Individual and social mechanisms regulating the dance activity within honey bee forager groups

A Thesis

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by

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Declaration

This thesis is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions.
The work was done under the guidance of Dr. Axel Brockmann at the Tata Institute of Fundamental Research, Mumbai.

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In my capacity as supervisor of the candidate's thesis, I certify that the above statements are true to the best of my knowledge.

Dr. Axel Brockmann
National Centre for Biological Sciences – TIFR, Bangalore

Date: 11 October 2019

Certificate

I certify that this thesis entitled "**Individual and social mechanisms regulating the dance activity within honey bee forager groups**" comprises research work carried out by **Ebi Antony George** at National Centre for Biological Sciences under the supervision of **Dr. Axel Brockmann** during the period 2012-2019 for the degree of Doctor of Philosophy of the Tata Institute of Fundamental Research (TIFR). The results presented in this thesis have not been submitted previously to this or any other University for a PhD or any other degree.

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Publications

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George EA, Bröger A-K, Thamm M, Brockmann A, Scheiner R. 2019 Inter-individual variation in honey bee dance intensity correlates with expression of the *foraging* gene. *Genes, Brain and Behavior*. 1– 11. (doi:10.1111/gbb.12592)

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Charles Darwin rightly said that "it is the long history of humankind (and animal kind, too) that those who learned to collaborate and improvise most effectively have prevailed". These words highlight an interesting parallel between the paradigm I studied (the foraging activity of the honey bee colony) and my own experience during my PhD at the National Centre for Biological Sciences, Bangalore. In both cases, any successful endeavour depended on the interaction between a diverse group of individuals, each supporting the other. Even though it is my name on the first page of this thesis, I am part of a multitude of people who have all contributed academically and otherwise to this document. I will attempt to enumerate the contributions of all these individuals below, even though space constraints will limit how much I can expound on their wonderful qualities.

First and foremost, I am deeply indebted to Dr. Axel Brockmann, mentor par excellence. When I wanted to take my project in a direction that was not a focus of the lab, he was still supportive of my ideas. From finding conferences that I should attend to potential labs that I should join, Axel has always been encouraging of my career as a scientist. The frankness of his criticism and his guidance with respect to framing questions has been instrumental in shaping my thought process. I could not have asked for a better supervisor in my first major stint in academia.

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I

Introduction

Chapter 1 – Introduction

Social Groups

The phenomenon of individuals living in groups is prevalent across the animal kingdom (Niko Tinbergen, 1990). Social groups are defined as groups of individuals which maintain relative spatial proximity over time (A. Ward & Webster, 2016). The turnover of individuals forming the group as well as the degree of association amongst these individuals can vary from one species to another (A. Ward & Webster, 2016). Group structure and cohesiveness depends on the interactions between the individuals, with 'social attraction' keeping these groups together (Beauchamp, Belisle, & Giraldeau, 1997; Fletcher Jr., 2006; Michelena, Sibbald, Erhard, & McLeod, 2009). Social groups can vary in size; larger groups like flocks and herds in vertebrates often comprise of smaller sub-groups (Aureli et al., 2008; Ramos-Fernández, Boyer, & Gómez, 2006; Silk, Croft, Tregenza, & Bearhop, 2014).

One of the main benefits of group living is the access to information from group members about resources like food and mates (Duboscq, Romano, MacIntosh, & Sueur, 2016; Morand-Ferron, Doligez, Dall, & Reader, 2010; Valone & Templeton, 2002; P. Ward & Zahavi, 1973). Individuals in a group need to expend much less energy searching for resources than they would have had to if they were searching in isolation (Giraldeau & Beauchamp, 1999). Groups can also buffer individuals against inter-group and inter-species competition by helping to acquire and defend resources (Clark, W. C., & Mangel, 1986). Finally, living in groups can also have anti-predatory benefits like an increase in the likelihood to detect predators and a decrease in the likelihood to be targeted by them (Treherne & Foster, 1980; A. J. W. Ward, Herbert-Read, Sumpter, & Krause, 2011; Wrona & Dixon, 1991).

