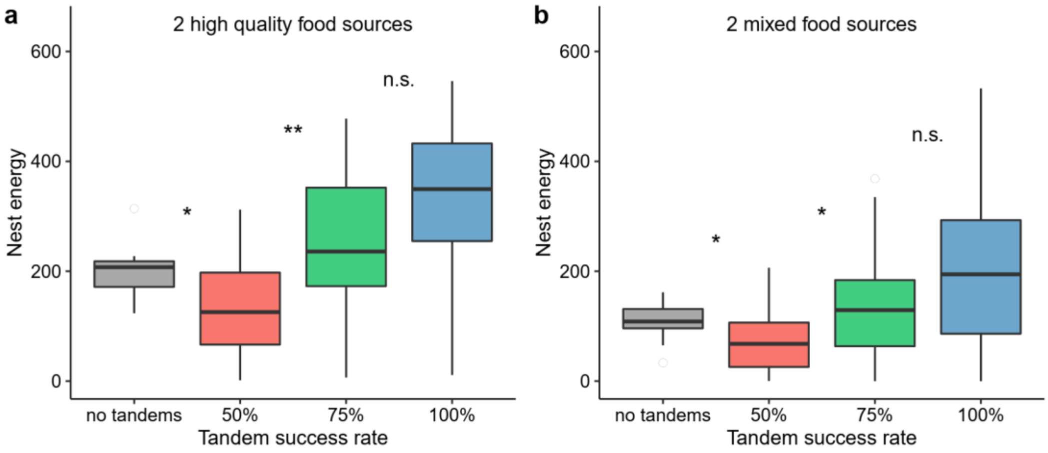

Figure 3.4: Nest energy with 2 food sources of high (**a**) and mixed (**b**) quality in relation to the tandem success rate. Adjacent treatment groups were compared, as indicated by asterisks or "n.s.". No tandems = only scouts. Default settings were used for the other parameters.

However, tandem runs with a high rate of failure (50%) are not always a disadvantage compared to having no tandem communication. When colonies can forage for longer (simulations of 21600 ticks), colonies that perform tandem runs with a ~50% break-up rate are more successful than colonies consisting of only scouts (Fig. 3.5) (high-quality, 50% success rate vs. no tandems: W = 866 p < 0.0001, mixed-quality, 50% success rate vs. no tandems: W = 689, p = 0.0003), highlighting the benefits of imperfect tandem runs over longer time periods.

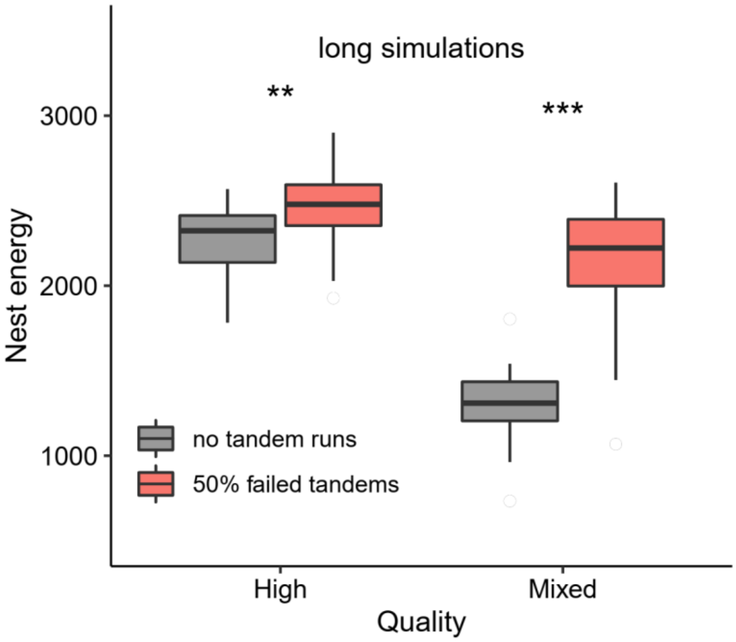

Figure 3.5: Nest energy with 2 food sources of high or mixed energetic quality and a long foraging duration. Colonies were scouting or could recruit with tandem runs that had a ~50% failure rate. Default settings were used for the other parameters.

Colony size and scout-recruit ratio

We tested various colony sizes ranging from 20 to 200 agents in an environment with few, mixed-quality food sources, *i.e.* an environment that favours tandem running under default conditions (see Fig. 3.2a).

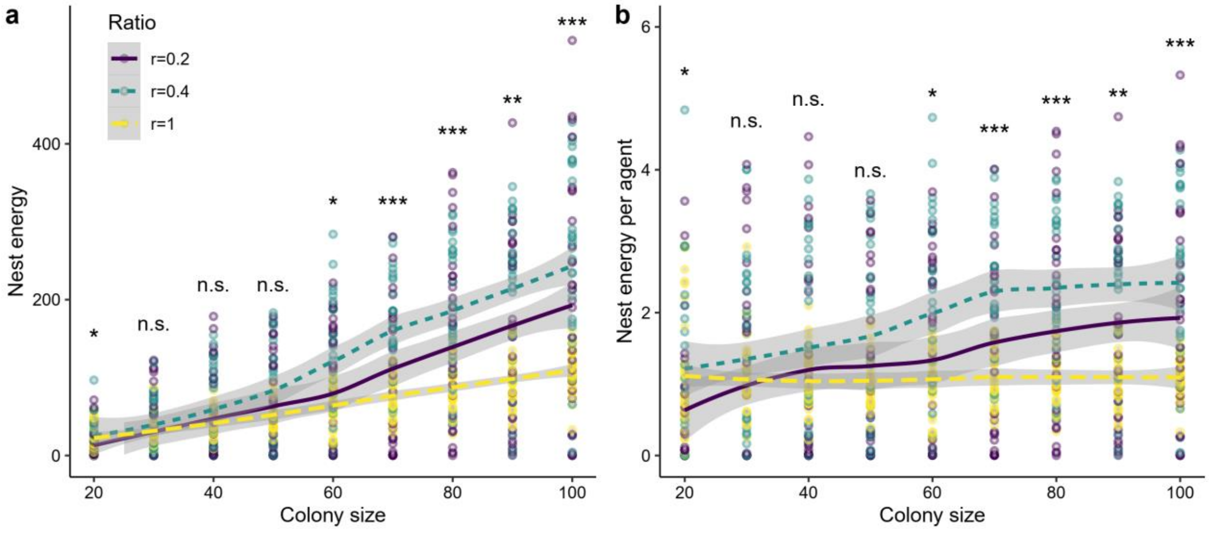

Figure 3.6: The relationship between colony size and nest energy (**a**) and nest energy per agent (**b**) in colonies with and without tandem runs in environments with two food sources of mixed quality. Three scout-recruit ratios were simulated, r = 0.2 and r = 0.4 and colonies consisting only of scouts, r = 1.0. Grey area indicates confidence intervals. Significance tests refer to comparisons among ratios, separately for each colony size. P-values for total nest energy (a) or energy per agent (b) are identical. Default settings were used for the other parameters.

Colony size had a strong effect on the total collected energy that was collected (Fig. 3.6). If colonies were very small (20 foragers), they were least successful if they performed tandem runs and had a default scout-recruit ratio of 0.2 (Table 3.3). There was no difference in foraging success when colony size ranged from 30 to 50 foragers. However, colonies with tandem recruitment were more successful if they had at least 60 agents (Table 3.3). The most successful colonies contained 40% scouts, suggesting that the scout-recruit ratio has a considerable impact on colony success. Fig. 3.6b shows the nest energy collected per agent (nest energy/colony size). In colonies with only scouts, individual agents collected a relatively constant amount of energy irrespective of colony size (Spearman rank correlation: rho = 0.1, p = 0.09). In colonies with tandem running, on the other hand, individual agents collected more energy on average as colony size increased from 20 to 100 agents (r = 0.2, rho = 0.34, $p < 0.0001$; r = 0.4, rho = 0.35, $p < 0.0001$).

Table 3.3: Effect of colony size on nest energy. Three conditions were tested: in two conditions, colonies performed tandem runs and had a scout-recruit ratio of 0.2 or 0.4. In the third condition, colonies consisted only of scouts (1.0). Pair-wise comparisons were performed if the overall $p < 0.05$ and p-values were corrected using sequential Bonferroni.

	Kruskal-Wallis Test		**p-value of pair-wise comparisons**		
Colony size	χ^2	p-value	0.2 vs. 0.4	0.2 vs. 1.0	0.4 vs. 1.0
20	12.7	0.002	0.018	0.002	0.42
30	2.33	0.31	NA	NA	NA
40	3.92	0.14	NA	NA	NA
50	1.81	0.41	NA	NA	NA
60	8.98	0.01	0.077	0.4	0.01
70	22.85	<0.0001	0.0007	0.35	<0.0001
80	20.6	<0.0001	0.13	0.007	<0.0001
90	15.86	0.0004	0.09	0.048	0.0002
100	19.17	<0.0001	0.053	0.027	<0.0001
200	46.59	<0.0001	0.006	0.0001	<0.0001

To explore this further, we simulated different scout-recruit ratios and different colony sizes to test how the balance between scouts and recruits affects colony foraging success. Simulations suggest that the optimal proportion of scouts is ~40% for the simulated environment, irrespective of colony size (Fig. 3.7). Interestingly, deviations from the optimal ratio have a larger negative impact in larger colonies (see "pointiness" of curves in Fig. 3.7). For example, there is no difference in success when colonies with 50 agents contain 40% or 80% of scouts ($W = 119$; $p = 0.54$). When colony size is 200, however, colonies with 80% scouts collect 31.5% less energy than colonies with 40% scouts ($W = 199$, $p = 0.0001$).

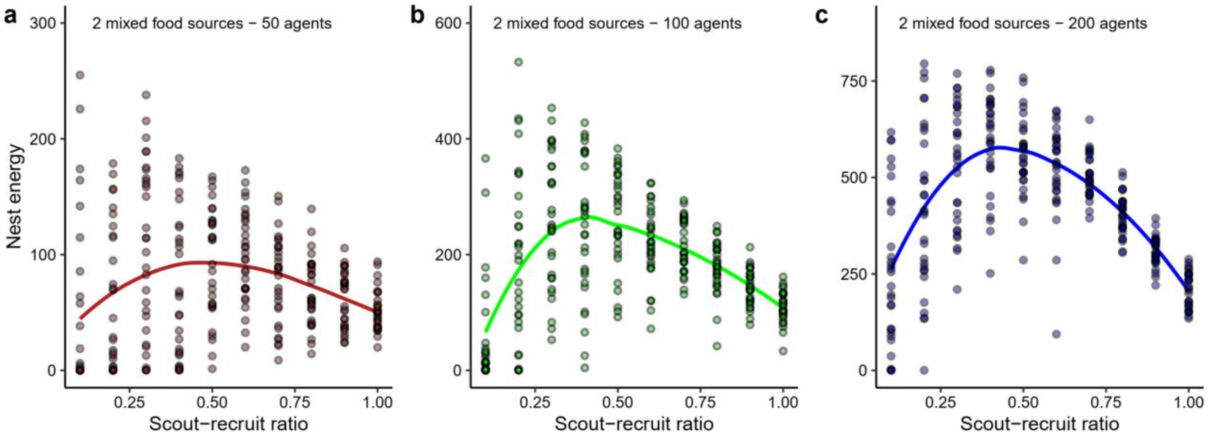

Figure 3.7: The effect of the scout-recruit ratio with three different colony sizes. The line shows the best fit line based on local polynomial regression using the LOESS method (locally estimated scatterplot smoothing). The smallest ratio was 0.1. A ratio of 1.0 refers to colonies containing only scouts.

Discovery times

Unsurprisingly, foragers needed more time to find their first food source in an environment with few food sources compared to when there were many food sources (Fig. 3.8). Recruits needed less time in an environment with few, high-quality food sources compared to scouts (Wilcoxon-signed rank test: W = 143, p-value < 0.0001), whereas there was no difference when food sources were mixed in energetic quality (Fig. 3.8a) (W = 348, p-value = 0.13). However, in an environment with many food sources, scouts did comparatively better and needed a similar amount of time to locate their first food source when food sources were all high-quality (W = 327, p-value = 0.07). With many, mixed-quality food sources, scouts were significantly faster than recruits (Fig. 3.8b) (W = 720, p-value < 0.0001).

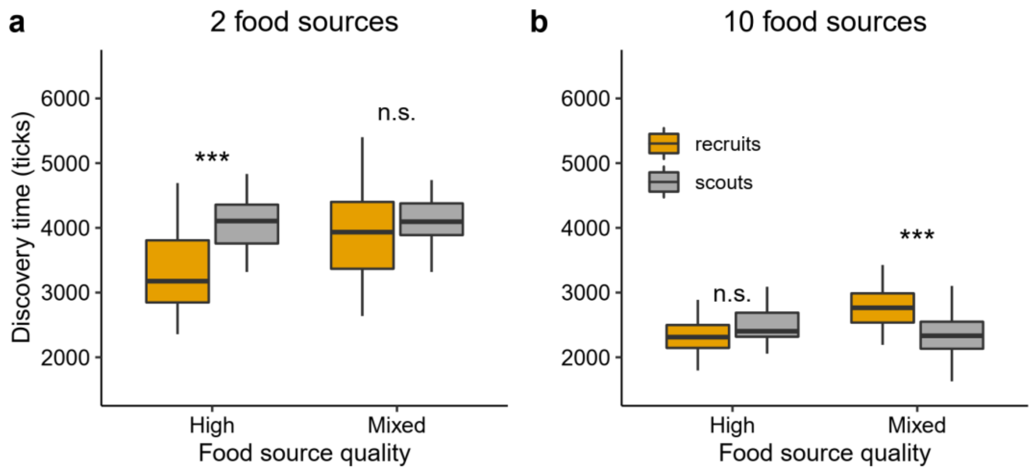

Figure 3.8: Time until agents located their first food source in environments with few (**a**) or many (**b**) food sources of constant or mixed quality. For recruits, the food discovery time consisted of the time waiting inside the nest and the tandem run duration. Default settings were used for the other parameters.

Discussion

Our simulations show that the spatio-temporal distribution of food sources greatly affects whether colonies with tandem communication are more successful than colonies that employ a scouting strategy. Tandem running was beneficial when colonies were in an environment with few food sources (+ 57-83% nest energy) and when food sources were of mixed energetic value (Fig. 3.2a,b). Colonies without tandem runs were more successful (~15%) in a rich environment that offered only high-quality food sources. This is in line with studies that simulated honeybee foraging and found that communicating food source locations by waggle dancing is most beneficial if food sources are hard to find and vary in their energetic value (Dornhaus *et al.*, 2006; Beekman & Lew, 2007; Schürch & Grüter, 2014; I'Anson Price *et al.*, 2019). Under such circumstances, the probability that scouts find high-quality food sources on their own is low and communicating the location of a relatively small number of high-quality patches becomes advantageous. As food source variability decreases and the number of high-quality food sources increases, scouts become more successful. Even though colonies with tandem communication also collect more energy in such an environment, the benefits of tandem communication no longer offset the costs of recruits waiting for information inside the nest. This highlights that communication often has considerable time and opportunity costs (Seeley, 1983; Seeley & Visscher, 1988; Dechaume-Moncharmont *et al.*, 2005; Schürch & Grüter, 2014; I'Anson Price *et al.*, 2019).

It has been hypothesised that recruitment communication is particularly beneficial in an ephemeral environment (Sherman & Visscher, 2002; Dornhaus & Chittka, 2004; Grüter & Ratnieks, 2011), *i.e.* when food sources last only for short time-periods and, thus, need to be exploited quickly. Counterintuitively, a simulation model of honeybee foraging has found that communication was less beneficial if food sources were shorter-lived (Schürch & Grüter, 2014). Our simulations support their findings by showing that tandem running was a very successful strategy in a stable environment with relatively long foraging durations (*i.e.* with longer simulations) and few, mixed-quality food sources (Fig. 3.3a). A long-lasting food source could be a large insect (Lanan, 2014), floral nectars or a group of honeydew secreting insects (Carroll & Janzen, 1973; Quinet & Pasteels, 1996; Völkl *et al.*, 1999; Mailleux *et al.*, 2003; Lanan, 2014). A very different pattern was observed when resources were shorter-lived: colonies without tandem communication were always more successful, irrespective of the foraging (simulation) duration (Fig. 3.3c,d). The most likely explanation is that colonies with tandem runs pay time costs without being able to take advantage of the benefits of this communication over longer time periods (see also Schürch & Grüter, 2014). Our model differs from theirs in that our food sources only disappeared if they were exploited, rather than with a constant probability. A food source that disappears after it has been exploited could be a droplet of honeydew that fell on vegetation. Honeydew droplets on leaf surfaces represent an important food source for the tandem recruiting *Temnothorax curvispinosus* (Lynch *et al.*, 1988).

Tandem runs occasionally break-up and success rates of ~50% to 90% are not uncommon (Wilson, 1959; Pratt, 2008; Kaur *et al.*, 2017; Glaser & Grüter, 2018; Grüter *et al.*, 2018). We simulated different success rates and found that colonies with more successful tandem runs collected more energy (Fig 3.4). If the success rate was about 50%, colonies consisting only of scouts collected more energy in an environment with few food sources, *i.e.* a virtual environment that normally favours tandem running. When foraging durations were longer, on the other hand, colonies with tandem runs gained the upper hand over scouting colonies even though half of all tandem runs failed (Fig. 3.5). Under these circumstances, even a relatively low number of successful recruitment events can be very important because the discovered high-quality food sources can be exploited for longer time periods by successful recruits. Additionally, tandem recruitment can lead to an exponential increase of ants at a feeder even if a leader recruits <1 follower per trip. With exponential growth, the impact of tandem runs will increase over time (Fig. 3.2e).

We found that colony size had a considerable effect on the value of tandem communication (Fig. 3.6). This contrasts with models of honeybee communication, where colony size did not greatly affect the benefits of communication (Dornhaus *et al.*, 2006; Schürch & Grüter, 2014), but is consistent with an empirical study on honeybee colony foraging success (Donaldson-Matasci *et al.*, 2013) and a mathematical model of ant communication (Planqué *et al.*, 2010). If colonies contained 60 or more foragers, tandem communication was usually beneficial. However, a pure scouting strategy was equally or more successful when colonies had 20 to 50 foragers, even in environments with few and mixed-quality food sources, *i.e.* a virtual environment that normally favours tandem running. This number of foragers could be expected in ant colonies with ~80-250 workers (assuming that foragers make up 20-30% of the worker population, e.g. Shaffer *et al.*, 2013), which is also the typical colony size of many ant species that use tandem running and species with solitary foraging (Beckers *et al.*, 1989). Our simulation results could explain why some species, e.g. in the genera *Diacamma* or *Neoponera*, do not perform tandem runs during foraging even though they use this recruitment method during migrations (Hölldobler, 1984; Traniello & Hölldobler, 1984; Maschwitz *et al.*, 1986). Whether colonies employ tandem running might depend on the food sources they collect (e.g. small or large items) and whether they are risk-averse or risk-prone because tandem recruitment was often associated with a more unpredictable outcome in our simulations (greater variation in nest energy gain among simulations of a particular situation, see Fig. 3.2). A better understanding of the natural history of these species and similar species that do perform tandem runs (e.g. *Neoponera* vs. *Pachycondyla*) is needed to understand why some species use this communication, while others forage solitarily.

Colony foraging performance depended on the proportions of scouts and recruits (Fig. 3.7). In our simulations with few food sources, colonies were most successful if scouts represented about 40% of the forager population, but this is likely to depend on the number and variability of food sources (see Fig. 3.2). Interestingly, having the right scout-recruit ratio is more important in larger colonies than in smaller ones, possibly because the foraging success of smaller colonies depends more on chance events, such as the discovery of a high-quality food source by a single scout. This suggests that larger colonies would benefit from having the ability to assess their current environment and adjust their use of communication accordingly. Our model assuming a fixed scout-recruit ratio may, therefore, be unrealistic. Whether and how colonies adjust the relative reliance on communication is not yet well known, but it has recently been reported that honeybees are able to assess the value of communication and reduce their reliance on waggle dances if dance information is not beneficial in the current environment (I'Anson Price *et al.*, 2019).

In the simulations, we measured the time recruits and scouts need to locate their first food source in environments with many or few food sources. We found that the food discovery time depends strongly on the environment. Recruits were faster in environments with few high-quality food sources, whereas scouts found a food source sooner in an environment with many, mixed-quality food sources. Our measurements also included the time that recruits wait inside the nest to find a tandem leader. Franks and Richardson (2006) found that tandem followers found a food source faster than scouts in their experiment with one food source, which, in combination with their other findings, indicated that tandem running fulfils the criteria for animal teaching set out by Caro and Hauser (1992; namely, a teacher [i] modifies its behaviour in the presence of a naïve observer, [ii] at some cost to the leader [iii] so that the observer can learn more quickly or efficiently). Our simulations suggest that this is the case only in certain environments, namely those with few, high-quality resources. In other situations, scouts are likely to learn food source locations quicker and tandem running might no longer fulfil the criteria for animal teaching (namely that a follower acquires knowledge or learns a skill more rapidly or efficiently than it might otherwise do, or that it would not learn at all, see Caro & Hauser, 1992).

Taken together, our simulations show that the value of tandem communication is highly dependent on the environment and the size and composition of the colony. Future studies should explore whether and how foragers can assess their foraging environment and modify their communication behaviour (see also Grüter & Czaczkes, 2019). It would also be desirable to test the conclusions from our simulations empirically, but so far it has been challenging to stop ants from performing tandem runs without affecting their behaviour.

Supplement

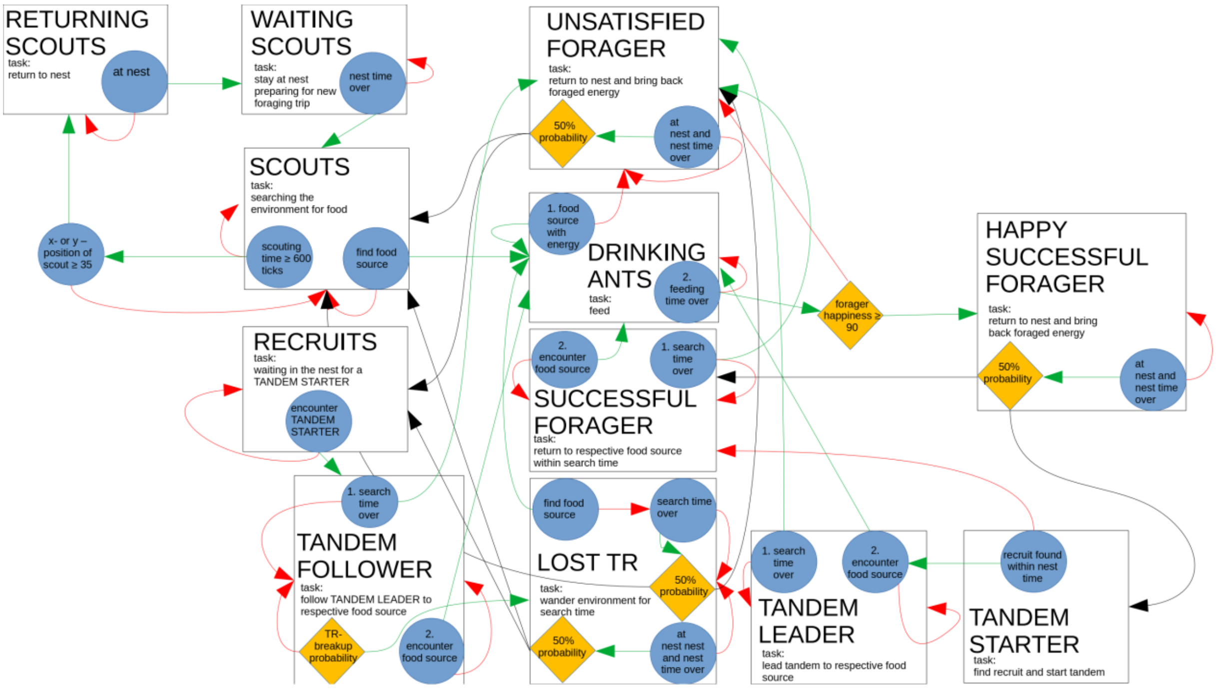

Figure S3.1: Detailed state diagram, showing the different states that agents could assume and probabilities to switch between states.

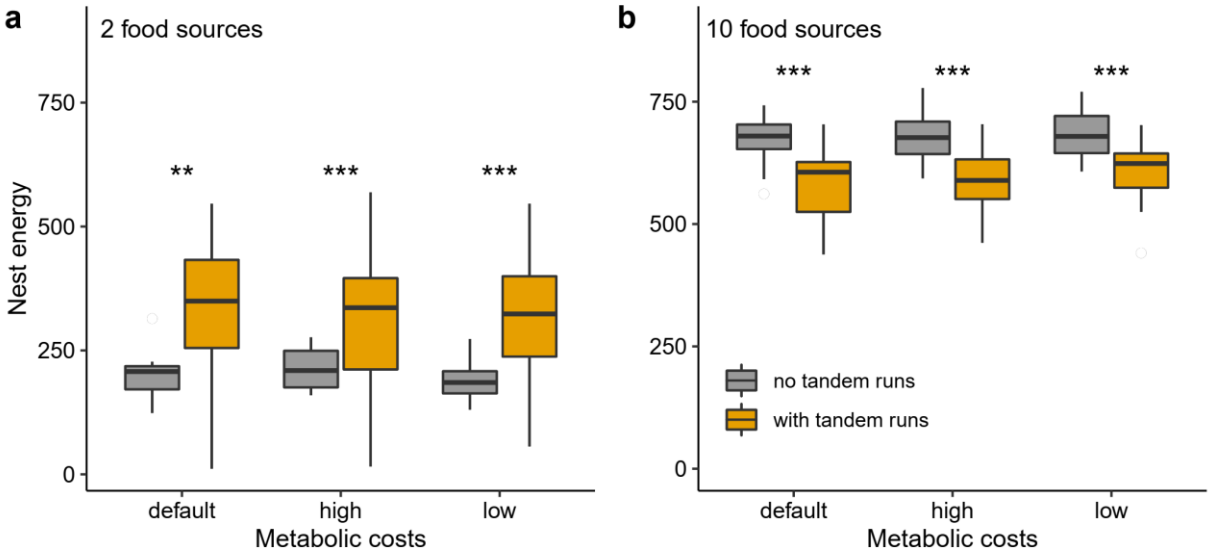

Figure S3.2: The effects of 10-times higher and 10-times lower metabolic costs on nest energy (see Table 3.1). Two (**a**) and ten (**b**) high-quality food sources were offered, default values were used for all other parameters. The default conditions match those shown in Fig. 3.2a and 3.2b. Mann-Whitney U tests, **p<0.001, ***p<0.0001.

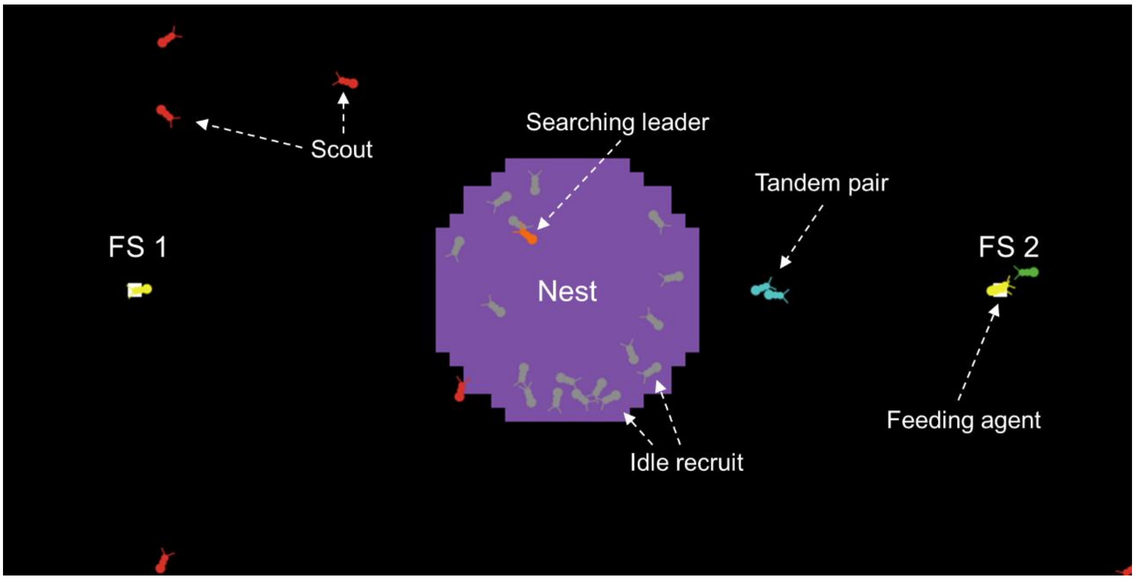

Figure S3.3: NetLogo interface showing some of the different agent types in different colours. In this situation, two food sources (FS 1 and FS 2) were offered.

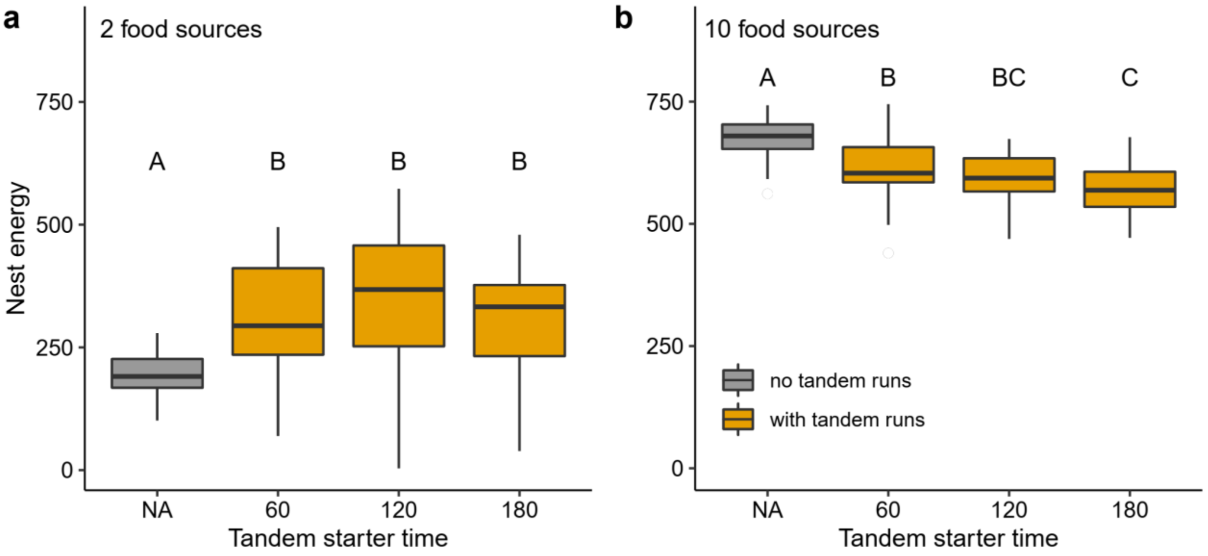

Figure S3.4: The effects of a shorter and longer recruitment period inside the nest ($t_{tandemstarter}$ = 60 and 180 ticks instead of 120 ticks) on nest energy. Two (**a**) and ten (**b**) high-quality food sources were offered, default values were used for all other parameters. Letters indicate significant differences ($p < 0.05$). The default condition did not differ from situations with longer or shorter $t_{tandemstarter}$, but differed from colonies without tandem runs.

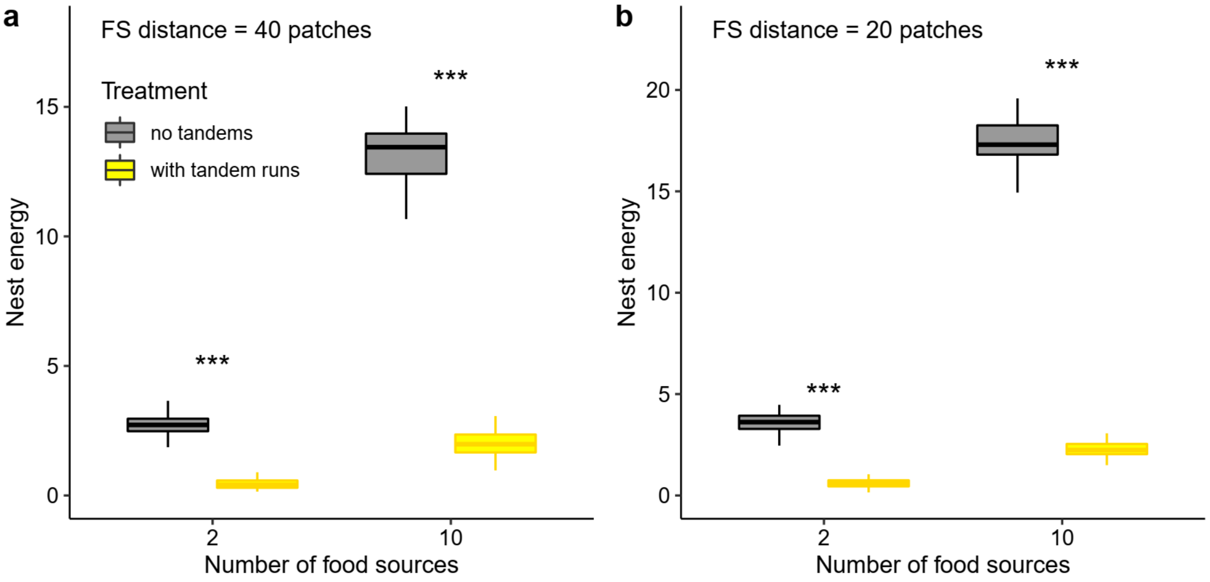

Figure S3.5: Nest energy of colonies with or without tandem runs when all food sources are of low quality. Two food source distances were simulated, 40 patches (**a**) or 20 patches (**b**). Default values were used for all other parameters. Mann-Whitney U tests, ***p<0.0001.

CHAPTER 4

Ants (*Temnothorax nylanderi*) adjust tandem running when food source distance exposes them to greater risks

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Abstract

Social insect colonies exploit food sources that vary in their profitability and riskiness. One factor that affects both profitability and riskiness is the foraging distance: more distant resources are both more costly to exploit and expose individuals to greater predation or navigational risks. *Temnothorax nylanderi* scouts use tandem running to recruit nestmates to resources, such as food or nest-sites. Tandem runs are often unsuccessful, leaving followers in potentially unknown or dangerous territory. Thus, as foraging distances increase communication mistakes are likely to be more costly. We tested if leaders and followers adjust their tandem running behavior in response to increasing foraging distances. We asked whether the success rate, the probability to recruit and the waiting time following a loss of contact depends on the foraging distances. We found that the success rate (75%-86%) of tandem runs does not decrease with increasing foraging distance, but rapidly increases with the leader's experience, from 67% for the 1st tandem run to 94% for the 4th. Pairs progressed faster and followers search longer for their partner after a loss of contact when visiting more distant food sources. The probability to perform a tandem run did not decrease with the foraging distance, but increased with foraging experience. Our results indicate that ants might attempt to reduce exposure to risks by progressing faster when visiting more distant food sources. As ants become more experienced, they lead more and better tandem runs. These findings suggest that both leaders and followers respond to the potential dangers posed by exploiting far-away resources.

Significance Statement

Foraging distance plays an important ecological role in animals as the foraging distance affects both energetic costs and predation risk. Ants have evolved several cooperative foraging strategies to exploit a food source as efficiently as possible, including a recruitment method called tandem running. Here, an informed leader guides a naïve follower to a valuable resource. We tested if tandem running behavior changes if food sources are more distant. Foraging distance indeed had an impact as leaders walked faster and followers searched for longer after a contact loss when food sources were more distant.

Introduction

Social insects have evolved various forms of communication that allow workers to share information with their nestmates about valuable resources, such as food or a new nest site. This allows an individual that has discovered such a resource to recruit more nestmates to it. Ants in particular use a variety of recruitment strategies, including different forms of group recruitment and mass-communication, *i.e.* the recruitment of large numbers of workers by means of pheromone trails (Czaczkes, Grüter, et al., 2015; Detrain & Deneubourg, 2008; Hölldobler & Wilson, 1990; Lanan, 2014; Traniello, 1989a). The benefit of a recruitment strategy depends on colony size: many species with larger colonies lay pheromone trails, whereas species with smaller colonies often use group recruitment or a recruitment strategy called tandem running (Beckers *et al.*, 1989; Planqué *et al.*, 2010). One explanation is that a relatively large number of workers is required to establish and maintain pheromone trails due to the volatility of trail pheromones (Beckers *et al.*, 1989; Beekman *et al.*, 2001).

Tandem running is common in species with very small colonies (Beckers *et al.*, 1989) and is based on tactile interactions between an informed leader and a usually naïve follower who tries to stay in contact with the leader (Fig. 4.1) (Hingston, 1929; Hölldobler *et al.*, 1974; Möglich *et al.*, 1974; Franks & Richardson, 2006; Franklin, 2014). Chemical signals may often play an important role, both when initiating a tandem run and to help followers maintain contact with the leader (Möglich *et al.*, 1974; Hölldobler & Traniello, 1980; Basari & Laird-Hopkins, 2014; Stuttard *et al.*, 2016). If contact is lost, leaders wait for followers, while followers search for leaders in order to re-establish contact (Hingston, 1929; Franks & Richardson, 2006; Richardson *et al.*, 2007; Franks *et al.*, 2010).

While tandem recruitment is likely to be beneficial for colonies because it accelerates the exploitation of good food sources (Shaffer *et al.*, 2013) or the migration to high-quality nest-sites (Franklin, 2014), tandem running also has costs. First, tandem pairs often have a greatly reduced walking speed compared to ants travelling alone (Franks & Richardson, 2006; Schultheiss *et al.*, 2015; Kaur *et al.*, 2017). Second, even if leaders know the location of a resource tandem runs are often unsuccessful (Pratt, 2008; Schultheiss et al., 2015; but see Kaur et al., 2017). For example, Wilson

(1959) observed tandem runs in *Cardiocondyla venustula* in the wild and found that 50% of the tandem runs broke-up and only 31% resulted in the discovery of a food source. Break-ups are not necessarily unsuccessful as lost followers occasionally find the resource on their own (Pratt, 2008; Franks *et al.*, 2010; Shaffer *et al.*, 2013; Schultheiss *et al.*, 2015).

Tandem recruitment is modulated by the value of the resource (Mallon *et al.*, 2001; Shaffer *et al.*, 2013). For example, *Temnothorax rugatulus* colonies perform significantly more tandem runs when offered a more concentrated sucrose solution (Shaffer *et al.*, 2013). A key factor affecting the value of a food source is the distance to it because more distant resources affect the pay-off gained from exploiting a resource, e.g. by affecting time, energy or predation costs (Traniello, 1989b). As a result, honeybees and several ant species recruit with lower intensity when foraging at more distant food sources (von Frisch, 1967; Taylor, 1977; Fewell *et al.*, 1992; Devigne & Detrain, 2006) and foragers are less likely to return to more distant foraging sites (Al Toufailia *et al.*, 2013b). Interestingly, distance does not seem to have the same effect in tandem running: *Temnothorax albipennis* and *T. curvispinosus* colonies performed more tandem runs after discovering a more distant nest-site (Pratt, 2008; O'Shea-Wheller *et al.*, 2016). Colonies might perform fewer tandem runs to nearby nest-sites because these are easy to discover by independent search. As a result, the threshold to switch to social carrying is reached earlier (Pratt, 2008). In *T. albipennis*, on the other hand, per capita recruitment to more distant nest-sites was higher, possibly because colonies can reduce the time of exposure to external risks by recruiting with higher intensity (O'Shea-Wheller *et al.*, 2016). However, it remains unclear how ants might adjust their recruitment intensity to increasing distances in a foraging context where energy and time costs might be valued differently than during emergency colony emigration.

Increasing foraging distances also increase the risks associated with foraging (Traniello, 1989b): ants travelling to more distant resources are more likely to get lost and they experience an increased exposure to predators and competitors (O'Shea-Wheller *et al.*, 2016). In *T. curvispinosus*, tandem runs to a nest-site at a 65 cm distance had a 70% chance of breaking up, whereas only 43% of all tandem runs broke-up when travelling to a nest-site at 11 cm (Pratt, 2008). If getting lost is indeed costly, then an increasing risk for followers to get lost could either select for a lower rate of tandem running to more distant food source or, alternatively, followers and leaders might adjust their behavior when travelling to more distant food sources in order to keep the break-up rate or predation risk low.

Here, we studied these two possibilities by offering *Temnothorax nylanderi* colonies food sources at different distances. In particular, we tested if the foraging distance affects the likelihood, success rate and progress rate of tandem runs. If break-ups occurred, we investigated whether the time that leader and followers spend waiting and searching for their partners depended on the foraging distance. Another factor that might affect tandem running is the experience of the involved ants, e.g. the navigational knowledge of an ant or the experience with leading tandem runs. Old and experienced *T. albipennis* workers were both more likely and more accurate tandem leaders during colony emigrations (Franklin *et al.*, 2012). Therefore, we also tested whether tandem run efficiency and probability increase with experience.

Material & Methods

Study site and study species

Fifteen *Temnothorax nylanderi* colonies were collected from acorns and decaying branches in the Lenneberger forest near Mainz in Germany in 2015. In the laboratory, colonies were kept in nests that consisted of two microscope slides (50 mm x 10 mm x 3 mm) and, between the two slides, a Plexiglas slide containing an oval cavity that provided a living space and an opening that functioned as a nest entrance. The nest was placed in a slightly larger box (100 mm x 100 mm x 30 mm) with paraffin oil-coated walls to prevent ants from escaping. The colonies were kept in a climate chamber at 22°C with a 12:12 h light/dark cycle. Colonies were fed twice a week with honey and a cricket and were provided with an *ad libitum* water source. The mean colony size (adult workers) was 130.9 ± 67.2 (± StDev) and all colonies had brood and a reproductive queen.

Experimental set-up and procedure

All experiments were conducted in the same climate chamber to ensure constant climatic conditions. Before each experimental trial, colonies were starved for 10 days to guarantee that ants were motivated to forage. On day eight of the starvation period the nests containing the colonies were placed in a foraging arena (30 cm x 23 cm x 6 cm) so they could get accustomed to the foraging environment. After each trial we cleaned the floor with ethanol to remove potential pheromone traces and foot prints. The walls of the arenas were also covered with Fluon to prevent ants from escaping. Each colony was tested three times with a 1M sucrose solution, placed either at 7 cm, 14 cm or 28 cm from the nest. Most foraging in this small ant occurs less than 0.5 m from the nest (Heinze *et al.*, 1996). The order of the tested distance was randomized and nests always occupied the same location on one side of the rectangular foraging arena.

On a test day, colonies were given access to a droplet of a sucrose solution at one of the three foraging distances. As soon as the first scout discovered the food source, arenas were video recorded for 90 minutes (Panasonic HC-V130). When foragers reached the food source for the first time, either alone or in a tandem pair, they were marked individually with a color dot (POSCA, Mitsubishi Pencil Co., UK) on their abdomen (Figure 4.1b). We tried to mark four scouts (*i.e.* ants that found the food on their own) and four recruits (*i.e.* ants that were followers in a tandem run), but this was not always possible. Eight different colors were used to be able to individually identify 8 focal foragers per trial. After each trial colonies were fed for one day before being starved again for 10 days. Thus, after 11 days colonies were tested again in a different distance set-up.

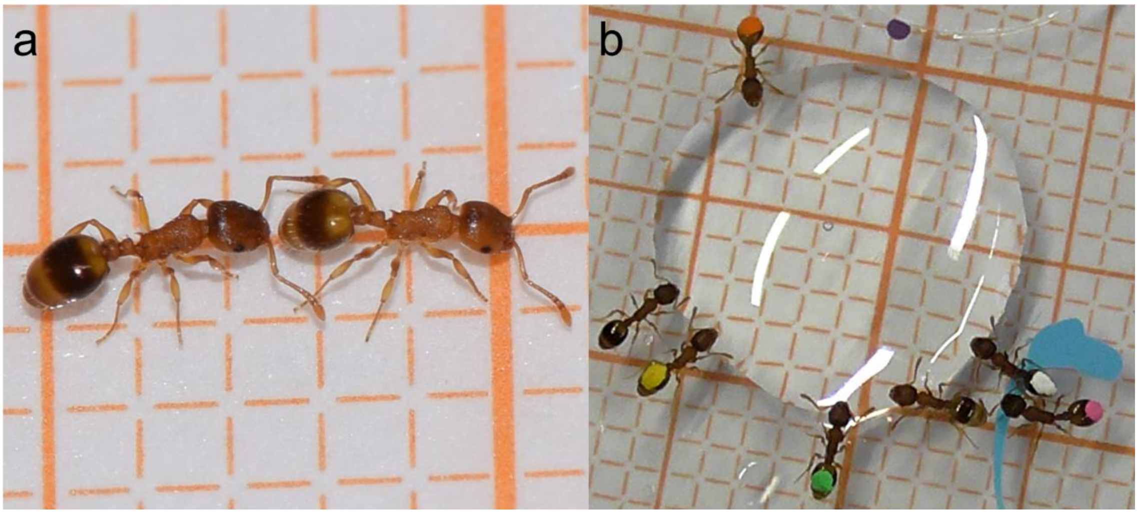

Figure 4.1: (**a**) A tandem run of two *Temnothorax nylanderi* workers (Photo by C. Grüter). (**b**) Marked workers collecting 1 M sucrose solution (Photo by S. Glaser).

Data collection

Different types of data were analyzed from the video recordings. For each visit of a marked ant we noted whether she visited the food source alone, as a leader or as a follower of a tandem run. We recorded whether tandem runs were successful: a tandem was considered successful (i) if the pair reached the food source together, (ii) if a follower was guided to within 1 cm from the food source or (iii) if the follower reached the food source less than one minute after a contact loss. When a tandem run was unsuccessful, we determined whether the leader or the follower was more likely to have caused the break-up. Therefore, we measured how long leaders waited and followers searched in the area of the break-up: leaders typically stood still or walked slowly a few millimeters in the area where they lost contact. When ants started to walk away from the break-up point at normal speed, we considered this as an ant giving-up trying to re-establish contact. See Basari et al. (2014) for a more detailed description. The ant that stopped waiting or searching first was considered to be responsible for the break-up.

The rate of progress (cm/sec) of the tandem pairs was determined using the object detection and tracking software AnTracks (www.antracks.org). We divided the total length of the trajectories (total walked distance) of the leader (example trajectory shown in Fig. S4.1) by the duration of the tandem run. The straightness of the trajectory was calculated by dividing the total walked distance by the food source distance. Since tandem leaders were occasionally followed by more than one ant we also noted the number of followers at the beginning and at the end for successful tandem runs.

Statistical analysis

All statistical tests were done in R 3.1.2 (R Core Team, 2016). We used linear mixed-effect models (LME) for normally distributed response variables and generalized linear mixed-effect models (GLMM) for response variables with a binomial or Poisson distribution (Zuur *et al.*, 2009). The variable "rate of progress" was transformed using a log transformation and then analyzed with a LME model. The variable "straightness" was transformed using the boxcox transformation (Crawley, 2007) to achieve normality. Colony ID and ant ID were used as hierarchically nested random effects to control for the non-independence of data points from the same colony and the same ant. We explored the role of two fixed effects, foraging distance and foraging experience. The effect of experience was tested either as the number of visits to the food source or as the number of performed tandem runs. We tested the effects of the fixed effects on the success rate, the probability of tandem runs, rate of progress and the straightness. Chi-square-tests were performed to test who caused the break-ups.

Results

Success rate

We marked 81 ants that performed 198 tandem runs. Overall, 160 (80.8%) tandem runs were successful. There was no difference between the three treatments in the success rate of tandem runs (Fig. 4.2a) (GLMM: $\chi^2 = 0.32$, df = 2, p = 0.85). We then explored whether the success rate of tandem runs depended on the foraging experience measured either as the number of foraging trips to the food source or as the number of tandem runs performed by an individual ant. Most ants (98.5%) performed fewer than 8 visits. Therefore, we focused on the first 7 visits. The success rate increased significantly (from 67% for the 2nd to 93% for the 7th visit) with an increasing number of visits (GLMM: slope = 0.4595, SE (standard error) = 0.1806, χ^2 = 8.95, df = 1, p = 0.0028). There was no interaction between the number of visits and distance (GLMM: χ^2 = 0.59, df = 2, p = 0.74).

We also used the number of tandem runs as a proxy for tandem running experience to explore whether this is linked to the success rate. The success rate increased significantly (from 67% for the 1st to 94% for the 4th tandem run) with an increasing number of tandem runs (Fig. 4.2b) (GLMM: slope = 1.046, SE = 0.2892, χ^2 = 18.23, df = 1, p < 0.001; interaction: no. tandem runs x distance: GLMM: χ^2 = 2.74, df = 2, p = 0.25).

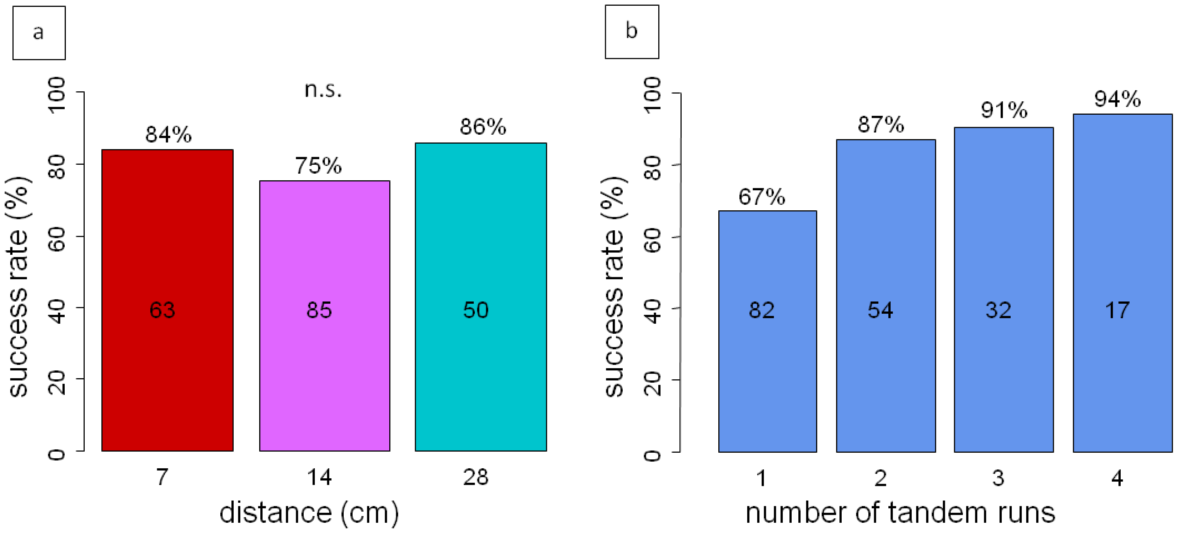

Figure 4.2: Success rate depending on distance and number of tandem run. (**a**) The distance had no influence on the success rate (Number in column match the amount of tandems in the treatment). (**b**) The number of tandem influenced the success rate positively (Number in column match the amount of ants who performed a tandem).

Probability to perform a tandem run

Overall, we recorded 1110 visits to a food source by 299 marked ants. We tested if the probability to perform a tandem run depended on the foraging distance and the foraging experience. There was a marginally non-significant tendency, that distance may influence the probability to perform a tandem run (GLMM: distance: χ^2 = 5.9855, df = 2, p = 0.0502). Thus, we did pair-wise comparisons for the three distances. The probability to perform a tandem run was significantly lower when food was at 7 cm compared to 14 cm (GLMM: 7 vs. 14 cm: slope = 0.5065, SE = 0.2080, z = 2.648, p = 0.00809; 7 vs. 28 cm: slope = 0.3031, SE = 0.2337, z = 0.953, p = 0.34; 14 vs. 28 cm: slope = 0.2034, SE = 0.2250, z = -1.469, p = 0.14), but the other comparisons were not significant (Fig. 4.3a). There was a significant increase in the probability to perform a tandem run with an increasing number of visits for all distances (Fig. 4.3b) (GLMM: slope = 0.4147, SE = 0.0501, χ^2 = 74.4, df = 1, p < 0.001; interaction: visit x distance: χ^2 = 5.1, df = 2, p = 0.078).