Intra-group competition for resources is one of the main costs experienced by group living individuals. The larger the group size, the lower the amount of resources each individual can obtain from a limited source (Pulliam & Caraco, 1984; Wrangham, Gittleman, & Chapman, 1993). This in turn might lead to aggression within individuals in the group and further costs associated with it (Dubois, Giraldeau, & Grant, 2003; C. A. Johnson, Grant, & Giraldeau, 2004). In groups with strong dominance hierarchies, subordinate individuals face costs related to limited opportunities for reproduction (Kleshchev & Osadchuk, 2014; von Rueden & Jaeggi, 2016). Further, larger or more dense groups may be more likely to be targeted by predators, thereby reducing some of the benefits associated with group living in species which are at a lower trophic-level (Curley, Rowley, & Speed, 2015; Fedigan, 1983; N. Tinbergen, Impekoven, & Franck, 1967). An increased likelihood of exposure to pathogens is another cost of group living (Loehle, 1995; Nunn, Jordan, McCabe, Verdolin, & Fewell, 2015).

Group living animals often engage in collective decision making, in which individuals have to incorporate environmental and social cues to make behavioural decisions (Sumpter, 2010). Collective behaviours are an emergent property of the group and arise from very local interactions between the individuals (Giardina, 2008). For example, in groups of starlings flying together, each individual interacts with only a few individuals in the space around them (Ballerini et al., 2008; Czirók, Vicsek, & Vicsek, 1999). These interactions are sufficient to produce cohesion within a group and lead to their synchronous large-scale flocking behaviour (Cavagna et al., 2015, 2014). By synchronising their behaviours, individuals remain part of the group, and thus collective behaviour is intricately associated with the benefits and costs of living in groups.

Eusociality

Eusocial groups represent one of the most elaborate forms of group living and are characterised by reproductive division of labour, the presence of multiple generations within the group, cooperative care of younger members of the group and a point of no-return to the solitary state as a consequence of anatomical differentiation of the non-reproductive members (Crespi & Yanega, 1994; E O Wilson, 1971; Edward O. Wilson & Hölldobler, 2005). Compared to most social groups, eusocial groups are made up of closely related individuals who are spatially together throughout their lives with very little turnover of group members (Hölldobler & Wilson, 2009; Jarvis, 1981). Colonies in eusocial species are typically made up of a large number of individuals most of whom are sterile. These workers show a further division of labour and partition all the tasks necessary for the maintenance of the colony amongst themselves (Hölldobler & Wilson, 2009; O'Riain, Jarvis, Alexander, Buffenstein, & Peeters, 2000).

The advantages of eusociality is reflected in the large biomass of eusocial groups amongst insect species, even though they only make up a tiny fraction of the total number of insect species (Edward O. Wilson & Hölldobler, 2005). Rapid and efficient foraging as well as defensive capabilities against competitors based on disposable workers allow these groups to thrive in a wide variety of environments (Hölldobler & Wilson, 2009). The main cost associated with eusocial groups can be hypothesised to be the large number of social cues and signals necessary to effectively organise the different worker groups (Leonhardt, Menzel, Nehring, & Schmitt, 2016; Seeley, 1998; Wittwer et al., 2017). In such groups, collective decision making, and associated behaviours, directly benefit the colony rather than the individual (Hölldobler & Wilson, 2009; Sober & Wilson, 2011; Edward O. Wilson & Hölldobler, 2005).