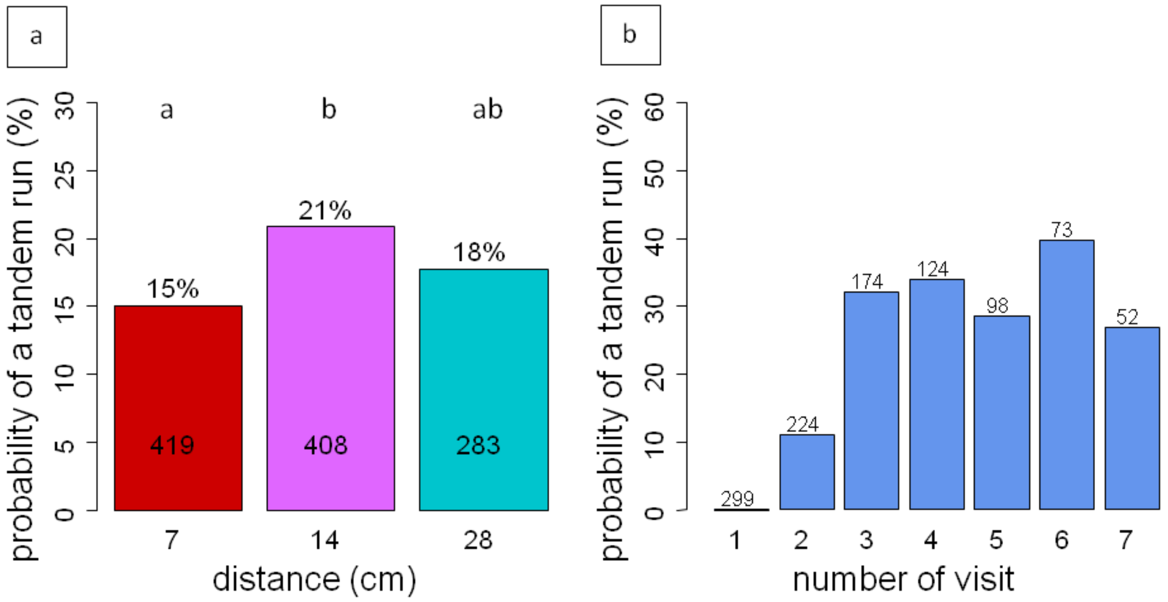

Figure 4.3: Probability to perform a tandem run. (**a**) Probability of a tandem depending on the distance (Number in column match the amount of ants that visited the food source). (**b**) The probability to perform a tandem increased with visits (Number above column match the amount of ants that started a tandem at a certain visit). Different letters indicate significant differences.

Break-ups and waiting time

Because 19.2% of all tandems were unsuccessful (tandems of marked and unmarked ants), we explored who might have caused the break-ups (Fig. 4.4a). Therefore, we evaluated who first left the area of contact loss. In ~70% (52 of 73) of the break-ups, the leader left the area of the break-up before the follower (chi-square-test: χ^2 = 27.7, df = 1, $p < 0.001$). In accordance with this, the waiting time of leaders was significantly shorter than the searching time of the follower and depended on the distance (GLMM: distance: $\chi^2 = 63.2$, df = 4, $p < 0.001$; 7 vs. 14: slope = 0.7450, SE = 0.1876, 7 vs. 28: slope = 1.027, SE = 0.1992, 14 vs. 28: slope = 0.2824, SE = 0.0846; leader/follower: slope = 1.986, SE = 0.0482, $\chi^2 = 2657.7$, df = 3, $p < 0.001$; interaction: $\chi^2 = 28.23$, df = 2, $p < 0.001$, 7 vs. 14: slope = 0.7405, SE = 0.1252, 7 vs. 28: slope = 0.7982, SE = 0.1368, 14 vs. 28: slope = 0.0577, SE = 0.1127). A more detailed examination of the distance effect revealed that the waiting time of the leader was shorter for 28 cm than for 14 cm (Fig. 4.4b) (GLMM: 7 vs. 14 cm: slope = 1.902, SE = 1.076, z = 1.8, p = 0.077; 7 vs. 28 cm: slope = 0.6792, SE = 1.076, z = -0.63, p = 0.53; 14 vs. 28 cm: slope = 1.223, SE = 0.1480, z = 8.3, $p < 0.001$). The searching times of the followers increased from 7 cm to 28 cm food distance, with 14 cm showing an intermediate searching time (Fig. 4.4c) (GLMM: 7 vs. 14 cm: slope = 0.9499, SE = 0.2116, z = 4.49, $p < 0.001$; 7 vs. 28 cm: slope = 0.7365, SE = 0.2310, z = 3.19, p = 0.0014; 14 vs. 28 cm: slope = 0.2134, SE = 0.1125, z = -1.9, p = 0.058).

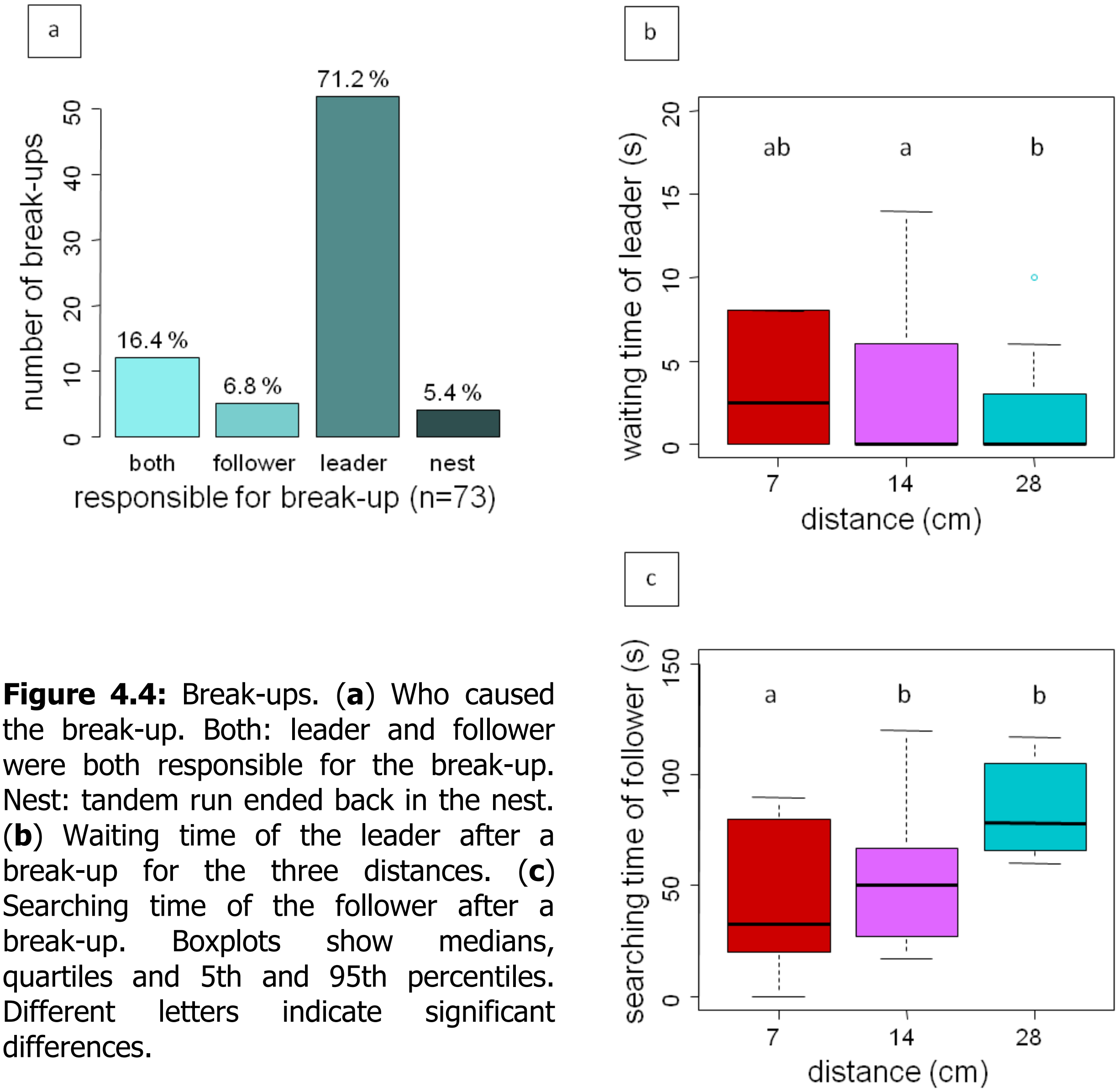

Figure 4.4: Break-ups. (**a**) Who caused the break-up. Both: leader and follower were both responsible for the break-up. Nest: tandem run ended back in the nest. (**b**) Waiting time of the leader after a break-up for the three distances. (**c**) Searching time of the follower after a break-up. Boxplots show medians, quartiles and 5th and 95th percentiles. Different letters indicate significant differences.

Tandem duration and rate of progress

We tested if the time tandem pairs need to reach the food source depended on experience measured as the number of tandem runs a leader had performed. We did this for each distance separately, because tandem durations differed greatly between distances. For the tandem runs to the food source at 7 cm, the ants needed less time with an increasing number of tandem runs performed (GLMM: 7cm: slope = 0.2866, SE = 0.0286, z = -10.03, $p < 0.001$). Interestingly, the opposite was found for tandem runs to either 14 cm or 28 cm as tandem runs of more experienced ants lasted longer (GLMM: 14 cm: slope = 0.1542, SE = 0.0107, z = -14.4, $p < 0.001$; 28 cm: slope = 0.0663, SE = 0.0100 z = 6.6, $p < 0.001$).

Ants progressed significantly faster if the distance to the food source was more than 7 cm (Fig. 4.5a). (LME: 7 vs. 14 cm: slope = 0.0945, SE = 0.0318, df = 101, t = 2.97, p = 0.0037; 7 vs. 28 cm: slope = 0.1316, SE = 0.0345, df = 101, t = 3.81, p < 0.001; 14 vs. 28 cm: slope = 0.0371, SE = 0.0345, df = 101, t = 1.07, p = 0.29). However, the number of the tandem runs a leader performed had no influence on the rate of progress (LME: df = 89, t = -0.27, p = 0.79, interaction: distance x number tandem: LRT = 0.79, p = 0.67). Whether a tandem run was successful or not had no influence on the rate of progress of the tandem runs (LME: df = 135, t = 0.02, p = 0.98, interaction: distance x success: LRT = 3.09, p = 0.21).

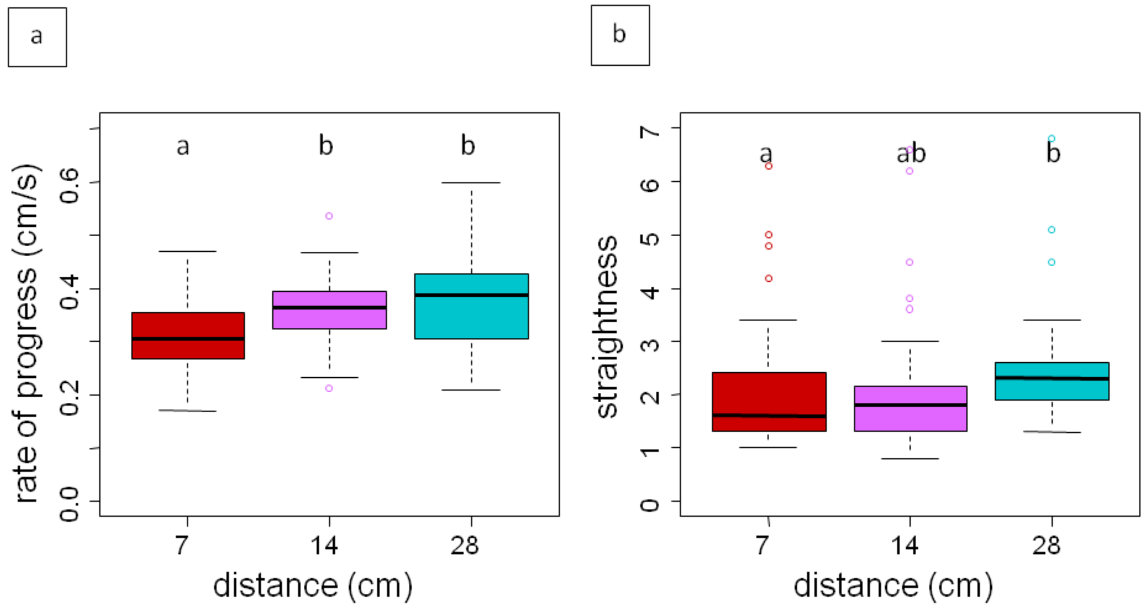

Figure 4.5: Rate of progress and straightness for the three foraging distances. (**a**) Rate of progress depending on the food distance. (**b**) The straightness of the tandem runs for the three food distances (straightness = walked distance / food distance).Boxplots show medians, quartiles and fifth and 95th percentiles. Different letters indicate significant differences.

Tandem trajectory

On their way to the food source tandem runners rarely walk in a straight line (see Fig. S4.1 for an example of a trajectory). We compared the straightness for the different foraging distances by dividing the walked distance through the foraging distance (Fig. 4.5b). We found that tandem runs were more straight when recruiting to the 7 cm feeder than when recruiting to the 28 cm feeder (GLMM: 7 vs. 14 cm: slope = 0.0533, SE = 0.0438, df = 70, t = -1.2, p = 0.23; 7 vs. 28 cm: slope = 0.1260, SE = 0.0472, df = 70, t = -2.67, p = 0.0095; 14 vs. 28 cm: slope = 0.0727, SE = 0.0471, df = 70, t = -1.54, p = 0.13). The number of the tandem runs performed had no influence on the straightness (LME: df = 70, t = 1.121, p = 0.2661; distance x number tandem: LRT = 2.292, p = 0.32).

Number of followers and foragers at food sources

We noticed that tandem leaders (of individually marked and unmarked ants) frequently had more than one follower (32.3% at beginning, 10.1% at end of tandem run). In such tandem runs, some followers got lost, whereas in other instances ants joined a tandem run that was already under way. As shown in Figure 4.6, some tandem runs started and ended with up to five followers.

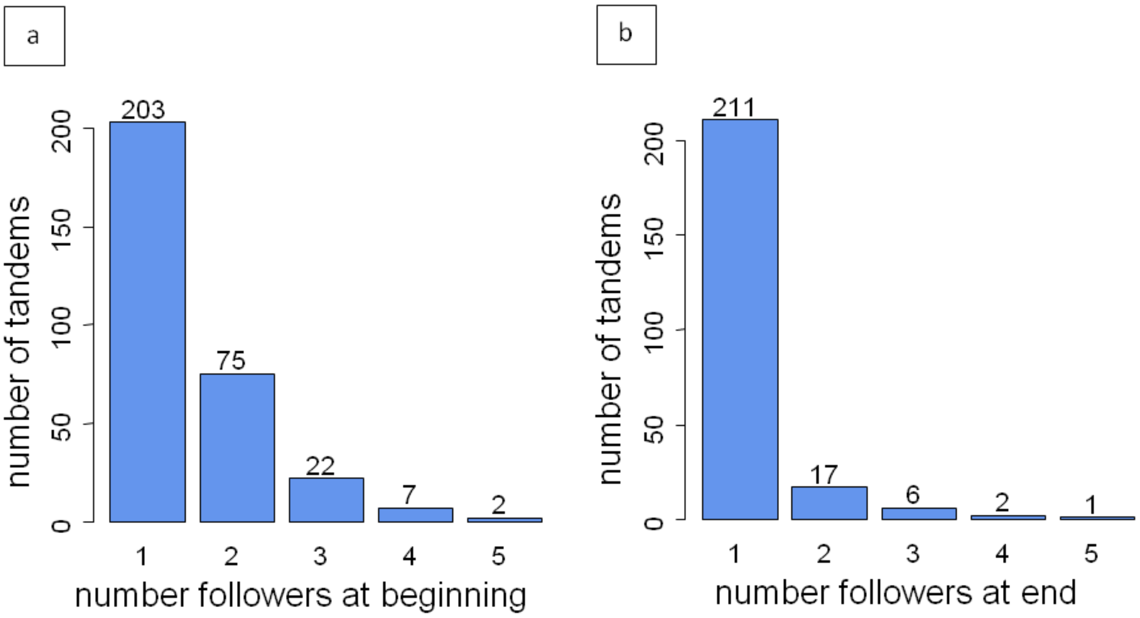

Figure 4.6: Number of followers at the (**a**) Beginning and (**b**) End of a tandem run.

Discussion

We found no effect of food distance on the success rate of tandem runs. The success rate of tandem runs to a nearby food source (7 cm) was almost identical to the success-rate to a food source four times further away (28 cm; 84% vs. 86%). This was unexpected given that tandem runs to more distant food sources last longer and probably provide more navigational challenges, e.g. because more visual information needs to be stored. These challenges could explain why tandem runs towards more distant food sources were less straight. Our results also differ from house-hunting *Temnothroax albipennis* where tandem runs to more distant nest-sites broke-up more often (Pratt, 2008). It is probable that testing greater foraging distances would have revealed a distance effect, but the foraging distances we used in our study are similar to natural foraging distances in *T. nylanderi* (Heinze *et al.*, 1996). Alternatively, tandem running ants might adjust their behavior when travelling further in order to maintain the error-rate and, therefore, the risk of exposing lost followers to dangers further from the colony. In accordance with this, we found that followers searched for 82% longer after losing contact with the leader when the food source was 28 cm compared to 7 cm (Fig 4.4c). There was no consistent effect of waiting time of the leaders in a particular direction (Fig. 4.4b), which is an interesting difference compared to *T. albipennis*, where leaders waited for longer if tandem runs have proceeded for longer (Richardson *et al.*, 2007). However, a similar effect was found in honeybees where foragers observe waggle dances longer if dances indicate more distant food sources and have a large angular noise (Al Toufailia *et al.*, 2013a). When break-ups did occur in our study, the leaders appeared to be responsible in 71% of all cases. Leaders often continued to travel towards the food source without waiting for their partner after losing contact, thereby leaving the searching follower behind.

After discovering the food source, foragers performed a tandem run in ~10% of all first returns to the food source. We found no effect of the foraging distance on the probability to perform a tandem run when comparing our short (7 cm) and our long (28 cm) treatment, but an increase with experience (see below). In contrast, a previous study found that tandem runs to new nest sites were more likely when nest sites were more distant possibly because emigrations over greater distances are more dangerous and therefore more tandem runs would be expected if they accelerate the migration process (O'Shea-Wheller *et al.*, 2016). Indeed, colony migrations might pose more risks because the whole colony, including brood and the queen, is exposed to threats. Thus, there might be a higher pay-off for being as fast as possible, compared to foraging. Many other social insects reduce the recruitment intensity as food sources are more distant, most likely because distant food sources are more costly to exploit (von Frisch, 1967; Taylor, 1977; Roces, 1990; Fewell *et al.*, 1992; Devigne & Detrain, 2006). These opposing pressures – increasing recruitment due to risk and difficulty of finding a resource vs. decreasing recruitment due to a decreasing profitability – might cancel each other out and explain why we found no consistent effect of foraging distance in our study. An increased exposure to risks could also help to explain the interesting finding that pairs progressed faster to more distant food sources (Fig. 4.5a). By progressing faster, tandem pairs might attempt to reduce exposure to predators.

Experience had a nearly immediate and positive effect on the success rate of tandem runs. While only 67% of all first tandem runs were successful, more than 90% were successful if the leader performed two tandem runs before (Fig. 4.2). It is possible that leaders improve their orientation abilities as they gain more experience navigating in the foraging box or that their ability to lead tandem runs increases with each tandem run. Also the probability to perform a tandem run increased with increasing experience, from ~10% to >30% after three visits to the food source (Fig. 4.3). Thus, tandem runs become more likely as tandem leaders become more successful, which is likely to reduce the risk of break-ups. Franklin et al. (2012) found that experienced ants were more likely to participate in tandem runs in *T. albipennis*. Old experienced leaders led slower and more direct tandem runs than young inexperienced leaders, suggesting an effect of age or experience (or both) on the characteristics of tandem runs in *T. albipennis* (Franklin *et al.*, 2012). Interestingly, experience had no effect on the rate of progress or the straightness of tandem runs in our study. Thus, how experience improves the success rate of tandem runs requires further examination. One possibility is that experience affects the behavior of ants during brief contact-losses.

Usually, followers in a tandem run are naïve (Franks & Richardson, 2006; Richardson et al., 2007; but see Schultheiss et al., 2015). In our experiments, it happened occasionally that followers (11 of 132 marked tandem followers in all trials) had already participated in a tandem run before. Possibly, some ants did not acquire sufficient navigational information and, therefore, followed another tandem run or foragers might have been dissatisfied with the food source and decided to follow another tandem run, not knowing that they will be led to the same food source again. Thirty-four percent of all tandem runs started with more than one follower (from 2-5; Figure 4.6) and 11% of all successful tandem runs reached the food source with more than one follower. How the cohesion between multiple followers is maintained and, in particular, whether contact pheromones are important for tandem cohesion in *T. nylanderi* remains to be investigated. In other species, pheromones help tandem pairs to maintain contact (Möglich *et al.*, 1974; Hölldobler & Engel, 1978; Traniello & Hölldobler, 1984; Basari & Laird-Hopkins, 2014). Our results, that leaders can be followed by several ants supports the statement that the evolution of group recruitment from tandem running represents a relatively small step (Beckers *et al.*, 1989).

Supplement

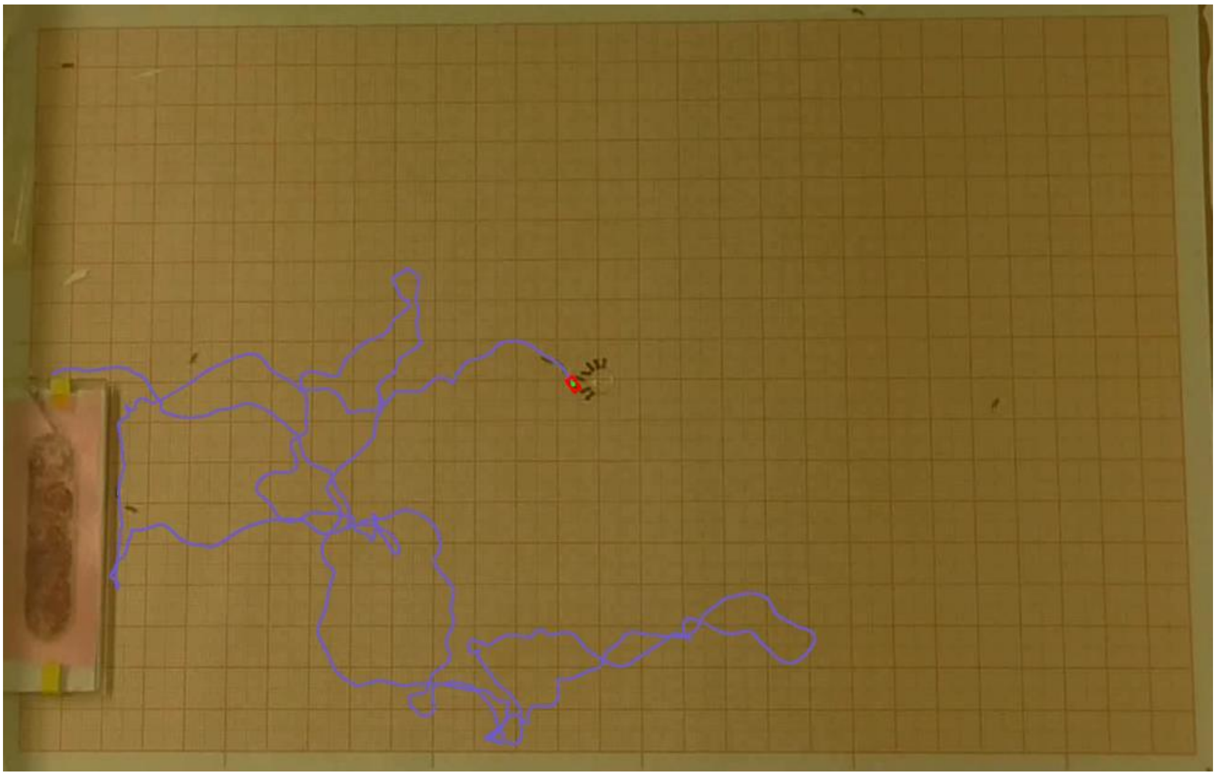

Figure S4.1: Trajectory (blue) of a tandem run to a food source at 14 cm distance. The leader (yellow dot in red rectangle) is detected and tracked by the AnTracks software.

CHAPTER 5

Large body size variation is associated with low communication success in tandem running ants

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Abstract

Diversity in animal groups is often assumed to increase group performance. In insect colonies, genetic, behavioral and morphological variation among workers can improve colony functioning and resilience. However, it has been hypothesized that during communication processes, differences between workers, e.g. in body size, could also have negative effects. Tandem running is a common recruitment strategy in ants and allows a leader to guide a nestmate follower to resources. A substantial proportion of tandem runs fail because leader and follower lose contact. Using the ant *Temnothorax nylanderi* as a model system, we tested the hypothesis that tandem running success is impaired if leader and follower differ in size. Indeed, we found that the success rate of tandem pairs drops considerably as size variation increases: tandem runs were unsuccessful when the leader-follower size difference exceeded 10%, whereas ~80% of tandem runs were successful when ants differed less than 5% in body length. Possible explanations are that size differences are linked to differences in walking speed or sensory perception. Ants did not choose partners of similar size, but extranidal workers were larger than intranidal workers, which could reduce recruitment mistakes because it reduced the chance that very large and very small ants perform tandem runs together. Our results suggest that phenotypic differences between interacting workers can have negative effects on the efficiency of communication processes. Whether phenotypic variation has positive or negative effects is likely to depend on the task and the phenotypic trait that shows variation.