Social organisation in Honey bees

Eusocial honey bee colonies are made up of workers which show an age-dependent non-reproductive division of labour (Robinson, 1992; Seeley, 1982). Younger workers are engaged in tasks within the hive, like brood care and comb construction (Hepburn, 2007; Robinson, 1992), while older workers are engaged in tasks outside the hive, like guarding and foraging (A. J. Moore, Breed, & Moor, 1987; Tenczar, Lutz, Rao, Goldenfeld, & Robinson, 2014; Visscher & Seeley, 1982). This division of labour has been extensively studied in *Apis mellifera*, and is regulated by strong physiological changes in the workers which in turn changes their behavioural responses to the various stimuli they experience (D. Moore, Angel, Cheeseman, Fahrbach, & Robinson, 1998; Robinson, Grozinger, & Whitfield, 2005; Robinson & Vargo, 1997). There is a certain degree of flexibility in the division of labour, with individuals able to rapidly change their work profile (from in-hive tasks to foraging and vice-versa) in response to abrupt changes in the colony's need (Z.-Y. Huang & Robinson, 1996; Z. Huang & Robinson, 1992; B. R. Johnson, 2010a).

Task allocation is a self-organised process in these colonies, with positive and negative feedback between different worker groups acting on age polyethism (B. R. Johnson, 2010a, 2010b; Seeley, 1982). Since the various stimuli experienced by an individual worker is always a subset of the stimuli that the colony needs to respond to, workers have to incorporate information from the cues and signals produced by other individuals into their behavioural responses (Grüter & Keller, 2016; Kocher & Cocroft, 2019). Cues refer to information obtained by individuals from either the environment or as a by-product of the action of other individuals in the group and are under no natural selection (Bradbury & Vehrencamp, 2011). Signals refer to mechanisms of information transfer between two individuals that are under natural selection (Seeley, 1998). Signals would therefore be costly, but contain relatively more appropriate information than cues, which may be more prevalent but context dependent. Both cues and signals can change the workers stimulus threshold for task initiation, the probability of task performance and also the intensity of the task performance (Beshers & Fewell, 2001; Beshers, Huang, Oono, & Robinson, 2001; Naug & Gadagkar, 1999; Weidenmüller, 2004). The division of labour in eusocial insect colonies is based on the interplay between individual response thresholds, social cues and signals and environmental conditions (Beshers & Fewell, 2001).

Foraging and Recruitment

Honey bee colonies need to gather a large amount of pollen and nectar on a daily basis, and hence foragers form a large proportion of the workers in the colony (Khoury, Barron, & Myerscough, 2013; Seeley, 1995; C Thom, Seeley, & Tautz, 2000). Foragers can be divided into scouts and recruits similar to producers and scroungers in other social groups (Aplin & Morand-Ferron, 2017; Page, Sweeney, Pilko, & Pinter-Wollman, 2018; Seeley, 1983). Scouts are explorers and show low food site fidelity towards the novel food sources they discover (Liang et al., 2012). Recruits are exploiters and show high food site fidelity, continuously foraging at a known food site till it becomes less rewarding (von Frisch, 1967). An appropriate proportion of scouts and recruits help the colony to efficiently explore and exploit food sources available to it and flexibly respond to changing food conditions (Anderson, 2001; Mosqueiro et al., 2017).

Scouts as well as previously recruited foragers returning from a rewarding food source recruit other nest mates in the hive to this food source using the waggle dance, one of the most unique form of communication amongst invertebrates (T. Sumpter & Pratt, 2003; von Frisch, 1967). The waggle dance increases the motivation of nest mates to forage and includes the spatial location of the food source (Dyer, 2002; C Grüter & Farina, 2009). A waggle dance consists of multiple dance circuits, each of which consists of a waggle phase and a return phase. The waggle phase, which encodes spatial information, is made up of a straight path during which the dancer shakes its abdomen back and forth (Gardner, Seeley, & Calderone, 2008; von Frisch, 1967). The return phase is made up of a semi-circular path which brings the dancer close to the point where it initiated the waggle phase and encodes information about the perceived profitability of the food source (Seeley, Mikheyev, & Pagano, 2000).