Significance statement

Diversity is often assumed to increase colony performance in social insects. However, phenotypic differences among workers could also have negative effects, e.g. during communication. Tandem running is a common recruitment strategy in ants, but tandem runs often fail when ants lose contact. We used the ant *Temnothorax nylanderi* to test the hypothesis that body size differences between tandem leader and follower impair tandem communication. We show that the success rate of tandem pairs drops considerably as size variation increases, possibly because ants of varying size also differ in walking speed. Our study supports the hypothesis that phenotypic variation

among workers might not always be beneficial and can negatively impact the efficiency of communication processes.

Introduction

Social groups consist of individuals that differ from each other in a number of ways. For instance, people working in a company may differ in experience, training, gender, ethnicity or skills and this diversity can affect group performance and success. Scientists interested in organizational theory have found that group diversity often has positive effects on group performance, most likely because diverse groups possess a broader range of knowledge, experience, personality, skills and abilities (Horwitz & Horwitz, 2007; van Knippenberg & Schippers, 2007). For instance, more diverse scientific collaborations produce publications of greater impact (Freeman & Huang, 2015; AlShebli *et al.*, 2018), more ethnically diverse markets show lower risks of price bubbles (Levine *et al.*, 2014) and companies with greater gender diversity in corporate leadership positions are more successful (Noland *et al.*, 2016). As a result, organizations may compose teams to incorporate differences in functional, ethnic or educational background (van Knippenberg & Schippers, 2007).

Insect colonies consist of many individuals that may appear similar or even identical to the casual observer, but closer examination readily reveals that the members of a colony differ in many ways, including their morphology, behavior, experience or genetic background (Wilson, 1971; Oster & Wilson, 1978; Hölldobler & Wilson, 2009). Several studies have found that differences among workers promote colony success. For example, colonies that show greater behavioral variation (e.g. because they are genetically more diverse) have been shown to collect food more successfully, respond better to environmental perturbations or produce more brood (Jones *et al.*, 2004; Mattila & Seeley, 2007; Oldroyd & Fewell, 2007; Modlmeier & Foitzik, 2011). Workers of many species also differ in their morphology and this morphological variation is closely tied to division of labor in many species (ants: Hölldobler & Wilson, 2009; bumblebees: Goulson et al., 2002; stingless bees: Baudier et al., 2019; Grüter et al., 2017; termites: (Tian & Zhou, 2014). Having different worker types for different tasks is likely to increase group performance because different worker types are more efficient at performing particular tasks (Oster & Wilson, 1978; Powell & Franks, 2005; Mertl & Traniello, 2009; Grüter *et al.*, 2012, 2017b; Powell, 2016). Even in species with gradual variation, *i.e.* without distinct morphological castes, colonies with a larger worker size range often seem more successful (Porter & Tschinkel, 1985; Beshers & Traniello, 1994; Billick, 2002; Billick & Carter, 2007 but see Jandt & Dornhaus, 2014; Colin *et al.*, 2017). In most cases, this intra-colonial variation is an example of phenotypic plasticity, where the phenotypic differences are generated by variation in environmental factors (e.g. food quantity, temperature), rather than differences in genotype (Oster & Wilson, 1978; Sumner *et al.*, 2006; Segers *et al.*, 2015; Molet *et al.*, 2017).

Group diversity can also have adverse effects on consensus decision-making, group functioning and increase intra-group conflicts (Horwitz & Horwitz, 2007; van Knippenberg & Schippers, 2007). In humans, for instance, individuals might prefer to interact with more similar individuals (Horwitz & Horwitz, 2007). In social insect colonies, there could also be circumstances where differences among cooperating workers have negative effects on group performance. Waddington et al. (1986) and Waddington (1989) argued that worker size variation reduces the efficiency of communication in social bees. If signal producers and receivers differ in morphology (and their sensory systems), the communication of misinformation or impaired information transfer could become more likely. For instance, honeybees of different sizes might judge distances to food sources differentially, which could lead to different interpretations of distance information provided during waggle dancing. There is indeed evidence that honeybee (*Apis mellifera*) colonies with larger body size variation collect less nectar (Waddington, 1989) and stingless bees with more sophisticated communication show lower within colony size variation (Waddington *et al.*, 1986). The former study also found that honeybee dancers tended to interact with bees of similar size. Waddington et al. (1986) argue that negative effects of size differences on communication could explain why colonies of the highly eusocial honeybees and stingless bees, which often use sophisticated communication during foraging, are less morphologically variable than the primitively eusocial bumblebees, which use simpler methods of communication (Dornhaus & Chittka, 1999). However, the potential disadvantages of worker size variation for communication processes in insect societies has received little attention and, as far as we know, we still lack evidence that body size differences among interacting individuals indeed affects communication efficiency.

A recruitment behaviour that relies on communication and that could be negatively affected by body size differences is tandem running, which is relatively common in ant species with small colony sizes (so far described in ~40 species) (Beckers *et al.*, 1989; Franklin, 2014; Grüter *et al.*, 2018); tandem running by reproductives is also found in some termites, e.g. Matsuura *et al.*, 2002). During a tandem run, a leader with information about the location of a resource slowly guides a nestmate to a food source or a nest site. Contact between the leader and the follower is maintained by frequent physical interactions and short-range pheromones (Möglich *et al.*, 1974; Basari & Laird-Hopkins, 2014). This behavior has been considered a case of animal teaching because leaders actively facilitate learning in a nestmate while also incurring costs (Franks & Richardson, 2006). Contact losses are common during tandem runs (and they might help followers to acquire navigational information), but break-ups are often prevented by leaders waiting for their partner while the latter searches for her leader (Franks & Richardson, 2006; Richardson *et al.*, 2007). Nonetheless, a substantial proportion of tandem runs break up before reaching the goal (e.g. ~19% in *Temnothorax nylanderi*, Glaser and Grüter 2018; ~23% in *Pachycondyla harpax*, Grüter et al. 2018; ~50% in *Cardiocondyla venustula*, Wilson 1959; up to 70% in *Temnothorax rugatulus*, Pratt 2008). Break-ups are costly in terms of time and could leave lost followers in dangerous areas.

We studied tandem running during colony emigrations in *Temnothorax nylanderi,* a species with moderate size variation (Molet *et al.*, 2017), and tested the hypothesis that the body size difference between leaders and followers affects the efficiency of recruitment. Specifically, we tested the prediction that size differences between interacting partners have a negative effect on the success rate. To obtain a better understanding of body size variation in this population, we also quantified the body size distribution in several colonies and tested whether extranidal workers (potential scouts for nest-sites or food) are larger than intranidal workers as was found in two other species from the same genus (Herbers & Cunningham, 1983; Westling *et al.*, 2014). Finally, we measured the relationship between body size and the walking speed of an ant.

Methods

Study site and species

Temnothorax nylanderi colonies were collected from acorns and decaying branches in the Lenneberg forest (50°00'44.2 N, 8°10'57.8 E) near Mainz in Germany in 2015 (part 1), 2016 (part 2), 2017 (part 4) and 2018 (part 3). Back in the laboratory, colonies were kept in nests made up of two microscope slides (50 mm x 10 mm x 3 mm) and, between the slides, an acrylic glass slide containing an oval cavity that created a living space. This nest was placed in a larger plastic box (100 mm x 100 mm x 30 mm) with paraffin oil-coated walls that prevented ants from escaping. Colonies had a reproductive queen and brood and were kept in a climate chamber at 25°C with a 12:12 h light/dark cycle. Colonies were fed twice a week with honey and a cricket and were provided with water *ad libitum*. *Temnothorax* colonies can occasionally contain queen-worker "intercastes", but they are very rare (Okada *et al.*, 2013) and none were found in our colonies.

Part 1: Measuring the body size distribution

All workers from four recently collected colonies (colony size: 59-76 workers) were individually transferred into a petri-dish covered with graph paper (square sizes of 1 mm^2), which served as a scale. Each ant was photographed three times with a Nikon D7000 camera (AF-S Micro Nikkor 105 mm lens), mounted on a tripod and at a constant distance of approx. 30 cm above the petri dish. We measured total body length and head width of ants using ImageJ 1.46 and averaged the values from the three photos. Body length and head width were highly correlated ($r = 0.84$, $N = 262$, Pearson correlation: $p < 0.0001$), but body length is used as our measure of body size in this study because it is easier to measure due to the small size of the ants.

Part 2: Body size and walking speed

We collected 3–4 large (2.75–3.25 mm body length) and 3–4 small (2.25–2.65 mm) workers from each of eight colonies as they were walking outside their artificial nest and put them in small groups in a plastic arena (17.8 x 11.8 x 4.7 cm). The walls were coated with Fluon to prevent ants from escaping. The floor was covered with graph paper that allowed us to measure walking speed. Body size was measured as described above. After a 10 min acclimatization period, ants were filmed (Canon Legria HF R706) from above (30 cm distance) for 10 min as they were exploring the arena. The walking speed (cm/sec) of ants was determined using the object detection and tracking software *AnTracks* (www.antracks.org). Three uninterrupted 20 sec sequences (*i.e.* periods when ants did not interact with another ant, stand still or walk along a wall) were averaged for each ant.

Part 3: Body size and tandem running

We used 22 colonies (range of 56-100 workers) and performed 1 (12 colonies) or 2 (10 colonies) emigrations per colony. One day before an emigration, nests containing colonies were placed in an emigration arena (31.2 x 22.3 x 4.7 cm). The walls were again coated with Fluon and the floor was covered with graph paper. On a test day, a new empty nest was placed in the emigration arena, 20 cm from the original nest. To motivate the colony to move to this new nest, the old nest was destroyed by gently removing the lid (see e.g. (Mallon & Franks, 2000; Pratt, 2008). We filmed emigrations with the camera (Panasonic HC-VX878 4K) positioned 70 cm above the arena. Filming ended when colonies stopped performing tandem runs, usually within 2 h after the destruction of the old nest.

We measured the body size of tandem leaders and followers by averaging three still images per ant taken from the video recordings (the correlation coefficients *r* among individual images was on average 0.93, *i.e.* measurement 1 vs. measurement 2 of the same ant, measurement 1 vs. measurement 3 of the same ant). The still images were taken at the beginning of a tandem run, so that the person taking the measurements was unaware whether a tandem run was going to be successful or not.

Additionally, we measured total duration of successful tandem runs, the pair's rate of progress ("speed", cm/sec) and the walked distance by the pair (cm) using *AnTracks*. The walked distance is a measure of how direct tandem runs are. A tandem was considered successful (*i*) if the pair reached the nest entrance together or (*ii*) if the follower reached the nest entrance less than one minute after a contact loss. The latter situation referred to breakups that were close to the new nest entrance, e.g. along the edge of the new nest, and was included because the tandem leader played a critical role in the discovery of the new nest by the follower. Only tandem runs from the old to the new nest were analyzed, *i.e.* forward tandem runs. Ants were not marked and to avoid measuring the same ant more than once (pseudoreplication), each pair was removed from the experiment immediately after the leader contacted the new nest. Ants that were part of unsuccessful tandem runs were also removed. On average, 8.6 ± 4.25 ants per colony were removed by the end of the experiment.

Part 4: body size and task

We measured the body size of five extranidal workers (presumably ants scouting for food or nest sites) and five intranidal workers (presumably nurses) from each of ten recently collected colonies. To make sure that some ants left the colony to explore the environment, colonies were starved 10-14 days. Extranidal workers were collected when they were encountered outside their artificial nest. Afterwards, the nest was opened and ants that sat on the brood pile or carried brood items were captured (intranidal workers). These ants were considered nursing workers. Each ant was photographed 3 times as described above.

Statistical analysis

All statistical analyses were performed in R 3.4 (R Development Core Team 2016). For part 1, we quantified the worker size distribution and explored if body length showed significant skewness or kurtosis. For this we used the methods described by Crawley (2007, pp. 285-289). The values of each colony were first centered (mean = 0) and then combined to test overall patterns. To test if ant size affects walking speed (part 2), we used a general linear mixed-effects model (LME) with colony as random effect to control for the non-independence of data points from the same colony (Zuur et al. 2009). Size class was used as the fixed-effect. To analyze the effects of leader and follower body size differences on tandem running (part 3), both generalized and general linear mixed-effects models (GLMM's and LME's) were used. GLMM's were used when the distribution of the response variable was binomial (success: yes or no), whereas LME's were used to test the effects on speed, duration and distance. We tested both the relative size difference (leader – follower, *i.e.* positive values mean that leaders were larger, negative values indicate that followers were larger) and the absolute size difference (|leader – follower|, *i.e.* values always positive) as the predictors. To confirm the findings of the mixed-model regarding tandem success while controlling for potential colony effects, we compared the body size differences of failed tandem runs with successful tandem runs from the same colony using a paired t-test. For this, all tandem runs from colonies that had both successful and failed tandem runs were included. Additionally, we tested if the order of a tandem run (e.g. first, second, third, etc. tandem run) during a trial affected tandem success.

Results

Part 1: body size distribution

Worker body length varied from 2.25 to 3.34 mm (2.68 ± 0.16, mean ± StDev, N = 262) in the four measured colonies (Fig. 5.1). Head width varied from 0.48 to 0.67 mm (0.58 ± 0.03). The within colony coefficient of variation (CV) for body length was 0.054 ± 0.01 (N = 4 colonies). Body size distribution was unimodal, suggesting that *T. nylanderi* does not have distinct physical castes (Fig. 5.1a). A Shapiro-Wilk test suggests a significant deviation from normality (W = 0.98472, p = 0.007). Body size also showed significant positive skew (t-value = 2.04, p = 0.02) and significant kurtosis (t-value = 3.55, p = 0.0002).

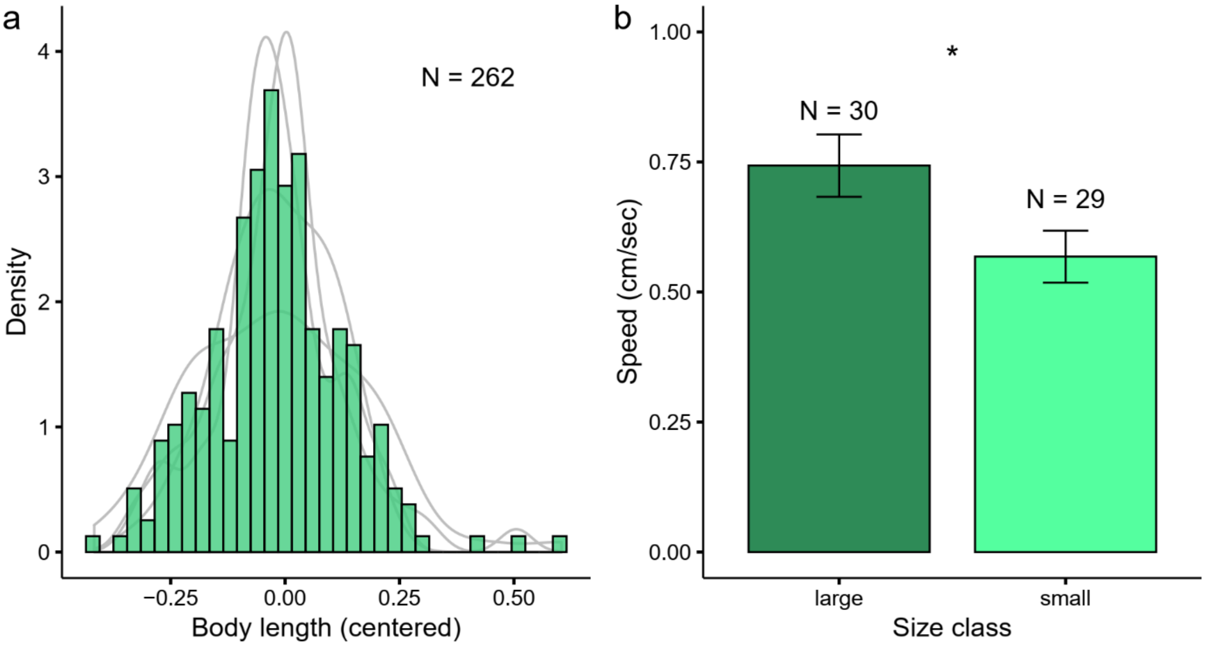

Figure 5.1: (**a**) Worker size (body length) density distribution in *T. nylanderi* colonies. Grey lines show the individual density distribution functions for the four colonies. (**b**) The walking speed of individual workers depending on their size. Bars show means ± 1 standard error.

Part 2: body size and walking speed

To test whether body size predicts walking speed, we quantified the walking speed of ants belonging to two size categories, large and small, as they explored a small arena. Ants from the large group were ~17% larger in body length than ants from the small group and walked significantly (+30%) faster than ants from the small group (Fig. 5.1b) (0.74 ± 0.3 cm/sec vs. 0.57 ± 0.26 cm/sec, LME: *t*-value = 2.32, $p = 0.025$). This indicates that larger ants walk faster in this particular experimental situation.

Part 3: Body size and tandem running

We analyzed 95 tandem runs from 22 colonies; 56% were successful. The size of tandem leaders did not correlate with the size of their followers (LME, values centered for each colony: *t*-value = -1.36, $p = 0.18$).

We then tested if the relative and absolute size difference predicted tandem success. Absolute size difference, but not relative size difference, significantly affected tandem success (Fig. 5.2) (absolute difference: GLMM, z-value = -4.22, $p < 0.0001$; relative difference: z-value = -0.7, $p = 0.49$). Thus, larger size differences were associated with a low chance of tandem success, but it did not matter if the larger ant was the leader or the follower (Fig. 5.2c). We also tested a model that included the absolute size difference and, in addition, the two predictors 'follower body length' and 'leader body length'. This would reveal if body length *per se* affects tandem success rate in addition to size differences. However, we found that only the absolute size difference, but not the body size of leaders and followers affected tandem success rate (GLMM, absolute difference: z-value = -4.12, $p < 0.0001$; leader body length: z-value = -0.21, $p = 0.85$; follower body length: z-value = 0.82, $p = 0.42$).

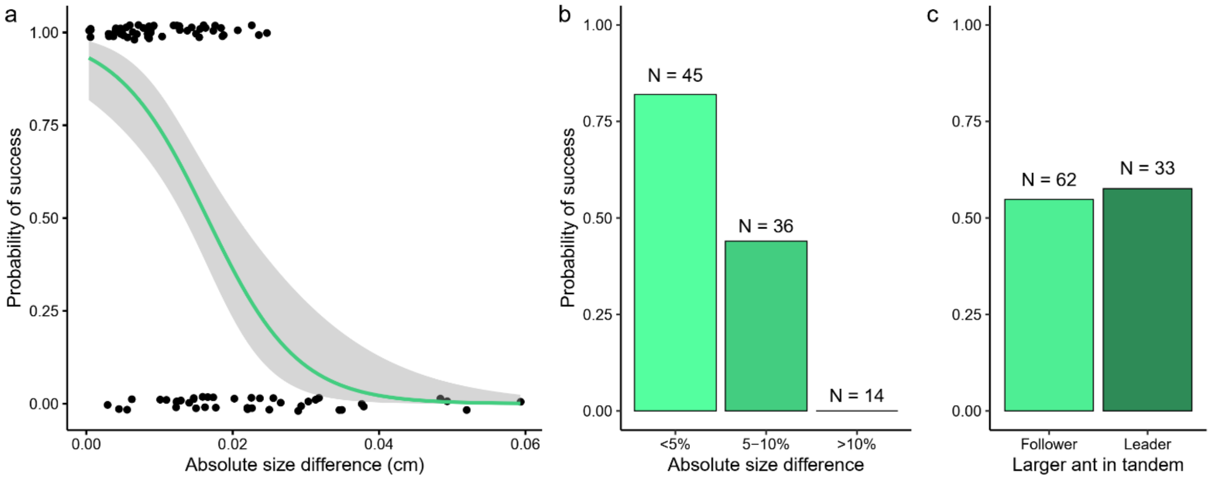

Figure 5.2: Probability that tandem runs were successful depending on the body length difference between leader and follower (**a**, **b**) and on whether the larger ant was the leader or the follower (**c**). (**a**) Note that all values are either 1 (success) or 0 (failure) but that jitter was used to better visualize the data points. Grey areas show the 95% confidence interval. (**b**) The same data as in (**a**), but the success probability for three different body length differences between the leader and the follower are shown. A value of 5% means that one of the ants was 5% smaller or larger than its partner.

A paired t-test confirmed that ant pairs of failed tandem runs differed more in size (0.2 ± 0.08 mm) than pairs of successful tandem runs (0.1 ± 0.05 mm) from the same colony (t-value = 3.64, df = 14, $p = 0.0027$, N = 15 colonies with both failed and successful tandem runs). The order of a tandem run during a trial did not affect success rate (GLMM, z-value = -0.19, $p = 0.85$).

We did not find any links between absolute and relative size difference and the speed, duration and walked distance of successful tandem runs (Table 5.1). We then explored whether the average size of ants in tandem (average of leader and follower) affects tandem success and tandem speed, but found no relationship (GLMM, success: z-value = 1.31, *p* = 0.19; LME, speed: t-value = 1.34, *p* = 0.18).

Table 5.1: The effects of relative and absolute size difference on the tandem success rate of all tandem runs (N = 95). Additionally, the speed (cm/sec), duration (sec, square root-transformed) and distance (cm, log-transformed) walked of successful tandem runs (N = 53) is shown. L = leader, F = follower. Linear mixed-effects models with colony as a random effect (GLMM's and LME's).

	Success rate	Duration (sec)	Distance (cm)	Speed (cm/sec)
Relative size difference: L-F	z-value = -0.7 *p* = 0.49	t-value = 0.57 *p* = 0.57	t-value = 0.38 *p* = 0.71	t-value = -0.92 *p* = 0.36
Absolute size difference: |L-F|	z-value = -4.22 *p* < 0.0001	t-value = 1.06 *p* = 0.30	t-value = 0.89 *p* = 0.38	t-value = -1.05 *p* = 0.30

Part 4: body size and task

We tested whether workers caught outside of their nest (potential scouts for food sources or nest sites) differ in size from workers that were captured inside their nest and in close proximity to brood (potential nurses). Extranidal workers (2.4 ± 0.2 mm; N = 50, CV = 0.083) were significantly larger than intranidal workers (2.32 ± 0.18 mm; N = 50, CV = 0.078) in ten recently collected colonies (LME: t-value = 2.12, p = 0.037). This is consistent with other *Temnothorax* species (see discussion).

Discussion

We found that larger body size differences among interacting ants were associated with a high probability of tandem run failure. Tandem runs were unsuccessful when the leader-follower size difference exceeded 10%, whereas ~80% of tandem runs were successful when ants differed less than 5% in body length (Fig. 5.2b). Speed, duration and distance travelled of the remaining successful tandem runs were not affected by body size differences. Our results suggest that size difference predict tandem success irrespective of whether the larger ant is the leader or the follower. One explanation could be that ants differing in size differ in their walking speed (Fig. 5.1b). Ants walking in a tandem run frequently need to accelerate and decelerate in order to maintain pair cohesion (Franks & Richardson, 2006). Body size could affect the speed at which ants perform these changes and, thus, the probability of short contact losses. In this case, body size differences would not affect communication or signaling *per se*, but the ability of ants to stay together during this recruitment communication process. Body size is likely to be linked to various sensory, physiological or cognitive traits (e.g. Waddington, 1989; Spaethe *et al.*, 2007; Grüter *et al.*, 2017b) that could affect the probability of tandem breakups. While our data do not allow us to identify the trait or traits that are responsible for breakups, they do suggest that failure is more common if leader and follower show phenotypic differences in the responsible trait or traits.

The tandem leaders in our study were probably relatively inexperienced because only the first tandem run of a pair was analyzed. Previous research has shown that tandem leaders perform more successful tandem runs with increasing experience (Franklin *et al.*, 2012; Glaser & Grüter, 2018) and it would be interesting to explore how size differences affect tandem runs of more experienced tandem leaders. It could be, for example, that leaders can learn how to guide tandem followers that differ greatly in size.

Even though some lost followers may discover the new nest by themselves (Pratt, 2008; Franks *et al.*, 2010), unsuccessful tandem runs often represent a loss of time because lost followers require more time to discover a resource or they have to return to their nest following a breakup (Franks & Richardson, 2006; Basari *et al.*, 2013; Grüter *et al.*, 2018). Furthermore, a breakup could leave followers in unknown and dangerous locations. Alternatively, repeated partial tandem runs could represent a strategy to gain information about the direction to a new nest-site. Breakups could also provide opportunities to discover different food sources or nest-sites, which could be beneficial in a dynamic environment (see e.g. Deneubourg *et al.*, 1983; Beekman & Dussutour, 2009). However, given the search time of *T. nylanderi* followers after a breakup (Glaser & Grüter, 2018) and the time ants have to wait inside the nest until they find a new leader, it is doubtful that this strategy would save time compared to performing one complete tandem run.

One observation that could indicate a strategy to reduce the risk of breakups is that extranidal workers were larger than intranidal workers. This is consistent with findings in two other *Temnothorax* species (Herbers & Cunningham, 1983; Westling *et al.*, 2014). Allocating larger workers to outside tasks is likely to decrease the probability that very large ants perform tandem runs with very small ants (the same could be achieved by allocating only small workers to outside tasks). Other potential advantages of larger extranidal workers could be an increased foraging rate due to their greater walking speed, greater foraging ranges (Ness *et al.*, 2004, but see Westling *et al.*, 2014) or a reduced risk of predation by certain predators.