The waggle dance acts as the main regulatory mechanism for the spatial and temporal distribution of the foraging activity of a honey bee colony. A foragers decision to recruit to a food source is based on not only the reward value of the food source but also on interactions with nest mates like receivers, who unload the nectar load brought in by foragers from different food sources (Anderson & Ratnieks, 1999; Seeley, 1989). Foragers, with limited knowledge about environmental and colony conditions, can use information from cues and signals associated with these interactions to ascertain the relative reward value of the food source they are exploiting for the colony and decide to recruit accordingly (W M Farina, 2000; Hart & Ratnieks, 2001; Seeley, 1986, 1989; Visscher & Seeley, 1982). The specific signal or cue involved could be the waiting time before unloading nectar, the time duration of trophallactic interactions with receivers, the number of trophallactic interactions and antennation with receivers or a combination thereof, although conclusive evidence for these are still lacking (De Marco, 2006; De Marco & Farina, 2001; Walter M Farina, 1996; Rivera, Donaldson-Matasci, & Dornhaus, 2015; Seeley, 1989).

Negative feedback also plays an important role in the regulation of foraging in honey bee colonies. This feedback involves the use of the stop signal in which foragers (or guard bees) continuously butt their heads against a target forager. This signal is produced in response to both overcrowded food sources (which would provide only diminishing returns with further recruitment) and cues and signals associated with the presence of predators (Lau & Nieh, 2010; Tan et al., 2016, 2013; C. Thom, 2003; Corinna Thom, Gilley, & Tautz, 2003). Foragers which have experienced either of these two contexts target other foragers dancing for the same food source (possibly using odour cues associated with the food source), thereby reducing their motivation to dance (D. Moore, Siegfried, Wilson, & Rankin, 1989; Nieh, 1993, 2010; Pastor & Seeley, 2005). Similarly, guards who have experienced cues and signals associated with predators at the colony entrance can inhibit the motivation of foragers to recruit and forage using the stop signal (Tan et al., 2016). Thus, the stop signal provides honey bee colonies with an effective mechanism to optimise recruitment and rapidly reduce the risk associated with foraging.

The intricate regulation of foraging activity leads to the presence of multiple forager groups in the colony, active at different food sources (Kirchner & Grasser, 1998; Kirchner & Lindauer, 1994; D. Moore & Doherty, 2009; Mujagic & Erber, 2009; Seeley, 1986, 1995; Shah, Jain, & Brockmann, 2018; Van Nest, Otto, & Moore, 2018). In contrast to the eusocial colony, forager groups lie at the other end of the social group spectrum and comprise of individuals who transiently occupy the same space and who have relatively weak associations with each other. Forager group sizes are proportionate to the relative reward value of the food source for the colony and can change rapidly based on changing food conditions (Seeley, 1986, 1995). Individual foragers show floral constancy and keep visiting the food source (till it gets less rewarding) leading to the formation of time memories associated with the food source (D. Moore & Doherty, 2009; Wagner, Van Nest, Hobbs, & Moore, 2013). On subsequent days, some of the foragers from the group fly out to this food source before the source becomes rewarding to inspect the source (Biesmeijer & Vries, 2001; Granovskiy, Latty, Duncan, Sumpter, & Beekman, 2012). Others in the forager group follow dances by these inspectors to get activated at the appropriate foraging time (D. Moore et al., 1989; Van Nest et al., 2018). The active forager group can be viewed as a recruitment hub for the particular food source, changing their foraging and recruitment activity in response to changing environmental conditions (Seeley, 1995). Interestingly, very little is known about the role of inter-individual variation within these forager groups, and the effect this variation might have on the functioning of the group (Seeley, 1994; Tenczar et al., 2014).

Consistent inter-individual differences

Individuals within groups vary in their responses to the same stimuli (Dall, Bell, Bolnick, & Ratnieks, 2012; Stamps, 2015). This variation can be due to simple stochastic differences between the response of similar individuals or can represent consistent inter-individual variation based on intrinsic differences in behavioural states. Consistent inter-individual differences in behaviour have been shown in a wide variety of behaviours and taxa (Bell, Hankison, & Laskowski, 2009; Wolf & Weissing, 2012) ranging from aggression in house crickets (Brown, Smith, Moskalik, & Gabriel, 2006) to nest construction in three spined-sticklebacks (Rushbrook, Dingemanse, & Barber, 2008) and consolation behaviour in chimpanzees (Webb, Romero, Franks, & De Waal, 2017). Individuals may also show correlated consistent differences in behavioural responses across contexts, a phenomenon referred to as behavioural syndromes or “animal personalities” (Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, & Ziemba, 2004).

Inter-individual variation in behaviour can be caused by a combination of genetic and epigenetic factors (Bult & Lynch, 2000; Dall et al., 2012; Dingemanse, Both, Drent, Van Oers, & Van Noordwijk, 2002; Van Oers, De Jong, Drent, & Van Noordwijk, 2004). Differences in the environmental stimuli, the pre-imaginal environment that an individual is exposed to and the social context can further contribute to inter-individual variation through epigenetic effects (Arenas & Farina, 2008; Daugherty, Toth, & Robinson, 2011; Krause, James, & Croft, 2010; Webster & Ward, 2011). Inter-individual variation in a population, and even in smaller groups can have far ranging consequences (Wolf & Weissing, 2012). It can lead to behavioural specialisation, affect various life history traits and population productivity and also lead to speciation (Biro & Stamps, 2008, 2010; Dall et al., 2012; Duckworth, 2008). In eusocial insects this variation is essential to the division of labour in the colony (Jeanson, Fewell, Gorelick, & Bertram, 2007; Tarapore, Floreano, & Keller, 2010).

Group responses to perturbations

Social groups can differ in their structure and in the dynamics of interactions amongst individuals in the group (Camazine et al., 2001; Fewell, 2003). In non-eusocial social groups, selection mainly acts at the individual level and hence individuals tend to maximise their own fitness (Dall et al., 2012; Sober & Wilson, 2011). In contrast, selection mostly acts at the colony level in eusocial species', and not at the individual level (Dall et al., 2012; Sober & Wilson, 2011; Waters & Fewell, 2012; Edward O. Wilson & Hölldobler, 2005). Individual behavioural decisions are hypothesised to maximise group benefits, and this can lead to different network of interactions between members in the two kinds of groups (Waters & Fewell, 2012). The number of social cues and signals present within eusocial groups can also be expected to be higher due to the selection at the colony level (Christoph Grüter & Keller, 2016; Kocher & Cocroft, 2019; Morand-Ferron et al., 2010; Seeley, 1998). Social cues and signals likely play a stronger role in regulating and maintaining inter-individual variation and division of labour in eusocial groups (Beshers & Fewell, 2001; Jeanson & Weidenmüller, 2013).

Irrespective of their structure and dynamics, all social groups must be flexible in responding to environmental and social perturbations (D. J. T. Sumpter, 2010). In social groups, network dynamics is highly dependent on the presence of predators. Individuals in high-risk groups showed stronger associations, which in turn influenced their behavioural responses (Hasenjager & Dugatkin, 2017; Kelley, Morrell, Inskip, Krause, & Croft, 2011; Micheletta et al., 2012). Similarly, changing food availability and gain or loss of members changed the dynamics of the social network, and sometimes even lead to instabilities in the network (Cantor et al., 2012; Formica, Wood, Cook, & Brodie III, 2016; Foster et al., 2012; Goldenberg, Douglas-Hamilton, & Wittemyer, 2016; Ilany, Barocas, Koren, Kam, & Geffen, 2013; Ilany, Booms, & Holekamp, 2015; Lusseau et al., 2003; Tanner & Jackson, 2012; Williams & Lusseau, 2006). Division of labour in eusocial insects relies on replacing lost individuals to maintain the workforce necessary to perform the various tasks in the colony (Charbonneau, Sasaki, & Dornhaus, 2017; O'Donnell, 1998; Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012). Loss of individuals within task groups can be offset either by individuals already involved in the task increasing their performance further (Gardner, Foster, & O'Donnell, 2007; Pendrel & Plowright, 1981; Pinter-Wollman et al., 2012) or by new individuals joining the task group (Breed, Williams, & Queral, 2002; O'Donnell, 1998).