Waddington (1989) hypothesized that size variation negatively affects waggle dance communication in honeybees because bees of different sizes may judge distances to food sources differentially. These costs of size variation could in turn select for low intra-colonial size variation (see also Waddington *et al.*, 1986; Sauthier *et al.*, 2017). While strong evidence for this is lacking, our finding that the size-frequency distribution in *T. nylanderi* colonies shows significant kurtosis (a leptokurtic distribution) indicates that there might be selection against body sizes that deviate strongly from the mean. Worker size variation in *T. nylanderi* (CV of ~ 5%) is similar to what has been found in *T. rugatulus*, which also performs tandem running (Westling *et al.*, 2014). A comparison of worker size variation in species that use tandem running *versus* closely related species without tandem running could provide further evidence to support or challenge the hypothesis that the need for efficient recruitment communication selects for low intra-colonial worker size variation. It would also be interesting to quantify worker size variation in ants with a very high success rate of tandem runs (~90%), such as *Diacamma indicum* (Kaur *et al.*, 2017), to explore whether worker size variation is particularly small in these species. Worker size in *T. nylanderi* is determined by food quantity, but also depends on rearing temperature and colony size (Molet *et al.*, 2017). The degree of worker size variation could be modified either by nurse workers providing more constant or variable conditions (e.g.

food amount) (Segers *et al.*, 2015), or by larvae modifying their developmental response to external factors (Rissing, 1987).

In *Apis mellifera*, dancing bees were more likely to be followed by bees of similar size (Waddington, 1989). We found no correlation between leader and follower size in *T. nylanderi*. This could be explained by an inability of ants to accurately estimate the size of potential partners or by time costs that result from waiting for tandem partners of similar size. Such waiting time costs are likely to be larger in small colonies, such as in *T. nylanderi*, than in larger colonies, such as in honeybees (Anderson & Ratnieks, 1999). Waggle dancing and tandem running are similar in that both require direct interactions between the signaler and the receiver. It is possible that behaviors involving direct interactions (e.g. cooperative transport, group recruitment or adult carrying) are more prone to being negatively affected by size variation than indirect forms of interactions, such as pheromone trails.

Body size variation is often linked to different roles in insect colonies. The most impressive examples are found in species with distinct physical worker castes (or sub-castes) performing different tasks (Wilson, 1971; Oster & Wilson, 1978; Hölldobler & Wilson, 2009). However, most species display moderate, continuous worker size variation (Oster & Wilson, 1978) and it is often not clear whether size variation has adaptive value at colony level or whether it could indicate constraints, such as seasonal variability in food availability. For example, bumblebees show moderate and continuous intra-colonial size variation (Goulson *et al.*, 2002; Couvillon *et al.*, 2010) and size is linked to the probability to perform certain tasks (Goulson *et al.*, 2002; Spaethe & Weidenmüller, 2002), but experimentally manipulating the body size variation in colonies did not affect colony performance in *Bombus impatiens* (Jandt & Dornhaus, 2014). Similarly, a recent study using *T. nylanderi* found that experimentally reducing worker size variation did not affect colony performance in the laboratory for several tasks (Colin *et al.*, 2017). This highlights that the effects of variation may not be straightforward and may remain hidden until colonies experience challenging conditions. Furthermore, while variation in some traits may be beneficial in the context of particular tasks, e.g. temperature regulation (Jones *et al.*, 2004), variation in other traits or in the context of other tasks (e.g. communication tasks) may be inconsequential or even negative. In the latter case, colonies would have to trade-off the costs and benefits of variation and, as a result, measuring colony traits such as biomass or brood production would miss important consequences of worker diversity because it averages positive and negative effects. Another problem of colony level analysis is that natural selection might affect phenotypic variation only in particular sub-sets of colony members, e.g. the foragers. Future research can help identify the conditions and contexts that make variation an asset or a problem for insect societies and those where worker variation is simply the result of naturally occurring variation in environmental conditions, without having any fitness consequences for colonies.

CHAPTER 6

Tandem communication improves ant foraging success in a highly competitive tropical habitat

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Abstract

Tropical ants experience intense intra- and interspecific competition for food sources, which influences their activity pattern and foraging strategies. Even though different ant species can coexist through spatial and temporal niche partitioning, direct competition for food cannot be avoided. Recruitment communication is assumed to help colonies to monopolize and exploit food sources successfully, but this has rarely been tested under field conditions. We studied if recruitment communication helps colonies of the Neotropical ant *Pachycondyla harpax* to be more successful in a highly competitive tropical environment. Additionally, we explored if temporal and spatial niche differentiation helps focal colonies to avoid competition. *Pachycondyla harpax* competed with dozens of ant species for food. Mass-recruiting competitors were often successful in displacing *P. harpax* from food baits. However, when foragers of *P. harpax* were able to recruit nestmates they had a 4-times higher probability to keep access to the food baits. Colonies were unlikely to be displaced during our observations after a few ants arrived at the food source. Competition was more intense after sunset, but a disproportionate increase in activity after sunset allowed focal colonies to exploit food sources more successfully after sunset. Our results support the hypothesis that recruitment communication helps colonies to monopolize food sources by helping them to establish a critical mass of nestmates at large resources. This indicates that even species with small colony size and a slow recruitment method, such as tandem running, benefit from recruitment communication in a competitive environment.

Introduction

The tropics are home to thousands of ant species, some forming colonies containing millions of ants. Collectively, their biomass exceeds the biomass of all wild mammals combined (Bar-On *et al.*, 2018) as they fulfil vital ecological roles as predators, herbivores or soil architects (Hölldobler & Wilson, 1990; Davidson *et al.*, 2003). Competition for food sources among colonies can be intense, but intra- and interspecific competition is often reduced through a variety of mechanisms (Hölldobler, 1981). Often species occupy particular dietary niches, e.g. by exploiting different food types (sugars, carrion, excrements, seeds and live prey) or food sizes (Torres, 1984; Houadria *et al.*, 2015). Additionally, species can show temporal activity patterns that allow them to avoid competitors (Torres, 1984; Stuble *et al.*, 2013; Houadria *et al.*, 2016; Rosumek, 2017). For instance, nocturnal species like some *Myrmecia* or *Polyrhachis* ants have adapted to low light levels by developing large eyes and efficient navigational strategies (Narendra *et al.*, 2013a; b, 2017). On the other hand, activity patterns of tropical species are also dependent on moisture gradients (Kaspari & Weiser, 2000).

Despite these mechanisms of niche differentiation, competition cannot be avoided entirely. The foraging strategies used by the different species are likely to have a strong impact on the ability of colonies to monopolize and exploit food sources fast and efficiently (Traniello, 1987, 1989b; Lach *et al.*, 2010; Drescher *et al.*, 2011; Grevé *et al.*, 2019). In particular, communication and recruiting other nestmates to food sources should increase a colony's chances of displacing competitors and gaining long-term access to food.

Communication among nestmates is widespread in ants and other social insects (Beckers *et al.*, 1989; Hölldobler & Wilson, 1990; Jarau & Hrncir, 2009; Almeida *et al.*, 2018; Glaser & Grüter, 2018; Grüter & Czaczkes, 2019). It allows the members of a colony to exchange information about their environment, such as the locations of food sources or the presence of predators and competitors (Kendal *et al.*, 2004; Dawson & Chittka, 2014; Lanan, 2014). During communication, ants may use tactile signals (e.g. antennation, drumming or vibrations (Hölldobler, 1999; Franklin, 2014) or chemical signals (e.g. alarm or trail pheromones (Hölldobler, 1976; Traniello, 1989a; Lach *et al.*, 2010; Czaczkes *et al.*, 2015b)). Ant species with large colony sizes often use chemical mass-recruitment with short- or long-lasting trails, trunk trails and mass-raids (Beckers *et al.*, 1989; Traniello, 1989b; Hölldobler & Wilson, 1990; Lanan, 2014; Czaczkes *et al.*, 2015b). Species with smaller colonies use recruitment strategies like tandem running or group recruitment (Möglich *et al.*, 1974; Beckers *et al.*, 1989; Liefke *et al.*, 2001).

In tandem running, an informed ant returns to the nest after finding a good nest site or a rewarding food source that is too large to be exploited by a single ant. In the nest she releases a pheromone to attract a potential recruit (Möglich *et al.*, 1974). When the pair walks towards the resource, the follower antennates the leader's gaster and hind legs to maintain cohesion (Franklin, 2014). Although ants in a tandem run walk slower than ants walking alone, tandem recruitment could save time if resources are hard to find by independent search (Franks & Richardson, 2006). Many species (e.g. in *Temnothorax* or *Pachycondyla*) use tandem running for both nest emigrations and foraging recruitment (Colin *et al.*, 2017; Grüter *et al.*, 2018), whereas others (e.g. *Neoponera* or *Diacamma*) perform tandem runs only during colony migrations, but not during foraging (Fresneau, 1985; Kaur *et al.*, 2017). This raises the question whether and how tandem running might improve foraging success and, in particular, whether tandem recruitment might improve foraging success when competition is intense.

Tropical ants with small colony sizes might be particularly prone to direct competition, so-called interference competition, because they have to compete with mass-recruiting species that use pheromone trails to recruit large numbers of foragers and soldiers that are specialized to fight (Dejean *et al.*, 2005; Czaczkes *et al.*, 2011b; Czaczkes & Ratnieks, 2012). It seems highly plausible that communication helps mass-recruiting colonies to monopolize food sources, but it remains unclear whether this is also the case in species with small colonies that use slower forms of communication, such as tandem running. So far, only very few studies have assessed the role of communication in foraging competition in ants. One such study by Dejean et al. (2005) found that *Pheidole megacephala* (Fabricius, 1793) increase recruitment when they perceive competitors near food sources. Most ponerine ants live in small colonies (often just a couple of hundred individuals or less), they are important generalist arthropod predators and show a diversity of foraging strategies (Nascimento *et al.*, 2012; Schmidt, 2013), one of which is tandem recruitment (Grüter *et al.*, 2018).

Here, we tested if recruitment communication helps colonies to be more successful in a highly competitive tropical environment. As a model system we studied *Pachycondyla harpax* (Fabricius, 1804), a common Neotropical species that usually uses tandem running to recruit nestmates to newly discovered food sources (Grüter *et al.*, 2018). A previous study observed that *P. harpax* foragers are often displaced at food sources by aggressive competitors, mainly *Pheidole* (Grüter *et al.*, 2018). First, we described and quantified the main competitors of *P. harpax* in a Brazilian environment. Second, we tested our main prediction that tandem recruitment helps colonies to access food sources that would otherwise be monopolized by competitors. Third, we tested if *P. harpax* might follow a strategy of temporal and spatial niche differentiation to avoid competition, e.g. by foraging in locations and during times when competition for food is lower.

Material & Methods

Study site and study species

Experiments were performed in March 2018 and February/March 2019 on the campus of the University of São Paulo in Ribeirão Preto, Brazil. Our study species *Pachycondyla harpax* forages for dead and living insects and plant seeds (Grüter *et al.*, 2018). If scouts detect food items, they cannot carry home by themselves, e.g. beetle larvae or caterpillars, they often initiate tandem runs to recruit nestmates to the food source. This species is common in the study area and nests underground (Grüter *et al.*, 2018). Colony sizes range from 15 to 100 individuals that aggressively defend food sources against many smaller ants (Wheeler, 1900).

Experimental setup and procedure

All experiments were conducted in the field in three different locations on the campus, where *P. harpax* occurred. We located and marked colonies by following foragers who return to their nest after offering them small pieces of cheese (mozzarella or "queijo minas").

Part 1: interspecific competition depending on food types, daytime and territory

We first attempted to identify competitors of *P. harpax* and explored whether our focal species has a preference for different baits. We consider other ant species that forage at the same time and collect the same food type to be competitors. This does not imply aggressive interactions, *i.e.* interference competition, since also non-aggressive species can reduce foraging success of focal colonies *via* exploitation competition (Human & Gordon, 1996; Lach, 2005). Furthermore, we checked if *P. harpax* face different levels of competition at different food types and distances from the colony entrance. We tested 15 colonies with four different food sources: we used honey and pieces of mango (approx. 1 cm^3) as carbohydrate baits and cheese cubes (approx. 1 cm^3) or meat (pieces of sausage or meal worms) as protein rich food sources. Food baits were placed at two different distances from the colony entrances: either at 30 cm ("inner territory") or at 100 cm ("outer territory"). Previous research has shown that most food items are collected <100 cm from the nest entrance (Grüter et al. 2018).

Furthermore, we tested if *P. harpax* face stronger competition during daytime or at night. We offered food baits (cubes of cheese, approx. 1 cm^3) during the morning (9.00-12.00 h) and after sunset (18.30-21.00 h). Due to low activity of *P. harpax* in the afternoon, we did not test for competition during that time. We again tested the influence of distance from the nest.

After providing a food source, we recorded the number of different ant species (competitors) at the food bait during and at the end of a 10-minute observation period. In case an ant species could not be identified by the observer, specimens were collected for later identification. Ants were identified by comparison with the species of the Entomological Collection Padre Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil (DZUP), where the vouchers were deposited.

Part 2: exploitation and recruitment depending on competition

We provided food baits to 42 individual foragers of *P. harpax* (31 colonies). After they accepted the food and, thus, were the first species at the food source (treatment bait, without competition at the beginning), we provided a second food source as a control bait approx. 30 cm from the first food source and at similar distance to the nest. The second food source was normally discovered quickly by competitors but could also be found by *P. harpax*. The second bait allowed us to perform a paired comparison between the two baits near a colony. As soon as the food sources were placed, we started filming (JVC GZ-GX1 camcorder) the immediate surroundings of the two food baits. In some trials, we made still images of the control bait. We recorded at least one image per minute. Additionally, both baits were regularly inspected. Each trial was filmed for approx. 60 minutes. Observations ended earlier if other ant species displaced *P. harpax* from the first bait. It was not possible to quantify the number of food that was removed from the bait without disturbing the experiments. We frequently observed single foragers, to breaking-off bits and to walk away and transporting it back to the nest.

During video analysis, we recorded the distance of each bait from the nest (whenever possible), the probability of recruitment by *P. harpax* colonies, the maximum number of *P. harpax* foragers at the bait (at the same time), whether and for how long *P. harpax* foragers had access to the bait, the time until competitors found the bait and if there was a takeover (within 60 min after initial discovery *P. harpax* did not have access anymore) by competitors. *P. harpax* foragers were considered to have access to the food source (yes or no) when the individuals of a colony had access to the food source for at least 15 min and were able to collect and exploit the food bait, even if there were other non-aggressive ant species present. We considered a takeover by another ant species to have occurred when *P. harpax* could no longer feed at the bait.

Part 3: daily activity of Pachycondyla harpax

We observed that *P. harpax* appeared to be more active after sunset (unpublished data; see also García-Pérez et al. 1997). To confirm this, we observed 15 colonies at various locations on the campus during several days (14 March 2019 – 21 March 2019), from 9.30 h in the morning until 20.00 h in the evening. Each colony was observed for one minute and all *P. harpax* individuals in an area of one meter around the nest entrance were recorded. If we found no focal ant, the colony was considered to be inactive. After sunset we explored the surrounding of the nest entrance using the light of head lamps.

Part 4: food exploitation success depending on daytime

To compare the foraging success of *P. harpax* during daytime (9.30-12.00 h) and after sunset (18.30-21.00 h), we tested 15 colonies in both time periods and provided cheese baits for individual *P. harpax* foragers. Every five minutes during a 45-minute observation period we performed a scan-sampling and checked whether foragers from the focal colony still had access and recorded the maximum number of foragers that were simultaneously present at the food source. This allowed us to explore the competitive performance of colonies during the day and at night.

Statistical analysis

All tests were performed in R 3.5.3 (R Core Team, 2019). We performed linear mixed-effect models (LMEs) for normally distributed response variables and generalized linear mixed-effect models (GLMMs) for response variables with a binomial or Poisson distribution (Zuur *et al.*, 2009). For the competition and food exploitation experiments, colony ID was used as random effect to account for the potential non-independence of observations from the same colony. Location was used as random factor for the recruitment experiments (Zuur *et al.*, 2009). For the activity analyzes, we used colony and date as random factors. In part 1, we tested the role of the fixed effects "daytime", "colony territory" (inner vs. outer) and "food type" on the presence of competitors and

P. harpax activity. In part 2, to test the influence of recruitment on the foraging success, we used the fixed effects "maximum number of *P. harpax* foragers at the baits" and "recruitment" (yes or no) to test if they predicted whether *P. harpax* had access (yes or no) to the food bait. Since these predictors are linked, we explored their importance separately. With a McNemar's test we analyzed if the probability to recruit was higher at the treatment than at the control feeder. To test for significant interactions among the fixed-effects, we used likelihood ratio tests (LRT) and compared the model without the interaction with the model containing the interaction. Non-significant interactions were removed from the model. In part 3, we tested if colony activity (ants vs. no ants) depended on time of the day. In part 4, we quantified the foraging activity of focal colonies depending on the time interval during the 45-minute observation period.

Results

Interspecific competition depending on food types and territory

Overall, we identified almost 40 different ant species at our baits during the 10-minute observation periods (Table 6.1). The most common competitors of *P. harpax* were mass-recruiting *Pheidole* species (mostly *Pheidole oxyops* Forel 1908), *Odontomachus* sp.; rarely, we found *Atta* or *Camponotus* at a food bait (Fig. 6.1). A similar number of ant species discovered the different types of food during the 10-minute observation period, irrespective of the distance from the *P. harpax* focal colony (range = 30-100 cm, 2.68 ± 1.20 species [mean ± sd]). There was a borderline significant difference between the total number of species that discovered honey compared to meat, with more species collecting honey than meat (GLMER: inner vs. outer territory: $z = -0.792$, $p = 0.428$; food sources: meat vs. honey: $z = 2.080$, $p = 0.038$; meat vs fruit: $z = 0.816$, $p = 0.415$; meat vs. cheese: $z = 0.931$, $p = 0.352$; cheese vs. honey: $z = 1.190$, $p = 0.234$; cheese vs. fruit: $z = -0.095$, $p = 0.924$; fruit vs. honey: $z = 1.252$, $p = 0.211$). At the end of the 10-min observation period, the number of ant species at the food baits decreased to 1.36 ± 0.76 species and there was no difference between food types (GLMER: inner vs. outer territory: $z = -0.643$, $p = 0.520$; food sources: meat vs honey: z = 1.500, p = 0.134; meat vs fruit: z = -0.067, p = 0.947; meat vs. cheese: z = 0.420, p = 0.675; cheese vs. honey: z = 1.104, p = 0.269; cheese vs. fruit: z = -0.479, p = 0.125; fruit vs. honey: z = 1.536, p = 0.211). This reduction is likely due to displacement of weakly competitive species by competitively superior species.

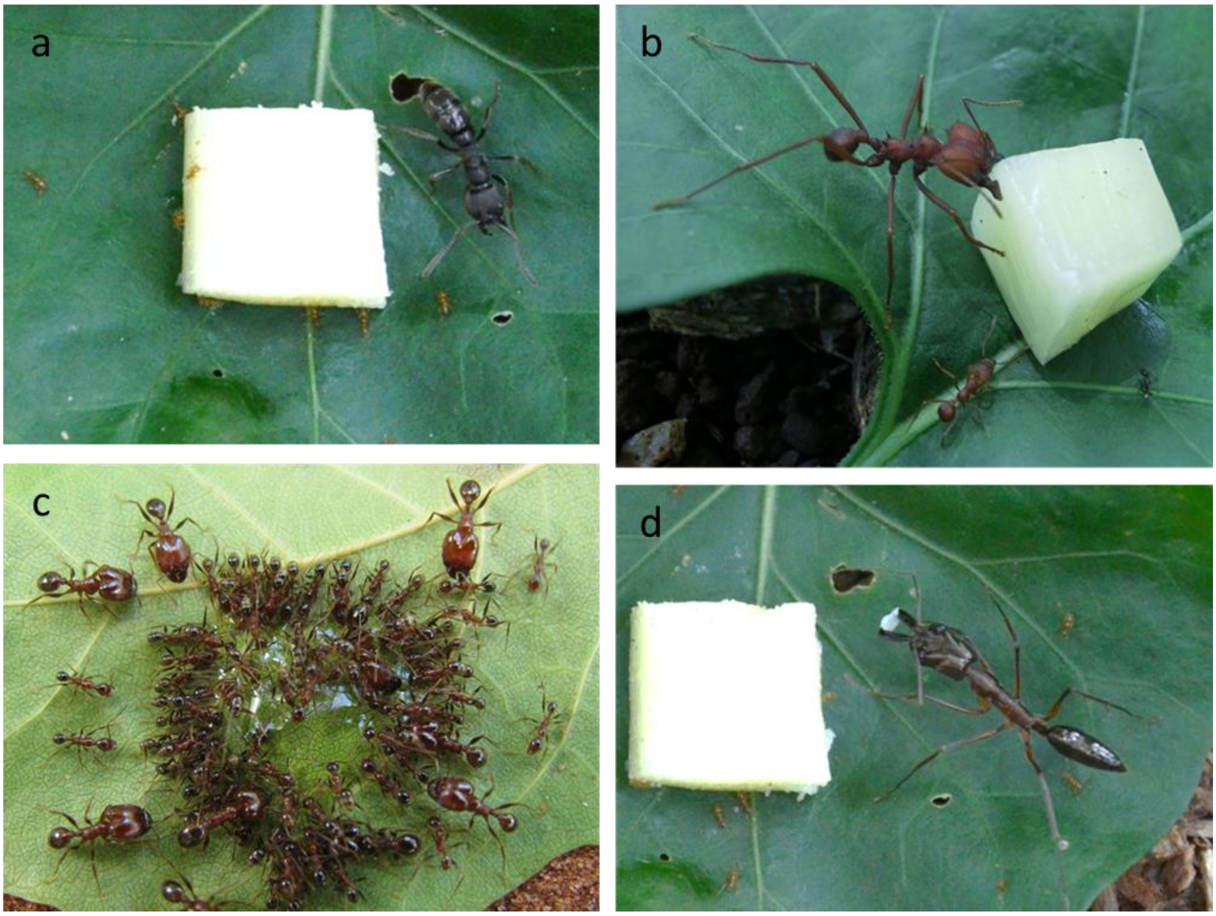


Figure 6.1: Study species and competitors at food baits. **(a)** *Pachycondyla harpax* defending the food source against *Wasmania auropunctata*. **(b)** *Atta sexdens* picking up and carrying away the cheese bait. **(c)** Workers and soldiers of mass recruiting *Pheidole oxyops (*Photo by Tomer Czaczkes*).* **(d)** *Odontomachus chelifer* carrying away a small piece of cheese.

Table 6.1 Competing ant species observed at different food baits during our observations.

Subfamily	**Species**
Dolichoderinae	*Dolichoderus bispinosus*
	Linepithema gallardoi
Formicinae	*Brachymyrmex* sp.
	Camponotus (*Myrmaphaenus*) sp. 1
	Camponotus (*Myrmaphaenus*) sp. 2
	Camponotus ager
	Camponotus atriceps
	Camponotus substitutus
	Sericomyrmex mayri
Myrmicinae	*Acromyrmex* sp.
	Apterostigma gr. *pilosum*
	Atta sexdens
	Crematogaster erecta
	Nesomyrmex sp.
	Pheidole angusta
	Pheidole gertrudae
	Pheidole oxyops
	Pheidole sensitiva
	Pheidole sp. 1
	Pheidole sp. 2
	Pheidole sp. 3
	Pheidole sp. 4
	Pheidole aff *radoszkowskii* sp. 1
	Pheidole aff *radoszkowskii* sp. 2
	Pheidole aff *subarmata*
	Solenopsis decipiens
	Solenopsis sp.
	Mycetomoellerius sp.
	Wasmannia auropunctata
Ponerinae	*Hypoponera* sp.
	Neoponera verenae
	Neoponera villosa
	Odontomachus chelifer
	Odontomachus sp.
	Pachycondyla striata

Exploitation & recruitment depending on competition

We found that after offering *P. harpax* foragers a bait, a substantial proportion of ants initiated tandem recruitment. In 42.9% (18 out of 42) of all trials, at least one forager started recruiting. The control bait was found in 38.1% (16 out of 42) of all trials by at least one *P. harpax* forager. Tandem recruitment to the control bait occurred in 37.5% (6 out of 16) of these trials (14.3% of all trials). Overall, tandem recruitment by *P. harpax* foragers was significantly more likely at the treatment bait than at the control bait (McNemar's test: $\chi^2 = 6.050$, df = 1, p = 0.014). The distance between the nest and the food source did not influence recruitment probability (GLMER: z = 1.052, p = 0.293). When comparing the number of *P. harpax* foragers at the two baits, we found no correlation between the maximum number of ants at the treatment and control feeder (GLMER: z = -0.653, p = 0.514). A positive correlation might have been expected if the number of ants at the two paired baits would have been the result of the colony size of the focal colony or reflected the level of competition in the area of the focal colony. Furthermore, competitors found the treatment and control feeder equally fast (treatment: 1.66 ± 2.28 min vs. control: 2.05 ± 1.79 min (mean ± sd); GLMER: z = -1.318, p = 0.187) and there was no correlation of time of discovery by competitors (GLMER: z = 1.526, p = 0.127).

We predicted that recruitment would lead to an increase in ants at the bait and, in turn, a higher probability to have access to the food bait. The results show that significantly more ants reached the food source when recruitment took place (Fig. 6.2a) (GLMER: z = 5.942, p < 0.001). Thus, when ants performed more tandem runs, the maximum number of ants that were present at a bait increased (GLMER: z = 4.486, p < 0.001). During a trial, individual foragers were often seen walking to the nest with small pieces of food and, subsequently, return to the food source in a tandem run or alone. We found that recruitment was associated with a more than 4-times higher probability to have access to the food source (no recruitment: 20.8% vs. recruitment: 88.9%) (Fig. 6.2b) (GLMER: z = 2.723, p = 0.006). Related to these two findings, we predicted that colonies are more likely to keep access if more ants are at the food source. We found that the probability of having access increased significantly with an increasing number of foragers at the food bait (Fig. 6.2c) (GLMER: z = 2.723, p =

0.006): *P. harpax* colonies had a 100% access chance when at least three ants were at the food source.

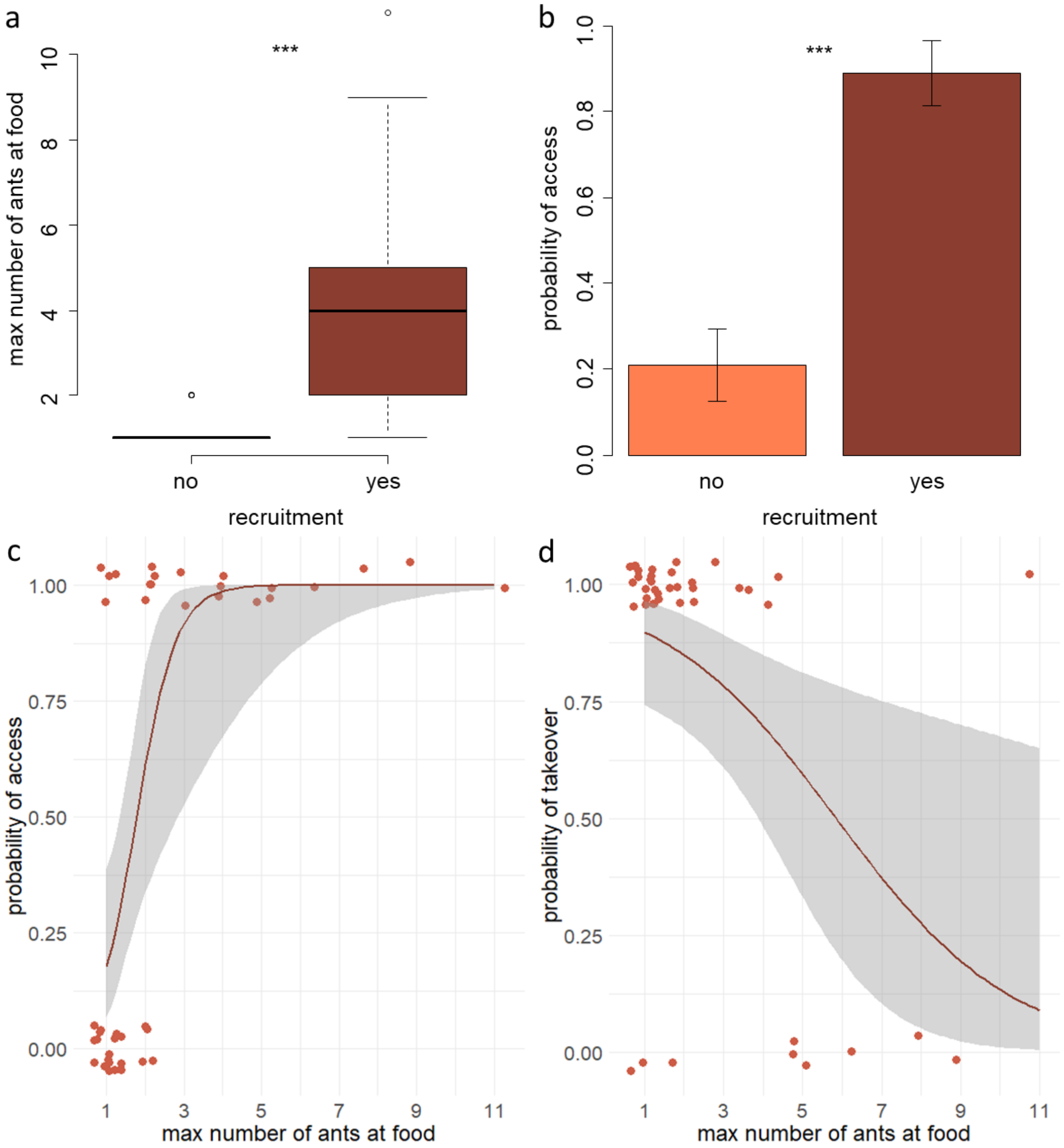
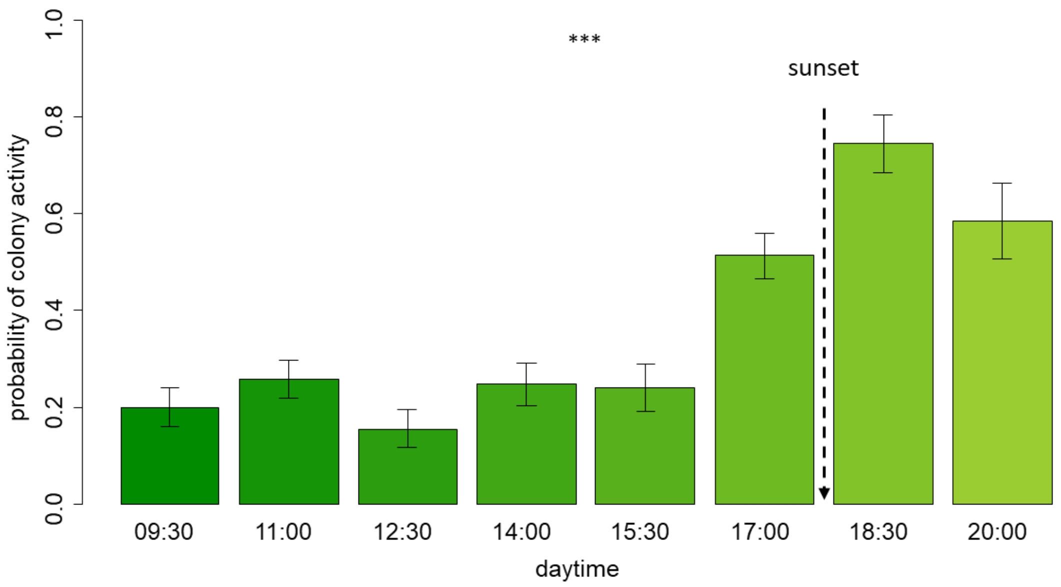


Figure 6.2: Maximum number of ants (**a**) and probability of ants at the food source (**b**) depending on whether our focal colony performed tandem recruitment. Probability of access (**c**) and of a food takeover by a competitive ant species (**d**) depending on the maximum number of ants at the food source. Access refers to a period of exploitation of at least 15 minutes during the observation period. Takeovers occurred when focal colonies were displaced during the 60 min observation period. Boxplots show median, 25th and 75th quartile and the 5th and 95th percentile. Bar plots show means and SE. Note that all values in (**c**) and (**d**) are either 1 or 0 but that jitter was used to better visualize the data points. Grey areas show the 95% confidence interval. *** $p < 0.001$

When competitors took over the food source at any time during the 60-minute observation period, *P. harpax* stopped recruiting. We tested if an increasing number of foragers at the food source lowered the chance of a takeover by another species and indeed found this to be the case (GLMER: $z = -3.087$, $p = 0.002$). When there were at least five ants of *P. harpax* at the food bait, takeover was extremely unlikely (Fig. 6.2d).

Activity cycle

We found in the first experiments that *P. harpax* has an increased activity after sunset (~6.30 pm) (see also García-Pérez et al. 1997). Therefore, we quantified the activity of 15 colonies from early morning (sunrise ~6.15 am) until after sunset (9:30h to 20:00h) over eight days (Fig. 6.3). There was substantial variation in activity throughout the day. As described by García-Pérez et al. (1997) in a different area, activity was generally low during the day and increased in the evening around 17.00 h up to 18.30 h (GLMER: $x^2 = 84.133$, $df = 7$, $p < 0.001$). While observing the colony activity, we also measured the temperature throughout the day. The morning counts ended around 11 am (26.88°C ± 1.63°C). The afternoon counts lasted from 12:30 pm until 5 pm (28.70°C ± 1.72°C). Sunset was typically around 6:30 pm. This is when our evening measurements started (24.70°C ± 1.34°C). Temperature was always measured a few cm above the ground in shaded areas.



Fiure. 6.3: The probability of colony activity of *P. harpax* foragers during daytime over eight days (n=15). Sunset was around 18.30.

Interspecific competition depending on daytime and territory

One explanation for the increased activity at night could be that colonies face less competition than during daytime. Thus, we compared the number of ant species at food baits in the morning vs. the evening. Contrary to our expectation, we observed that significantly more competitor species discovered the bait after sunset (Fig. 6.4a) (LME: t = -2.355, p = 0.024). There was also a tendency for more ant species to discover the bait closer to the nest entrance (LME: t = -1.753, p = 0.087).

The probability that *P. harpax* foragers would discover the baits during the 10-minute observation period was higher at night than during daytime (Fig. 6.4b) (GLMER: z = -2.332, p = 0.020). This probability did not depend on the distance of the cheese bait from the nest entrance of the focal colonies (GLMER: z = -0.354, p = 0.724). There was a borderline non-significant trend that *P. harpax* were more likely to still have access to the food at night compared to daytime at the end of the 10-min observation period, (GLMER: daytime: z = 1.910, p = 0.056; territory: z = -0.363, p = 0.716). The most frequent and successful competitor was the mass-recruiting *Pheidole oxyops* (present in 62.2% experiments) (Fig. 6.1c).

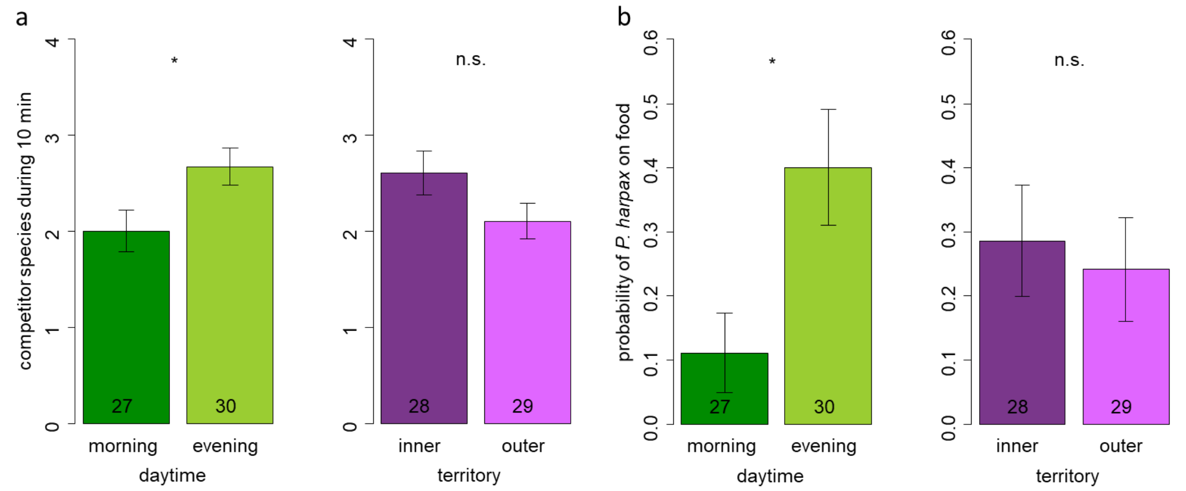


Figure 6.4: Number of different competitors (**a**) and the probability (**b**) that *P. harpax* would discover the bait during 10 min depending on the time of day and the distance from the entrance of the focal colony (territory). Sample sizes refer to number of trials. n.s.: not significant, * $p < 0.05$

Food exploitation and access depending on daytime

We then presented individual *P. harpax* foragers with cheese baits to explore whether time of day affected their ability to maintain access to the food source during a 45-minute observation period. During daytime, the number of foragers at the bait remained constant and low, whereas the number of *P. harpax* at the food increased after sunset (Fig. 6.5a & 6.5b) (GLMER: morning vs. evening: $z = -8.056$, $p < 0.001$; time: morning: $x^2 = 10.033$, df = 8, $p = 0.263$; evening: $x^2 = 27.66$, df = 8, $p < 0.001$).

Not all colonies were able to keep access to the food bait over the 45 minutes. After sunset, more colonies kept access to the food source than in the morning (morning: 18.5% vs. evening: 58.6%) (Fig. 6.5c & 6.5d (GLMER: morning vs. evening: $z = -8.056$, $p < 0.001$; time: morning: $x^2 = 3.2*10^{10}$, df = 8, $p < 0.001$; evening: $x^2 = 801.82$, df = 8, $p < 0.001$).

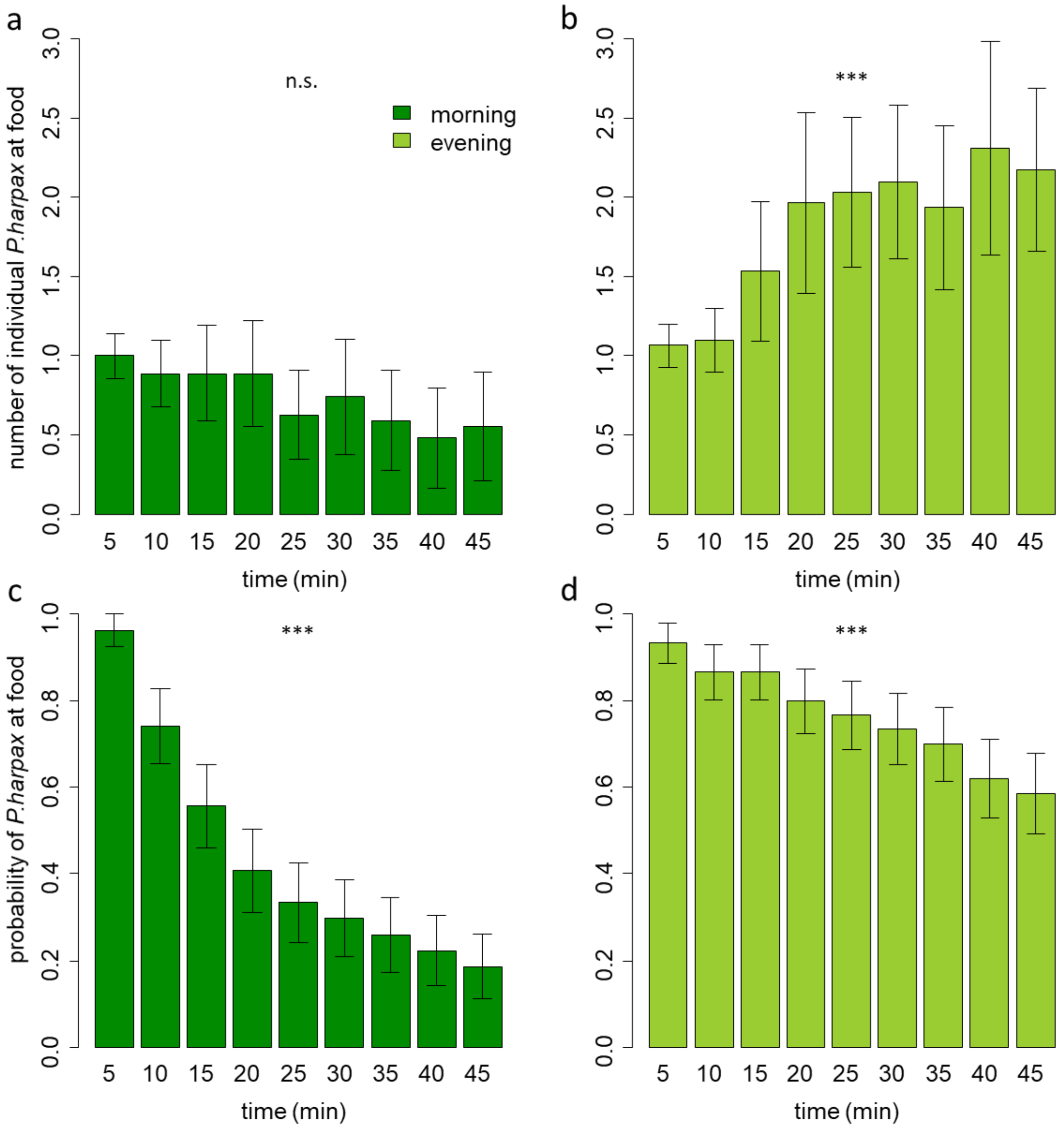


Figure 6.5: Number of individual *P. harpax* workers and the probability of colony presence at food baits during the 45-minute period in the morning (**a** & **c**) and evening (**b** & **d**). n.s.: not significant, *** p < 0.001

Discussion

Our results support the hypothesis that recruitment communication by tandem running increases access to food sources and, thus, foraging success in *Pachycondyla harpax*. Competition in this Brazilian habitat was intense for all types of food as almost 40 different ant species discovered and exploited our baits during our observations. This intense competition is not surprising given that *P. harpax* is a generalist forager, like many other Neotropical ant species. Confronted with big food items, foragers attempted to cut off small pieces of food and transport them back to the nest. However, they were frequently displaced by competitor species. Although several different species can discover a food source, only a few of them remain at the food bait over longer time periods. This reduction is likely due to displacement by competitively superior species. The most abundant competitors were *Pheidole* spp. (Table 6.1; 94.6 % of all observations), which are very efficient and aggressive mass-recruiters (Czaczkes *et al.*, 2011b; Czaczkes & Ratnieks, 2012). While it was not possible to collect data on the number of competing individuals at baits, the observations on the dominance of *Pheidole* spp. suggest that the number of competing ants affects the ability of *P. harpax* to access and defend food items. In some cases, however, a single individual of *Odontomachus* or *Neoponera* could banish their competitors, demonstrating that there is not always a straightforward relationship between the number and dominance of competitors. Different competitors could also fight against each other and thereby give a third party the opportunity to have access and exploit a food bait.

It is often assumed that mass-recruitment via pheromone trails helps colonies to monopolize food sources (de Biseau *et al.*, 1997; Detrain & Deneubourg, 2008; Drescher *et al.*, 2011). Accordingly, *Pheidole megacephala* scouts start recruiting more nestmates and soldiers to a food source if pheromones from competing colonies are encountered (Dejean *et al.*, 2005). Our results support and extend this view by showing that also species with small colonies employing relatively slow recruitment mechanisms are likely to benefit from recruitment communication. When *P. harpax* foragers had an opportunity to recruit nestmates to a food source, their foraging success was much higher: focal colonies with recruitment were 4 times more likely to keep access to the food during our observations. This is likely to allow more foragers to recruit further ants, leading to a positive feedback and a larger number of ants at the food. The bait was unlikely to be taken over by competitors when about five or more *P. harpax* foragers were at the food source (Fig. 6.2d). When *P. harpax* foragers found the food source by chance, they were often not able to exploit it for longer time periods if competitors were already present and prevented other *P. harpax* ants to recruit nestmates. When focal foragers discovered the bait before competitor species, as was the case at the treatment bait, tandem recruitment was much more frequently observed compared to the control bait (43% vs. 14%), which was randomly placed in the vicinity of the same nest. Thus, finding a food source first, before competitor species, is of critical importance to foraging success. The recruitment probability in *P. harpax* is comparable or higher than in other species that use recruitment methods other than chemical trails. For example, about 10% of returning honeybee foragers perform a waggle dance (Seeley, 1995) and ~10 to 30% of ant foragers lead tandem runs to food sources in *Cardiocondyla venustula* and *Temnothorax nylanderi* (Wilson, 1959; Glaser & Grüter, 2018).

Competition might be less intense in some areas, allowing more *P. harpax* foragers to have access for longer and to recruit nestmates to exploit the food source. A large number of *P. harpax* at a bait might also indicate a large colony size. In both cases, we would expect a positive correlation between the paired treatment and control baits in terms of the number of *P. harpax* foragers exploiting them. In other words, the two baits in proximity of a particular nest should be similar in the number of *P. harpax* foragers. However, this was not the case in our study as the number of *P. harpax* foragers at the treatment feeder was unrelated to the number at the paired control feeder. Additionally, there was no correlation in how quickly competitors found the treatment and control feeder. This finding, in combination with our other findings, suggests that the number of ants at a bait is largely the result of discovering a food source first, followed by successful recruitment. Colony size might still be an important factor for competitiveness. When a colony is larger, more individuals can scout or be recruited to a food source. Hence, larger colonies are probably more successful during foraging compared to smaller colonies (Dornhaus *et al.*, 2012). In addition, foragers from larger colonies might also be more aggressive (Oster & Wilson, 1978), which would further affect access to food sources.

One possibility to avoid competition might be to forage during different times of the day, as is the case with nocturnal and diurnal species (Rosumek, 2017). In a study on grassland ants, for example, different species foraged at different times of the day depending on air temperature (Albrecht & Gotelli, 2001). This temporal niche partitioning can help subdominant species to avoid dominant species (Stuble *et al.*, 2013). Stingless bees, likewise, shift their activity to avoid the presence of other, mainly aggressive species (Nagamitsu & Inoue, 1997; Keppner & Jarau, 2016). In the tropics, species also can adapt to different seasons, e.g. by being more active during the rainy seasons (Baumgartner & Roubik, 1989), whereas others are active during both seasons. In accordance with a previous study (García-Pérez *et al.*, 1997), we observed that the activity of *P. harpax* increases in the afternoon and is highest after sunset. Thus, we hypothesized that this shift in activity is due to competition being less intense after sunset. Contrary to this expectation we found that competition seemed to be even stronger after sunset than during the morning/daytime: we found a 33% increase in the number of competitor species at the food baits after sunset. A possible explanation might be that most ant species start becoming more active when temperatures are going down and humidity increases to prevent water loss (Schilman *et al.*, 2007). The >100% increase in *P. harpax* activity after sunset (Fig. 6.3) more than compensated for the increase in competitor species after sunset (Fig. 6.4) and, as a result, *P. harpax* colonies were better able to defend and exploit food sources after sunset (Fig. 6.5). After 45 minutes, only 18.5% of the colonies maintained access to the food during the morning compared to 56.6% after sunset. This suggests that *P. harpax* might be more successful during the night because they increase their activity at a higher rate than other ant species. As a result, we observed an increase in the number of foragers at the food bait over time after sunset, but not during daytime. This increase is most likely due to ants being recruited via tandem runs (personal observation) in combination with a higher rate of independent discoveries at night.

Conclusions

It is often assumed that recruitment communication in social insects is beneficial for foraging success (but see Dechaume-Moncharmont *et al.*, 2005; I'Anson Price *et al.*, 2019). One key benefit could be that recruitment allows colonies to monopolize food sources in a competitive environment by building up a critical mass of nestmates to defend a large resource. However, evidence for this has been scarce. In our study we show that *Pachycondyla harpax* are likely to improve their foraging success thanks to tandem recruitment. The results also indicate that foragers have better access to food sources at night, most likely aided by recruiting nestmates. This highlights the potential influence of communication on foraging success and efficiency in ants, including those species with small colony sizes and slow, direct recruitment communication.

GENERAL DISCUSSION

Simone M. Glaser

Communication in eusocial insects is widespread and probably one of the reasons why they are so successful. Information sharing can lead to fast information flows in colonies which allows them to recruit nestmates and exploit resources quickly. There has been an ongoing discussion about the ancestral recruitment strategies in ants and which strategies developed with time in descendant species (Jaffe, 1984; Beckers *et al.*, 1989; Reeves & Moreau, 2019). Our results strongly indicate that mass-recruitment or group-recruitment were most likely used for recruitment by the ancestors of current ants (Chapter 1). This finding is supported by a recent study that has found that the ancestral ants probably had medium colony sizes with up to several hundred to thousands of individuals in one colony (Burchill & Moreau, 2016). Ant species with colonies in this size range tend to use group- or mass-recruitment (Beckers *et al.*, 1989). In all subfamilies we found transitions and switches between recruitment strategies from group- or mass-recruitment to no recruitment and back from no recruitment to tandem running or mass-recruitment. This also indicates that strategies are a flexible and reversible trait in ants. Surprisingly, most transitions arose from mass-recruitment to group-recruitment or no recruitment, which is probably connected to the appearance of ant species with smaller colony sizes in several subfamilies (e.g. Amblyoponinae, Myrmeciinae, Ponerinae and Pseudomyrmecinae). In ant species with smaller colonies, individuals tend to recruit nestmates via tandem running or they exploit resources solitarily (Beckers *et al.*, 1989). This finding could be explained by the fact that species with smaller colony size may have switched to a food diet that does not demand mass recruitment.

There have been several observations that species recruit via tandem running to new nest sites, but do not use tandem runs to recruit to food sources (e.g. *Neoponera* or *Diacamma*) (Fresneau, 1985; Kaur *et al.*, 2017). This indicates that during foraging the ant workers actively choose not to recruit, either because they are hunting and foraging for smaller food items where it is not necessary to recruit nestmates or because there are just a few foragers in the colony and thus they cannot maintain pheromone trails (Beckers *et al.*, 1989). In contrast, during nest relocations every individual of a colony has to move. This can be very dangerous under harsh conditions (Franks *et al.*, 2003), since also the brood and queen have to move, thus it is advantageous just to move to nest sites of higher quality (Dornhaus *et al.*, 2004) and to move as fast as possible, which is ensured by recruitment of nestmates. Thus, during the literature research we focused on the general ability of species to recruit nestmates independent of the context. This could also explain the different findings compared to a recent study of Reeves & Moreau (2019). In their study they concluded that solitary foraging, rather than mass-recruitment, is the ancestral recruitment strategy in ants. It is noteworthy that they focused exclusively on recruitment during foraging. To find out why some species evolved different recruitment strategies or lost the ability to recruit nestmates it is important to understand under which circumstances it is useful for individuals and colonies to recruit nestmates. Often it can be more efficient to use other information strategies.

The influence of food source distribution on the use of social information

In nature the environment is constantly changing and the distribution of food sources and new nest sites is shifting steadily (Chapter 2, 3 & 4). To be up to date individuals have to collect and share information continuously.