Contemporary studies

Inter-individual behavioural differences affect the responses of populations and groups to perturbations (Flack, Girvan, De Waal, & Krakauer, 2006; Krause et al., 2010; McCann, 2000; Pike, Samanta, Lindström, & Royle, 2008; Noa Pinter-Wollman et al., 2012; Schindler et al., 2010; Weidenmüller, 2004; Wolf & Krause, 2014). Thus, it is likely that the social context has a strong effect on maintaining consistent inter-individual variation in behavioural responses (Dall et al., 2012; Jeanson & Weidenmüller, 2013; Krause et al., 2010). Consistency in individual differences can lead to predictable group level responses. However, flexibility in individual responses is necessary to enable the group to adapt to changing conditions. Social groups must therefore trade-off consistency and flexibility of individual responses. This trade-off can either be through a change in activity of the individuals already responding to the stimulus or through a change in the members of the group who are responding to the stimulus. Understanding how individual consistency is regulated by the social environment is thus essential to comprehend how these groups would respond to perturbations.

Unfortunately, most studies on behavioural syndromes in group living animals have either neglected the social context completely or limited it to the presence of a single conspecific (Krause et al., 2010). Bringing together the separate fields of animal personalities and social networks is difficult due to experimental constraints. These experiments would involve multiple observations of identified individuals over long time periods followed by carefully controlled manipulative experiments and complex analysis to tease apart changes in the dynamics of social networks (Andersson et al., 2017; Arganda, Hinz, de Polavieja, Pérez-Escudero, & Vicente-Page, 2014; Crall, Gravish, Mountcastle, & Combes, 2015; Farine & Whitehead, 2015; N. Pinter-Wollman et al., 2013; Sih, Hanser, & McHugh, 2009; Sridhar, Roche, & Gingins, 2019; Wild et al., 2018).

In parallel to the work in this thesis, there has been an increase in the number of studies that focussed on the role of consistent individual differences in forming and maintaining social groups (L. M. Aplin et al., 2013; Crall et al., 2018; Firth et al., 2017; Formica et al., 2016; Garrison, Kleineidam, & Weidenmüller, 2018; Rudin, Tomkins, & Simmons, 2018). In great tits (Parus major) a strong link between personalities of the individuals and their positions in the social network was demonstrated. More exploratory individuals associated with more conspecifics but in a more transient manner as compared to less exploratory individuals who associated with fewer conspecifics over longer time periods (L. M. Aplin et al., 2013). A subsequent study identified consistent 'social phenotypes' of individuals like strength of associations with conspecifics, the interaction rate and group size choice (Lucy M. Aplin et al., 2015). Firth et al. (2017) built on these results by looking at how perturbations affect the individuals and the social network. On removing some individuals, flock mates compensated by increasing the number of associations and the strength of already existing associations. Those individuals which were closest to the removed individuals occupied their network positions and associated connections.

A similar interaction between consistent inter-individual differences and individual responses to perturbations was shown in bumblebees (*Bombus impatiens*). Crall *et al.* (2018) observed bumblebee colonies to identify how individuals respond to a loss of foragers in the colony. Foragers showed consistent differences in both their spatial distribution in the nest and their foraging activity. On the removal of active foragers, individuals which were more spatially central initiated foraging. There was a strong link between the spatial fidelity of individual workers and the amount of information (from nestmates, brood and waxpots) that they were exposed to. These asymmetric exposure to various relevant stimuli within the colony caused the differential response of individuals to perturbations.