We manipulated food sources in empirical experiments and tested with an agent-based simulation model how the adaptive significance of social learning was influenced by the food source distribution. Our results indicate that following tandem runs is beneficial when food sources are overexploited and scarce (Chapter 2 & Chapter 3). Scouts can find the most valuable resources by discovering new food patches and resource switching. While discovering more and more food sources they can return to the most profitable ones. Thereby they can increase the average food quality uptake over time and afterwards lead tandem runs to the most beneficial resources (Chapter 2). During the tandem runs, recruits receive via social learning the information about the food sources with the highest reward(Sasaki *et al.*, 2016). Similar results have been found in honeybees. Successful foragers start to dance more often for high quality food sources (Schürch & Grüter, 2014). In environments with just a few food sources, scouts as well as recruits foraged on food sources of average quality (Figure 2.1b). This is contrary to what was expected, since in *T. rugatulus*, foragers were recruiting to the qualitatively higher food source when there were unequal feeders available (Shaffer *et al.*, 2013). When there is a high supply of food sources, they are easily found by foragers (Seeley, 1986; Schürch & Grüter, 2014). In general, scouts perform better and can find the resources faster than recruits, but only if they are all of high quality. Subsequently they can recruit nestmates to food sources of higher quality. Thus, tandem followers can save time by using social learning instead of individual learning when food sources are scarce (Chapter 2). When food sources are abundant, foragers should try individual learning when they can find food sources faster than by social learning and save time. Interestingly, we found that recruits performed fewer foraging trips when they spent more time in the nest waiting to be recruited. With computer simulations we found that when the food source quality varies, tandem runs to more profitable food sources help to increase energy uptake of the colony. This was found independent of food source distribution. Combining the waiting time inside of the nest with the duration of a tandem run, recruits need less time to reach a food source than scouts, when food sources are scarce (Fig. 2.4b).

This demonstrates that there could be hidden costs as recruit while waiting inside the nest (Dechaume-Moncharmont *et al.*, 2005). While waiting to be recruited, they do not have the opportunity to find new food sources. These findings just count for stable food sources, when ants collect e.g. honeydew or find large dead insects. When food sources are unstable or ephemeral and are exploited fast, the use of individual learning should be more beneficial (Chapter 3, Figure 3.3), since the resource could have disappeared until a nestmate is recruited.

Another factor that influences the use of social learning is food distance (Chapter 4). With increasing food distance, tandem pairs walk less straight to the resource. At the same time, they increase their walking speed, possibly to shorten the time while being exposed to risks and dangers further from the colony (Figure 4.5). Interestingly, Franklin et al. (2012) found that experienced leaders led slower and more straight tandem runs than young, inexperienced tandem leaders. In general, a higher walking speed can lead to more break-ups of the tandem pair (Richardson *et al.*, 2007). Unexpectedly, the success rate of tandem runs was similar, independent of food distance. This could be explained by the leader and follower waiting and searching for each other for longer to reunite and accomplish the tandem run when food source distances increase. Indeed, when a break-up occurred, tandem followers waited longer after losing contact with a leader foraging at increasing food distances. Interestingly, we did not find any effect of waiting time of the leader depending on the food distance. This is contrary to what has been found in the closely related species *T. albipennis*, where tandem leaders wait longer when they have invested more time in a tandem run (Franks *et al.*, 2010). When a tandem follower did not maintain contact again after a contact loss she still had the chance to find another, valuable resource. Many other social insects decrease the recruitment strength with an increasing food distance (Dornhaus, 2002), probably because distant food sources are more risky and costly to exploit. In our study we found that food distance did not have any effect on the probability of starting a tandem run. An important factor that influenced the probability of tandem runs was the experience of the tandem leader. When tandem leader had successful tandem runs in the past, the probability to start the next tandem increased significantly (Chapter 4, Fig 4.2).

Colony size and how individuals influence the foraging success

Using a simulation model, we found that colony size had a major effect on the benefits of tandem communication (Chapter 3). Species that perform tandem runs live in rather small colonies (Beckers *et al.*, 1989). Additionally, it is expected that just 20-30% workers, thus a fragment of the colony are foragers (Shaffer *et al.*, 2013). If colonies exceed a threshold of about 60 foragers in our simulation, tandem communication was usually advantageous. When colonies had fewer foragers, a strategy of individual learning was more beneficial. These results could explain why some species with very small colony size, eg. *Diacamma* (Kaur *et al.*, 2017) do not perform tandem runs during foraging. Recruiting nestmates can still be helpful during colony migrations (O'Shea-Wheller *et al.*, 2016)(O'Shea-Wheller *et al.*, 2016), since the whole colony, including the queen and brood are exposed to higher risks during the move. Besides colony size and the number of foragers also the information-use strategy plays an important role. Interestingly, the effects of the ratio of scouts and recruits seems to be dependent on colony size and food distribution. When colonies are small or food sources are scarce, it is probably more important to have more scouts to be able to find a high-quality food source. In larger colonies or with abundant food sources, more recruits could be more beneficial, since the scouts can find the food sources more easily and perform the tandem runs to the most beneficial resources. In nature, the scout-recruit-ratio seems to be quite complex and flexible (Seeley, 1983; Mailleux *et al.*, 2003). Colonies can adapt their intensity of recruitment behaviour to changing environmental conditions and individual colony needs (Mailleux *et al.*, 2003).

Even though foragers can change their information-use strategy depending on the environment, it seems that there are two distinct types of foragers in *T. nylanderi*. They differ in whether they use individual or social learning to find new food sources. Our results suggest that original scouts lead most of the tandem runs and thus are the main providers of social information. Furthermore, they more often discover new resources by using individual learning or they return to known resources. In contrast, recruits use social learning by following a tandem run and thereafter use mainly private information to return to the learned resource. After leading a tandem run, foragers get more experience and the following tandem runs are more successful (Franklin *et al.*, 2012). Similar foraging behaviour has been found in honeybees. Old foragers provide social information and tend to perform more waggle dances, whereas young, unexperienced foragers follow the waggle runs (Jaffe & Deneubourg, 1992; Biesmeijer & Seeley, 2005). However, it is unclear, how experience is linked to age in ants, since the workers of *T. nylanderi* can live for several years (Beros *et al.*, 2021).

Another factor that influences the success of tandem runs is the body size difference of tandem partners (Chapter 5). When the body length difference exceeded 10%, the probability of a break-up increased and thereafter a failure of the tandem run occurred often (Figure 5.2b). An explanation could be that body size in ants is connected to walking speed. Different speeds of leader and follower can cause irregluarities and lead to connection problems during the tandem run. To prevent a contact loss, the partners try to accelerate and decelerate to maintain cohesion (Franklin *et al.*, 2011). This could further lead to short contact losses, which could increase the likelihood of a break-up. When tandem partners had equal body length, the success rate of tandem runs was significantly higher. Similar results have been found in honeybees. When body size variance is higher between dancer and dance follower, the amount of misinformation communicated increased and foraging became less successful (Waddington, 1989). However, a tandem break-up does not automatically indicate a failure. The recruit could still find the intended resource or discover a new resource in a changing environment. Otherwise, after a break-up, the follower is often left alone in an unknown and dangerous environment and thereafter returns to the nest.

Not only food source distribution but also the distribution of competitive insects and ant species can influence the behaviour of tandem running ant species. Our results suggest that the use of social information increases the access and leads to faster exploitation of food sources. Especially in the tropics, competition for nest sites and food sources is intense (Houadria *et al.*, 2015). Quite often, several ant species find the same resource and the more aggressive or faster recruiting ant species is more likely to monopolize the resource. *P. harpax* had a higher chance of exploiting the resource after recruiting nestmates (Figure 6.2). Especially mass recruiting ant species like *Pheidole* spp. are common competitors of *P. harpax*. They mass-recruit via pheromone trails. The soldier caste then defends the food source while the smaller foragers exploit the resource (Czaczkes *et al.*, 2011b; Czaczkes & Ratnieks, 2012). The presence of competitors could also affect the intensity of recruitment as foragers might recruit more and faster to expoit the resource. To avoid competition, colonies can also forage for specific food types or during different times of the day (Grevé *et al.*, 2019). We observed that *P. harpax* increased their foraging activity in the afternoon and had their highest activity after sunset (Figure 6.3, see also García-Pérez *et al.*, 1997). Also competitors increased their activity after sunset, when temperatures decrease and humidity increases to prevent water loss (Stuble *et al.*, 2013; Houadria *et al.*, 2015; Grevé *et al.*, 2019). Astonishingly, *P. harpax* colonies were able to compensate for the higher competition by increasing their own activity by more than 100%. When they had the ability to recruit nestmates, colonies were better able to exploit and defend the food resources.

Outlook

We have explored the evolution of recruitment and studied the influence of environmental and colony factors on the information-use strategy during foraging in tandem running ants. However, while observing the scouts and tandem runs during the experiments, new questions evolved. In chapter 1 we analysed the evolution of different recruitment strategies. We found that the ability to use pheromone trails to recruit nestmates was lost in several ant species. However, it is unclear if and how ant species that use tandem runs for recruitment also use pheromones. Basari et al. (2014) found that in *Temnothorax albipennis* tandem leaders deposit pheromone trails on the ground on their way to a valuable resource. In the next experiments I would analyse if scouts or tandem leaders of *T. nylanderi* use contact pheromones or deposit them on the ground to support and guide their nestmates to the resources.

In chapter 2 and chapter 3 we studied the use of social information during recruitment. We found that scouts use primarily individual information and are the main providers of social information, leading most of the tandem runs. In contrast, tandem followers receive social information and thereafter use mainly private information. This leads to the question if ant workers keep their information-use strategies or if foraging strategies change with age or experience.

We tracked the trajectories of tandem runs in chapter 4 to analyse the walked distance from the nest to the resource. So far, it is unclear, what exactly the followers learn during a tandem run to a resource. It has been found that tandem followers have the ability to learn landmarks on their way to a resource and can thereafter orient their way on it (Basari & Laird-Hopkins, 2014), but it is still unclear whether they either learn the trajectory to the resource or the location and use path integration to remember the resource. For *Temnothorax albipennis* it has been shown that tandem followers learn specific routes during the tandem run (Sasaki *et al.*, 2020). Thus, the next step would be to provide different path for foragers in a flexible set up. Scouts and tandem pairs would have different options compared to the follower when she is returning to the nest.

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REFERENCES

Al Toufailia, Couvillon, M.J., Ratnieks, F.L.W. & Grüter, C. 2013a. Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour. *Behav. Ecol. Sociobiol.* **67**: 549–556.

Al Toufailia, H., Grüter, C. & Ratnieks, F.L.W. 2013b. Persistence to unrewarding feeding locations by honeybee foragers (*Apis mellifera*): The effects of experience, resource profitability and season. *Ethology* **119**: 1096–1106.

Albrecht, A.M. & Gotelli, N.J. 2001. Spatial and temporal niche partitioning in grassland ants. *Oecologia* **126**: 134–141.

Almeida, C.S., Cristaldo, P.F., Desouza, O., Bacci, L., Florencio, D.F., Cruz, N.G., *et al.* 2018. Resource density regulates the foraging investment in higher termite species. *Ecol. Entomol.* **43**: 371–378.

AlShebli, B.K., Rahwan, T. & Woon, W.L. 2018. The preeminence of ethnic diversity in scientific collaboration. *Nat. Commun.* **9**: 1–10.

Anderson, C. & Ratnieks, F.L.W. 1999. Task partitioning in insect societies. I. Effect on colony size on queueing delay and colony ergonomic efficiency. *Am. Nat.* **154**: 536–548.

Bar-On, Y.M., Phillips, R. & Milo, R. 2018. The biomass distribution on earth. *PNAS* **115**.

Barry, S.C., Brooks, S.P., Catchpole, E.A. & Morgan, B.J.T. 2003. The analysis of ring-recovery data using random effects. *Biometrics* **59**: 54–65.

Basari, N., Bruendl, A.C., Hemingway, C.E., Roberts, N.W., Sendova-Franks, A.B. & Franks, N.R. 2013. Landmarks and ant search strategies after interrupted tandem runs. *J. Exp. Biol.* 944–954.

Basari, N. & Laird-Hopkins, B. 2014. Trail laying during tandem-running recruitment in the ant *Temnothorax albipennis*. *Naturwissenschaften* **101**: 549–556.

Baudier, K.M., Ostwald, M.M., Grüter, C., Segers, F.H.I.D., Roubik, D.W., Pavlic, T.P., *et al.* 2019. Changing of the guard: mixed specialization and flexibility in nest defense (*Tetragonisca angustula*). *Behav. Ecol.* **30**: 1041–1049.

Baumgartner, D.L. & Roubik, D.W. 1989. Ecology of necrophilous and filth-gathering stingless bees (Apidae: Meliponinae) of Peru. *J. Kansas Entomol. Soc.* **62**: 11–22.

Beckers, R., Goss, S., Deneubourg, J.L. & Pasteels, J.M. 1989. Colony size, communication and ant foraging strategy. *Psyche A J. Entomol.* **96**: 239–256.

Beekman, M. & Dussutour, A. 2009. How to Tell Your Mates Recruitment Mechanisms. In: *Food exploitation by social insects: ecological, behavioral, and theoretical approaches*, pp. 105–124. CRC Press, Taylor & Francis Group, Boca Raton.

Beekman, M., Gloag, R.S., Even, N., Wattanachaiyingchareon, W. & Oldroyd, B.P. 2008. Dance precision of *Apis florea* - Clues to the evolution of the honeybee dance language? *Behav. Ecol. Sociobiol.* **62**: 1259–1265.

Beekman, M. & Lew, J. Bin. 2007. Foraging in honeybees — when does it pay to dance ? *Behav. Ecol.* **19**: 255–261.

Beekman, M., Sumpter, D.J. & Ratnieks, F.L. 2001. Phase transition between disordered and ordered foraging in Pharaoh's ants. *Proc. Natl. Acad. Sci. U. S. A.* **98**: 9703–9706.

Bengston, S.E. & Dornhaus, A. 2013. Colony size does not predict foraging distance in the ant *Temnothorax rugatulus*: a puzzle for standard scaling models. *Insectes Soc.* **60**: 93–96.

Beros, S., Lenhart, A., Scharf, I., Negroni, M.A., Menzel, F. & Foitzik, S. 2021. Extreme lifespan extension in tapeworm-infected ant workers. *R. Soc. Open Sci.* **8**.

Beshers, S.N. & Traniello, J.F.A. 1994. The Adaptiveness of Worker Demography in the Attine Ant *Trachymyrmex septentrionalis*. *Ecology* **75**: 763–775.

Biesmeijer, J.C. & Seeley, T.D. 2005. The use of waggle dance information by honey bees throughout their foraging careers. *Behav. Ecol. Sociobiol.* **59**: 133–142.

Billick, I. 2002. The relationship between the distribution of worker sizes and new worker production in the ant *Formica neorufibarbis*. *Oecologia* **132**: 244–249.

Billick, I. & Carter, C. 2007. Testing the importance of the distribution of worker sizes to colony performance in the ant species *Formica obscuripes* forel. *Insectes Soc.* **54**: 113–117.

Boyd, R. & Richerson, P.J. 1995. Why does culture increase human adaptability? *Ethol. Sociobiol.* **16**: 125–143.

Branstetter, M.G., Longino, J.T., Ward, P.S. & Faircloth, B.C. 2017. Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods Ecol. Evol.* **8**: 768–776.

Brown, C. & Laland, K.N. 2003. Social learning in fishes : a review. *Fish Fish.* **4**: 280–288.

Burchill, A.T. & Moreau, C.S. 2016. Colony size evolution in ants: macroevolutionary trends. *Insectes Soc.* **63**: 291–298. Springer International Publishing.

Caro, T.M. & Hauser, M.D. 1992. Is There Teaching in Nonhuman Animals ? *Q. Rev. Biol.* **67**: 151–174.

Carroll, C.R. & Janzen, D.H. 1973. Ecology of foraging by ants. *Annu. Rev. Ecol. Syst.* **4**: 231–257.

Colin, T., Doums, C., Péronnet, R. & Molet, M. 2017. Decreasing worker size diversity does not affect colony performance during laboratory challenges in the ant *Temnothorax nylanderi*. *Behav. Ecol. Sociobiol.* **71**.

Collett, M., Chittka, L. & Collett, T.S. 2013. Spatial memory in insect navigation. *Curr. Biol.* **23**: R789–R800. Elsevier.

Collignon, B. & Detrain, C. 2010. Distributed leadership and adaptive decision-making in the ant *Tetramorium caespitum*. *Proc. R. Soc. B Biol. Sci.* **277**: 1267–1273.

Couvillon, M.J., Jandt, J.M., Duong, N. & Dornhaus, A. 2010. Ontogeny of worker body size distribution in bumble bee (*Bombus impatiens*) colonies. *Ecol. Entomol.* **35**: 424–435.

Crawley, M.J. 2007. *The R Book*. John Wiley & Sons Ltd, West Sussex, England.

Czaczkes, T.J., Czaczkes, B., Iglhaut, C.I. & Heinze, J. 2015a. Composite collective decision-making. *Proc. R. Soc. B Biol. Sci.* **282**.

Czaczkes, T.J., Grüter, C., Jones, S.M. & Ratnieks, F.L.W. 2011a. Synergy between social and private information increases foraging efficiency in ants. *Biol. Lett.* **7**: 521–524.

Czaczkes, T.J., Grüter, C. & Ratnieks, F.L.W. 2015b. Trail pheromones: An integrative view of their role in social insect colony organization. *Annu. Rev. Entomol.* **60**: 581–599.

Czaczkes, T.J., Nouvellet, P. & Ratnieks, F.L.W. 2011b. Cooperative food transport in the Neotropical ant, *Pheidole oxyops*. *Insectes Soc.* **58**: 153–161.

Czaczkes, T.J. & Ratnieks, F.L.W. 2012. Pheromone trails in the Brazilian ant *Pheidole oxyops*: extreme properties and dual recruitment action. *Behav. Ecol. Sociobiol.* **66**.

Czaczkes, T.J., Salmane, A.K., Klampfleuthner, F.A.M. & Heinze, J. 2016. Private information alone can trigger trapping of ant colonies in local feeding optima. *J. Exp. Biol.* **219**: 744–751.

Davidson, D.W. 1977. Foraging ecology and community organization in desert seed-eating ants. *Ecology* **58**: 725–737.

Davidson, D.W., Cook, S.C., Snelling, R.R. & Chua, T.H. 2003. Explaining the abundance of ants in lowland tropical rainforest canopies. *Science (80-.).* **300**: 969–973.

Dawson, E.H., Avarguès-Weber, A., Chittka, L. & Leadbeater, E. 2013. Learning by observation emerges from simple associations in an insect model. *Curr. Biol.* **23**: 727–730.

Dawson, E.H. & Chittka, L. 2014. Bumblebees (*Bombus terrestris*) use social information as an indicator of safety in dangerous environments. *Proc. R. Soc. B Biol. Sci.* **281**.

de Biseau, J.C., Quinet, Y., Deffernez, L. & Pasteels, J.M. 1997. Explosive food recruitment as a competitive strategy in the ant *Myrmica sabuleti* (Hymenoptera, Formicidae). *Insectes Soc.* **44**: 59–73.

Dechaume-Moncharmont, F.X., Dornhaus, A., Houston, A.I., McNamara, J.M., Collins, E.J. & Franks, N.R. 2005. The hidden cost of information in collective foraging. *Proc. R. Soc. B Biol. Sci.* **272**: 1689–1695.

Dejean, A., Delabie, J.H.C., Corbara, B., Azémar, F., Groc, S., Orivel, J., *et al.* 2012. The ecology and feeding habits of the arboreal trap-jawed ant *Daceton armigerum*. *PLoS One* **7**.

Dejean, A., Le Breton, J., Suzzoni, J.P., Orivel, J. & Saux-Moreau, C. 2005. Influence of interspecific competition on the recruitment behavior and liquid food transport in the tramp ant species *Pheidole megacephala*. *Naturwissenschaften* **92**: 324–327.

Deneubourg, J.L., Pasteels, J.M. & Verhaeghe, J.C. 1983. Probabilistic behaviour in ants: A strategy of errors? *J. Theor. Biol.* **105**: 259–271.

Detrain, C. & Deneubourg, J.-L. 2008. Collective decision-making and foraging patterns in ants and honeybees. *Adv. In Insect Phys.* **35**: 123–173.

Devigne, C. & Detrain, C. 2006. How does food distance influence foraging in the ant *Lasius niger*: The importance of home-range marking. *Insectes Soc.* **53**: 46–55.

Donaldson-Matasci, M.C., DeGrandi-Hoffman, G. & Dornhaus, A. 2013. Bigger is better: honeybee colonies as distributed information-gathering systems. *Anim. Behav.* **85**: 585–592. Elsevier Ltd.

Dornhaus, A. 2002. Significance of honeybee recruitment strategies depending on foraging distance (Hymenoptera: Apidae: *Apis mellifera*). *Entomol. Gen.* **26**: 93–100.

Dornhaus, A. & Chittka, L. 1999. Evolutionary origins of bee dances. *Nature* **401**: 38.

Dornhaus, A. & Chittka, L. 2004. Why do honey bees dance? *Behav. Ecol. Sociobiol.* **55**: 395–401.

Dornhaus, A., Franks, N.R., Hawkins, R.M. & Shere, H.N.S. 2004. Ants move to improve: colonies of *Leptothorax albipennis* emigrate whenever they find a superior nest site. *Anim. Behav.* **67**: 959–963.

Dornhaus, A., Klügl, F., Oechslein, C., Puppe, F. & Chittka, L. 2006. Benefits of recruitment in honey bees: effects of ecology and colony size in an individual-based model. *Behav. Ecol.* **17**: 336–344.

Dornhaus, A., Powell, S. & Bengston, S. 2012. Group size and its effects on collective organization. *Annu. Rev. Entomol.* **57**: 123–141.

Drescher, J., Feldhaar, H. & Blüthgen, N. 2011. Interspecific aggression and resource monopolization of the invasive ant *Anoplolepis gracilipes* in Malaysian Borneo. *Biotropica* **43**: 93–99.

Dunlap, A.S., Nielsen, M.E., Dornhaus, A. & Papaj, D.R. 2016. Foraging Bumble Bees Weigh the Reliability of Personal and Social Information. *Curr. Biol.* **26**: 1195–1199. Elsevier Ltd.

Fewell, J.H. 1988. Energetic and time costs of foraging in harvester ants, *Pogonomyrmex occidentalis*. *Behav. Ecol. Sociobiol.* **22**: 401–408.

Fewell, J.H., Harrison, J.F., Stiller, T.M. & Breed, M.D. 1992. Distance effects on resource profitability and recruitment in the giant tropical ant, *Paraponera clavata*. *Oecologia* **92**: 542–547.

Foitzik, S. & Heinze, J. 1998. Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. *Behav. Ecol.* **9**: 367–375.

Franklin, E.L. 2014. The journey of tandem running: the twists, turns and what we have learned. *Insectes Soc.* **61**: 1–8.

Franklin, E.L., Richardson, T.O., Sendova-Franks, A.B., Robinson, E.J.H. & Franks, N.R. 2011. Blinkered teaching: tandem running by visually impaired ants. *Behav. Ecol. Sociobiol.* **65**: 569–579.

Franklin, E.L., Robinson, E.J.H., Marshall, J. a R., Sendova-Franks, A.B. & Franks, N.R. 2012. Do ants need to be old and experienced to teach? *J. Exp. Biol.* **215**: 1287–

1292.

Franks, N.R., Dornhaus, A., Fitzsimmons, J.P. & Stevens, M. 2003. Speed versus accuracy in collective decision making. *Proc. R. Soc. B Biol. Sci.* **270**: 2457–2463.

Franks, N.R. & Richardson, T. 2006. Teaching in tandem-running ants. *Nature* **439**: 153.

Franks, N.R., Richardson, T.O., Keir, S., Inge, S.J., Bartumeus, F. & Sendova-Franks, A.B. 2010. Ant search strategies after interrupted tandem runs. *J. Exp. Biol.* **213**: 1697–1708.

Freeman, R.B. & Huang, W. 2015. Collaborating with people like me: Ethnic coauthorship within the United States. *J. Labor Econ.* **33**: S289–S318.

Fresneau, D. 1985. Individual foraging and path fidelity in a ponerine ant. *Insectes Soc.* **32**: 109–116.

Galef, B. & Laland, K.N. 2005. Social learning in animals : Empirical studies and theoretical models. *Bioscience* **55**.

García-Pérez, J.A., Blanco-Piñón, A., Mercado-Hernández, R. & Badii, M. 1997. El comportamiento depredador de *Pachycondyla harpax* Fabr. sobre *Gnathamitermes tubiformans* Buckley en condiciones de cautiverio. *Southwest. Entomol.* **22**.

Glaser & Grüter, C. 2018. Ants (*Temnothorax nylanderi*) adjust tandem running when food source distance exposes them to greater risks. *Behav. Ecol. Sociobiol.* **72**: 1–8. Behavioral Ecology and Sociobiology.

Glaser, S.M., Feitosa, R.M., Koch, A., Goß, N., do Nascimento, F.S. & Grüter, C. 2021. Tandem communication improves ant foraging success in a highly competitive tropical habitat. *Insectes Soc.*, doi: 10.1007/s00040-021-00810-y. Springer International Publishing.

Goulson, D., Peat, J., Stout, J.C., Tucker, J., Darvill, B., Derwent, L.C., *et al.* 2002. Can alloethism in workers of the bumblebee, *Bombus terrestris*, be explained in terms of foraging efficiency? *Anim. Behav.* **64**: 123–130.

Goy, N., Glaser, S.M. & Grüter, C. 2021. The adaptive value of tandem communication in ants: Insights from an agent-based model. *J. Theor. Biol.* **526**: 110762. Elsevier Ltd.

Grevé, M.E., Houadria, M., Andersen, A.N. & Menzel, F. 2019. Niche differentiation in rainforest ant communities across three continents. *Ecolony Evol.* **9**: 8601–8615.

Grooters, H.J. 1987. Influences of queen piping and worker behaviour on the timing of emergence of honey bee queens. *Insectes Soc.* **34**: 181–193.

Grüter, C. 2020. *Stingless Bees*. Springer International Publishing.

Grüter, C., Balbuena, M.S. & Farina, W.M. 2008. Informational conflicts created by the waggle dance. *Proc. R. Soc. B Biol. Sci.* **275**.

Grüter, C. & Czaczkes, T.J. 2019. Communication in social insects and how it is shaped by individual experience. *Anim. Behav.* **151**: 207–215. Elsevier Ltd.

Grüter, C., Czaczkes, T.J. & Ratnieks, F.L.W. 2011. Decision making in ant foragers (*Lasius niger*) facing conflicting private and social information. *Behav. Ecol. Sociobiol.* **65**: 141–148.

Grüter, C. & Farina, W.M. 2009. The honeybee waggle dance: can we follow the steps ? *Cell Press* **24**: 242–247.

Grüter, C. & Leadbeater, E. 2014. Insights from insects about adaptive social information use. *Cell Press* **29**: 177–184.

Grüter, C., Leadbeater, E. & Ratnieks, F.L.W. 2010. Social learning: the importance of copying others. *Curr. Biol.* **20**: 683–685.

Grüter, C., Menezes, C., Imperatriz-Fonseca, V.L. & Ratnieks, F.L.W. 2012. A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proc. Natl. Acad. Sci. U. S. A.* **109**: 1182–1186.

Grüter, C. & Ratnieks, F.L.W. 2011. Honeybee foragers increase the use of waggle dance information when private information becomes unrewarding. *Anim. Behav.* **81**: 949–954.

Grüter, C., Segers, F.H.I.D., Menezes, C., Vollet-Neto, A., Falcón, T., von Zuben, L., *et al.* 2017a. Repeated evolution of soldier sub-castes suggests parasitism drives social complexity in stingless bees. *Nat. Commun.* **8**: 6–13.

Grüter, C., Segers, F.H.I.D. & Ratnieks, F.L.W. 2013. Social learning strategies in honeybee foragers: do the costs of using private information affect the use of social information ? *Anim. Behav.* **85**: 1443–1449. Elsevier Ltd.

Grüter, C., Segers, F.H.I.D., Santos, L.L.G., Hammel, B., Zimmermann, U. & Nascimento, F.S. 2017b. Enemy recognition is linked to soldier size in a polymorphic stingless bee. *Biol. Lett.* **13**.

Grüter, C., Wüst, M., Cipriano, A.P. & Nascimento, F.S. 2018. Tandem recruitment and foraging in the ponerine ant *Pachycondyla harpax* (Fabricius). *Neotrop. Entomol.* **47**: 742–749.

Harmon, L.J. 2019. *Phylogenetic Comparative Methods*.

Heinze, J., Foitzik, S., Hippert, A. & Hölldobler, B. 1996. Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. *Ethology* **102**: 510–522.

Herbers, J.M. & Cunningham, M. 1983. Social Organization in *Leptothorax longispinosus* Mayr. *Anim. Behav.* **31**: 759–771.

Heyes, C. 2012. What's social about social learning? *J. Comp. Physiol.* **126**: 193–202.

Hingston, R. 1929. *Instinct and intelligence*. Macmillan, New York.

Hölldobler, B. 1984. Communication During Foraging and Nest-Relocation in the African Stink Ant, *Paltothyreus tarsatus* Fabr. (Hymenoptera, Formicidae, Ponerinae). *Z. Tierpsychol.* **65**: 40–52.

Hölldobler, B. 1981. Foraging and spatiotemporal territories in the honey ant *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **9**: 301–314.

Hölldobler, B. 1999. Multimodal signals in ant communication. *J Comp Physiol A* **184**: 129–141.

Hölldobler, B. 1971. Recruitment behavior in *Camponotus socius* (Hym. Formicidae). *Z. Vgl. Physiol.* **75**: 123–142.

Hölldobler, B. 1976. Tournaments and slavery in a desert ant. *Science (80-.).* **192**: 912–914.

Hölldobler, B. & Engel, H. 1978. Tergal and sternal glands in ants. *Psyche (Stuttg).* **85**: 285–330.

Hölldobler, B., Möglich, M. & Maschwitz, U. 1974. Communication by tandem running in the ant *Camponotus sericeus*. *J. Comp. Physiol.* **90**: 105–127.

Hölldobler, B. & Traniello, J. 1980. Tandem running pheromone in ponerine ants. *Naturwissenschaften* **67**: 360.

Hölldobler, B. & Wilson, E.O. 1990. *The Ants*. Harvard University Press, Springer-Verlag Berlin Heidelberg.

Hölldobler, B. & Wilson, E.O. 2009. *The Superorganism*. W. W. Norton & Company.

Hoppitt, W. & Laland, K.N. 2013. *Social learning: An Introduction to Mechanisms, Methods, and Models*. Princeton University Press, Princeton, New Jersey.

Horner, V., Proctor, D., Bonnie, K.E., Whiten, A. & de Waal, F.B.M. 2010. Prestige affects cultural learning in chimpanzees. *PLoS One* **5**: e10625.

Horwitz, S.K. & Horwitz, I.B. 2007. The effects of team diversity on team outcomes: A meta-analytic review of team demography. *J. Manage.* **33**: 987–1015.

Houadria, M., Blüthgen, N., Salas-Lopez, A., Schmitt, M.-I., Arndt, J., Schneider, E., *et al.* 2016. The relation between circadian asynchrony, functional redundancy, and trophic performance in tropical ant communities. *Ecology* **97**: 225–235.

Houadria, M., Salas-Lopez, A., Orivel, J., Blüthgen, N. & Menzel, F. 2015. Dietary and temporal niche differentiation in tropical ants - can they explain local ant coexistence? *Biotropica* **47**: 208–217.

Hrncir, M. & Maia-Silva, C. 2013. On the diversity of foraging-related traits in stingless bees. In: *Pot-Honey: A Legacy of Stingless Bees*, pp. 1–654.

Human, K.G. & Gordon, D.M. 1996. Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia* **105**: 405–412.

I'Anson Price, R., Dulex, N., Vial, N., Vincent, C. & Grüter, C. 2019. Honeybees forage more successfully without the "dance language " in challenging environments. *Sci. Adv.* **5**.

I'Anson Price, R. & Grüter, C. 2015. Why, when and where did honey bee dance communication evolve? *Front. Ecol. Evol.* **3**: 1–7.

Jaffe, K. 1984. Negentropy and the evolution of chemical recruitment in ants (Hymenoptera: Formicidae). *J. Theor. Biol.* **106**: 587–604.

Jaffe, K. & Deneubourg, J.L. 1992. On foraging, recruitment systems and optimum number of scouts in eusocial colonies. *Insectes Soc.* **39**: 201–213.

Jandt, J.M. & Dornhaus, A. 2014. Bumblebee response thresholds and body size: Does worker diversity increase colony performance? *Anim. Behav.* **87**: 97–106. Elsevier Ltd.

Jarau, S. & Hrncir, M. 2009. *Food exploitation by social insects*. Taylor & Francis Group, LLC.

Jessen, K. & Maschwitz, U. 1986. Orientation and recruitment behavior in the ponerine ant *Pachycondyla tesserinoda* (Emery): laying of individual-specific trails during tandem running. *Behav. Ecol. Sociobiol.* **19**: 151–155.

Jones, J.C., Myerscough, M.R., Graham, S. & Oldroyd, B.P. 2004. Honey bee nest thermoregulation: Diversity promotes stability. *Science (80-.).* **305**: 402–404.

Kaspari, M. & Weiser, M.D. 2000. Ant activity along moisture gradients in a neotropical forest. *Biotropica* **32**: 703.

Kaur, R., Joseph, J., Anoop, K. & Sumana, A. 2017. Characterization of recruitment through tandem running in an Indian queenless ant *Diacamma indicum*. *R. Soc. Open Sci.* **4**: 160476.

Kendal, R.L., Coolen, I., Bergen, Y. Van & Laland, K.N. 2005. Trade-offs in the adaptive use of social and asocial learning. *Adv. Study Behav.* **35**: 333–379.

Kendal, R.L., Coolen, I. & Laland, K.N. 2004. The role of conformity in foraging when personal and social information conflict. *Behav. Ecol.* **15**: 269–277.

Keppner, E.M. & Jarau, S. 2016. Influence of climatic factors on the flight activity of the stingless bee *Partamona orizabaensis* and its competition behavior at food sources. *J. Comp. Physiol. A* **202**: 691–699. Springer Berlin Heidelberg.

Lach, L. 2005. Interference and exploitation competition of three nectar-thieving invasive ant species. *Insectes Soc.* **52**: 257–262.

Lach, L., Parr, C.L. & Abbott, K.L. 2010. *Ant ecology*. Oxford University Press Inc., New York.

Laland, K.N. 2004. Social learning strategies. *Anim. Learn. Behav.* **32**: 4–14.

Lanan, M.C. 2014. Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecological News* **20**: 53–70.

Langridge, E.A., Franks, N.R. & Sendova-Franks, A.B. 2004. Improvement in collective performance with experience in ants. *Behav. Ecol. Sociobiol.* **56**: 523–529.

Leadbeater, E. & Chittka, L. 2005. A new mode of information transfer in foraging bumblebees? *Curr. Biol.* **15**: 447–448.

Leadbeater, E. & Chittka, L. 2007. Social learning in insects - from miniature brains to consensus building. *Curr. Biol.* **17**: 703–713.

Levine, S.S., Apfelbaum, E.P., Bernard, M., Bartelt, V.L., Zajac, E.J. & Stark, D. 2014. Ethnic diversity deflates price bubbles. *Proc. Natl. Acad. Sci. U. S. A.* **111**: 18524–18529.

Liefke, C., Hölldobler, B. & Maschwitz, U. 2001. Recruitment behavior in the ant genus *Polyrhachis* (Hymenoptera, Formicidae). *J. Insect Behav.* **14**: 637–657.

Lynch, J.F., Johnson, A.K. & Balinsky, E.C. 1988. Spatial and temporal variation in the abundance and diversity of ants (Hymenoptera: Formicidae) in the soil and litter layers of a Maryland forest. *Am. Midl. Nat.* **119**: 31–44.

Mailleux, A.-C., Deneubourg, J.-L. & Detrain, C. 2003. Regulation of ants' foraging to resource productivity. *Proc. R. Soc. B Biol. Sci.* **270**: 1609–1616.

Mallon, E.B. & Franks, N.R. 2000. Ants estimate area using Buffon's needle. *Proc. R. Soc. B Biol. Sci.* **267**: 765–770.

Mallon, E.B., Pratt, S.C. & Franks, N.R. 2001. Individual and collective decision-making during nest site selection by the ant *Leptothorax albipennis*. *Behav. Ecol. Sociobiol.* **50**: 352–359.

Maschwitz, U., Hölldobler, B. & Möglich, M. 1974. Tandemlaufen als Rekrutierungsverhalten bei *Bothroponera tesserinoda* Forel (Formicidae: Ponerinae). *Z. Tierpsychol.* **35**: 113–123.

Maschwitz, U., Jessen, K. & Knecht, S. 1986. Tandem Recruitment and Trail Laying in the Ponerine Ant *Diacamma rugosum*: Signal Analysis. *Ethology* **71**: 30–41.

Maschwitz, U. & Schönegge, P. 1983. Forage communication, nest moving recruitment, and prey specialization in the oriental ponerine *Leptogenys chinensis*. *Oecologia* **57**: 175–182.

Matsuura, K., Kuno, E. & Nishida, T. 2002. Homosexual tandem running as selfish herd in *Reticulitermes speratus*: Novel antipredatory behavior in termites. *J. Theor. Biol.* **214**: 63–70.

Mattila, H.R. & Seeley, T.D. 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science (80-.).* **317**: 362–364.

Menzel, R., Kirbach, A., Haass, W.-D., Fischer, B., Fuchs, J., Koblofsky, M., *et al.* 2011. A common frame of reference for learned and communicated vectors in honeybee navigation. *Curr. Biol.* **21**: 645–650. Elsevier Ltd.

Mertl, A.L. & Traniello, J.F.A. 2009. Behavioral evolution in the major worker subcaste of twig-nesting *Pheidole* (Hymenoptera: Formicidae): does morphological specialization influence task plasticity? *Behav. Ecol. Sociobiol.* **63**: 1411–1426.

Modlmeier, A.P. & Foitzik, S. 2011. Productivity increases with variation in aggression among group members in *Temnothorax* ants. *Behav. Ecol.* **22**: 1026–1032.

Möglich, M., Maschwitz, U. & Hölldobler, B. 1974. Tandem calling: a new kind of signal in ant communication. *Science (80-.).* **186**: 1046–1047.

Molet, M., Péronnet, R., Couette, S., Canovas, C. & Doums, C. 2017. Effect of temperature and social environment on worker size in the ant *Temnothorax nylanderi*. *J. Therm. Biol.* **67**: 22–29. Elsevier Ltd.

Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B. & Pierce, N.E. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. *Science (80-.).* **312**: 101–104.

Nagamitsu, T. & Inoue, T. 1997. Aggressive foraging of social bees as a mechanism of floral resource partitioning in an Asian tropical rainforest. *Oecologia* **110**: 432–439.

Narendra, A., Alkaladi, A., Raderschall, A., Robson, S.K.A. & Ribi, W.A. 2013a. Compound eye adaptations for diurnal and nocturnal lifestyle in the intertidal ant, *Polyrhachis sokolova*. *PLoS One* **8**: 4–9.

Narendra, A., Kamhi, J.F. & Ogawa, Y. 2017. Moving in dim light: Behavioral and visual adaptations in nocturnal ants. *Integr. Comp. Biol.* **57**: 1104–1116.

Narendra, A., Reid, S.F. & Raderschall, C.A. 2013b. Navigational efficiency of nocturnal myrmecia ants suffers at low light levels. *PLoS One* **8**: 1–7.

Nascimento, F.S., Souza, D.I.S.A., Tannure-Nascimento, I.C. & Dantas, J.O. 2012. Social facilitation and food partitioning in the queenless ant *Dinoponera quadriceps* (Hymenoptera : Formicidae). *J. Nat. Hist.* **46**.

Ness, J.H., Bronstein, J.L., Andersen, A.N. & Holland, J.N. 2004. Ant body size predicts dispersal distance of ant-adapted seeds: Implications of small-ant invasions. *Ecology* **85**: 1244–1250.

Noland, M., Moran, T. & Kotschwar, B. 2016. Is Gender Diversity Profitable? Evidence from a Global Survey. *Work. Pap. Ser.*

O'Shea-Wheller, T.A., Sendova-Franks, A.B. & Franks, N.R. 2016. Migration control: a distance compensation strategy in ants. *Sci. Nat.* **103**: 66. The Science of Nature.

Ogle, D.H., Wheeler, P. & Dinno, A. 2020. FSA: Fisheries Stock Analysis.

Okada, Y., Plateaux, L. & Peeters, C. 2013. Morphological variability of intercastes in the ant *Temnothorax nylanderi*: pattern of trait expression and modularity. *Insectes Soc.* **60**: 319–328.

Oldroyd, B.P. & Fewell, J.H. 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* **22**: 408–413.

Oster, G.F. & Wilson, E.O. 1978. *Caste and ecology in the social insects*. Princeton University Press, Princeton, New Jersey.

Paradis, E. & Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**: 526–528.

Planqué, R., van den Berg, J.B. & Franks, N.R. 2010. Recruitment strategies and colony size in ants. *PLoS One* **5**: e11664.

Porter, S.D. & Tschinkel, W.R. 1985. Fire ant polymorphism: the ergonomics of brood production. *Behav. Ecol. Sociobiol.* **16**: 323–336.

Powell, S. 2016. A comparative perspective on the ecology of morphological diversification in complex societies: nesting ecology and soldier evolution in the turtle ants. *Behav. Ecol. Sociobiol.* **70**: 1075–1085. Behavioral Ecology and Sociobiology.

Powell, S. & Franks, N.R. 2005. Caste evolution and ecology: a special worker for novel prey. *Proc. R. Soc. B Biol. Sci.* **272**: 2173–2180.

Pratt, S.C. 2008. Efficiency and regulation of recruitment during colony emigration by the ant *Temnothorax curvispinosus*. *Behav. Ecol. Sociobiol.* **62**: 1369–1376.

Quinet, Y. & Pasteels, J.M. 1996. Spatial specialization of the foragers and foraging strategy in *Lasius fuliginosus* (Latreille) (Hymenoptera, Formicidae). *Insectes Soc.* **43**: 333–346.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reeves, D.D. & Moreau, C.S. 2019. The evolution of foraging behavior in ants (Hymenoptera: Formicidae). *Arthropod Syst. Phylogeny* **77**: 351–363.

Rendell, L., Boyd, R., Cownden, D., Enquist, M., Eriksson, K., Feldman, M.W., *et al.* 2010. Why copy others? Insights from the social learning strategies tournament. *Science (80-.).* **328**: 208–213.

Revell, L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**: 217–223.

Richardson, T.O., Sleeman, P. a., McNamara, J.M., Houston, A.I. & Franks, N.R. 2007. Teaching with evaluation in ants. *Curr. Biol.* **17**: 1520–1526.

Rieucau, G. & Giraldeau, L.A. 2011. Exploring the costs and benefits of social information use: an appraisal of current experimental evidence. *Philos. Trans. R. Soc. B Biol. Sci.* **366**: 949–957.

Rissing, S.W. 1987. Annual cycles in worker size of the seed-harvester ant *Veromessor pergandei* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **20**: 117–124.

Roces, F. 1990. Leaf-cutting ants cut fragment sizes in relation to the distance from the nest. *Anim. Behav.* **40**: 1181–1183.

Rosumek, F. 2017. Natural history of ants: What we (do not) know about trophic and temopral niches of neotropical species. *Sociobiology* **64**: 244–255.

Sasaki, T., Danczak, L., Thompson, B., Morshed, T. & Pratt, S.C. 2020. Route learning during tandem running in the rock ant *Temnothorax albipennis*. *J. Exp. Biol.* **223**.

Sasaki, T., Janssen, M.A., Shaffer, Z. & Pratt, S.C. 2016. Exploration of unpredictable environments by networked groups. *Curr. Zool.* **62**: 207–214.

Sasaki, T. & Pratt, S.C. 2013. Ants learn to rely on more informative attributes during decision-making. *Biol. Lett.* **9**.

Sauthier, R., I'Anson Price, R. & Grüter, C. 2017. Worker size in honeybees and its relationship with season and foraging distance. *Apidologie* **48**: 234–246.

Schilman, P.E., Lighton, J.R.B. & Holway, D.A. 2007. Water balance in the Argentine ant (*Linepithema humile*) compared with five common native ant species from southern California. *Physiol. En* **32**: 1–7.

Schmidt, C. 2013. Molecular phylogenetics of ponerine ants (Hymenoptera: Formicidae: Ponerinae). *Zootaxa* **3647**: 201–250.

Schultheiss, P., Raderschall, C. a & Narendra, A. 2015. Follower ants in a tandem pair are not always naïve. *Sci. Rep.* **5**: 10747. Nature Publishing Group.

Schürch, R. & Grüter, C. 2014. Dancing bees improve colony foraging success as long-term benefits outweigh short-term costs. *PLoS One* **9**: e104660.

Seeley, T.D. 1983. Division of labor between scouts and recruits in honeybee foraging. *Behav. Ecol. Sociobiol.* **12**: 253–259.

Seeley, T.D. 1986. Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behav. Ecol. Sociobiol.* **19**: 343–354.

Seeley, T.D. 1989. Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. *Behav. Ecol. Sociobiol.* **24**: 181–199.

Seeley, T.D. 1995. *The wisdom of the hive: The social physiology of honey bee colonies*. Harward University Press, Cambridge, Massachusetts.

Seeley, T.D. & Visscher, P.K. 1988. Assessing the benefits of cooperation in honeybee foraging: search costs, forage quality, and competitive ability. *Behav. Ecol. Sociobiol.* **22**: 229–237.

Segers, F.H.I.D., Menezes, C., Vollet-Neto, A., Lambert, D. & Grüter, C. 2015. Soldier production in a stingless bee depends on rearing location and nurse behaviour. *Behav. Ecol. Sociobiol.* **69**: 613–623.

Shaffer, Z., Sasaki, T. & Pratt, S.C. 2013. Linear recruitment leads to allocation and flexibility in collective foraging by ants. *Anim. Behav.* **86**: 967–975. Elsevier Ltd.

Sherman, G. & Visscher, P.K. 2002. Honeybee colonies achieve fitness through dancing. *Nature* **419**: 920–922.

Shettleworth, S.J. 2010. *Cognition, evolution, and behavior*. University Press Oxford.

Silvestre, R., Brandão, C.R.F. & Paiva, R.V.S. 1999. Ant guilds in the Brazilian "Cerrados." In: *Ant guilds in the brazilian "Cerrados."*

Slagsvold, T. & Wiebe, K.L. 2011. Social learning in birds and its role in shaping a foraging niche. *Philos. Trans. R. Soc. B Biol. Sci.* **366**: 969–977.

Smolla, M., Alem, S., Chittka, L. & Shultz, S. 2016. Copy-when-uncertain: bumblebees rely on social information when rewards are highly variable. *Biol. Lett.* **12**.

Smolla, M., Gilman, R.T., Galla, T. & Shultz, S. 2015. Competition for resources can explain patterns of social and individual learning in nature. *Proc. R. Soc. B Biol. Sci.* **282**.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry, Third edition*. W. H. Freeman and company, New York.

Spaethe, J., Brockmann, A., Halbig, C. & Tautz, J. 2007. Size determines antennal sensitivity and behavioral threshold to odors in bumblebee workers. *Naturwissenschaften* **94**: 733–739.

Spaethe, J. & Weidenmüller, A. 2002. Size variation and foraging rate in bumblebees (*Bombus terrestris*). *Insectes Soc.* **49**: 142–146.

Stroeymeyt, N., Joye, P. & Keller, L. 2017. Polydomy enhances foraging performance in ant colonies. *Proc. R. Soc. B Biol. Sci.* **284**.

Stuble, K.L., Rodriguez-Cabal, M.A., McCormick, G.L., Juric, I., Dunn, R.R. & Sanders, N.J. 2013. Tradeoffs, competition, and coexistence in eastern deciduous forest ant communities. *Oecologia* **171**: 981–992.

Stuttard, J.P., Gottlieb, D. & Franks, N.R. 2016. Ants incommunicado: collective decision-making over new nest sites by ants with reduced communication. *Behav.*

Ecol. Sociobiol. **70**: 145–155.

Sumner, S., Pereboom, J.J.M. & Jordan, W.C. 2006. Differential gene expression and phenotypic plasticity in behavioural castes of the primitively eusocial wasp, *Polistes canadensis*. *Proc. R. Soc. B Biol. Sci.* **273**: 19–26.

Sumpter, D.J.T. 2010. *Collective Animal Behavior*. Princeton University Press, Princeton, New Jersey.

Sumpter & Pratt, S.C. 2003. A modelling framework for understanding social insect foraging. *Behav. Ecol. Sociobiol.* **53**: 131–144.

Taylor, F. 1977. Foraging behavior of ants: Experiments with two species of myrmecine ants. *Behav. Ecol. Sociobiol.* **2**: 147–167.

Thornton, A. & McAuliffe, K. 2006. Teaching in wild meerkats. *Science (80-.).* **313**: 227–229.

Tian, L. & Zhou, X. 2014. The soldiers in societies: Defense, regulation, and evolution. *Int. J. Biol. Sci.* **10**: 296–308.

Torres, J.A. 1984. Niches and coexistence of ant communities in Puerto Rico: Repeated patterns. *Biotropica* **16**: 284–295.

Traniello, J.F.A. 1989a. Chemical trail systems, orientation, and territorial interactions in the ant *Lasius neoniger*. *J. Insect Behav.* **2**: 339–354.

Traniello, J.F.A. 1987. Comparative foraging ecology of north temperate ants: The role of worker size and cooperative foraging in prey selection. *Insectes Soc.* **34**: 118–130.

Traniello, J.F.A. 1989b. Foraging strategies of ants. *Annu. Rev. Entomol.* **34**: 191–210.

Traniello, J.F.A. & Hölldobler, B. 1984. Chemical communication during tandem running in *Pachyconcyla obscuricornis* (Hymenoptera: Formicidae). *J. Chem. Ecol.* **10**: 783–794.

van Knippenberg, D. & Schippers, M.C. 2007. Work group diversity. *Annu. Rev. Psychol.* **58**: 515–541.

Villet, M.H. 1990. Social organization of *Platythyrea lamellosa* (Roger) (Hymenoptera: Formicidae): II. Division of labour. *South African J. Zool.* **25**: 254–259.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W. & Hoffmann, K.H. 1999. Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**: 483–491.

von Frisch, K. 1946. Die Tänze der Bienen. *Ölsterreichische Zool. Zeitschrift* **1**: 1–48.

von Frisch, K. 1967. *The dance language and orientation of bees*. Harvard University Press, Cambridge, Massachusetts.

Waddington, K.D. 1989. Implications of variation in worker body size for the honey bee recruitment system. *J. Insect Behav.* **2**: 91–103.

Waddington, K.D., Herbst, L.H. & Roubik, D.W. 1986. Relationship between Recruitment Systems of Stingless Bees and Within-Nest Worker Size Variation. *J. Kansas Entomol. Soc.* **59**: 95–102.

Ward, P.S. 2014. The phylogeny and evolution of ants. *Annu. Rev. Ecol. Evol. Syst.* **45**: 23–43.

Westling, J.N., Harrington, K., Bengston, S. & Dornhaus, A. 2014. Morphological differences between extranidal and intranidal workers in the ant *Temnothorax rugatulus*, but no effect of body size on foraging distance. *Insectes Soc.* **61**: 367–369.

Wheeler, W.M. 1900. A study of some texan Ponerinae. *Biol. Bull.* **2**: 1–31.

Wilensky, U. 1999. NetLogo. Center for Connected Learning and Computer-Based Modeling. Northwestern University, Evanston, IL.

Wilensky, U. & Rand, B. 2015. *An introduction to agent-based modeling: Modeling natural, social and engineered complex systems with NetLogo*. The MIT Press Cambridge, Massachusetts London, England.

Wilson, E.O. 1962. Chemical communication among workers of the fire ant *Solenopsis saevissima* (Fr. Smith). *Anim. Behav.* **10**: 159–164.

Wilson, E.O. 1959. Communication by tandem running in the ant genus *Cardiocondyla*. *Psyche (Stuttg).* **66**: 29–34.

Wilson, E.O. 1971. *The insect societies*. Belknap Press of Harvard University Press.

Worden, B.D. & Papaj, D.R. 2005. Flower choice copying in bumblebees. *Biol. Lett.* **1**: 504–507.

Zuur, A., Ieno, E., Walker, N., Saveliev, A. & Smith, G. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

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