Garrison *et al.* (2018) also studied the effect of social cues on consistency in bumblebees (*Bombus terrestris*). They focussed on the fanning behaviour of bumblebee workers in response to changing brood temperature in a solitary and a social context. Previous work had revealed consistent inter-individual variation in the response thresholds, probability and duration of fanning behaviour of workers

(Weidenmüller, 2004). Interestingly, there was a mismatch between the thresholds, probability and duration in the solitary and the social context, with individuals responding at a higher threshold with decreased probability and duration in the social context. A combination of individual differences and behavioural flexibility associated with the social context produced consistent group level responses to changing brood temperature in bumble bees.

Experiments in crickets (*Teleogryllus oceanicus*) showed varying effects of the social context and environmental stimuli on consistent inter-individual differences (Rudin et al., 2018). Individual crickets showed consistent inter-individual differences, with more dominant individuals being bolder, more active and more exploratory (Rudin, Tomkins, & Simmons, 2016). The presence of social information (in the form of acoustic conspecific signals) made individuals less bold and active. In contrast, an environmental disturbance (in the form of physical shaking of the containers housing the individuals) made individuals less exploratory but had no effect on their boldness or activity levels. The social context had a stronger effect on the consistency in individual behavioural responses in comparison to the environmental context.

Purpose of this thesis

In this thesis, I studied the consistency in inter-individual differences in the waggle dance activity of honey bees within a forager group. Behavioural studies on honey bee foragers have a long history, providing robust experimental techniques for long term (relative to the life of a honey bee worker) observations and controlled manipulations (Scheiner et al., 2013; von Frisch, 1967). The waggle dance behaviour integrates both social and environmental cues and plays a vital role in regulating the colonies foraging activity. Hence, it is an excellent behavioural paradigm to study the role of these different cues in regulating inter-individual variation in eusocial insect groups.

Each chapter in the thesis (except the Introduction and Conclusion) is structured in the form of a manuscript, with its own introduction and discussion. Further, after the discussion, a 'future directions' section describes possible follow up experiments that can expand on the results in the chapter. Chapters 2, 3 and A1 are already published as part of a single manuscript (George & Brockmann, 2019). Chapter 4 has been published as a separate manuscript and the entire manuscript is provided here (George, Bröger, Thamm, Brockmann, & Scheiner, 2019). A brief overview of the various chapters is provided below:

Chapter 2 – Consistency

The second chapter reports studies that quantify the consistency of inter-individual differences in the waggle dance activity of honey foragers from the same forager group. All the foragers were exposed to the same environmental conditions, and no manipulation of the social context was done. The results of repeated observations of the same individuals highlighted strong consistent inter-individual differences in all parameters of the waggle dance behaviour within forager groups. I end this chapter by discussing the role that consistent inter-individual differences can play in the regulation of foraging at the colony level.

Chapter 3 – Removal

In the third chapter, the manipulation experiments done to look at the effect of a change in the social environment on inter-individual differences in dance activity are described. The manipulation involved the removal of foragers from the forager group. The pre and post-removal dance activity of the remaining individuals were compared to quantify the change in activity. Changing the forager group size, and a likely change in associated social cues, caused individuals which were more active to change their dance activity, while other foragers in the group were unaffected. Further there was a greater increase in the dance probability, as compared to the dance intensity of individual foragers. I then discuss how the results of these manipulation experiments increase our knowledge of forager group (and the honey bee colony in general) responses to perturbations.

Chapter 4 – Thresholds

In the fourth chapter, the link between inter-individual variation in dance activity and in gustatory response thresholds is explored. Individuals were first observed foraging under free-flying conditions and their dance activity was quantified. Following this, the response thresholds of the individuals were quantified using the proboscis extension reflex assay. The results show a weak correlation between response thresholds and dance intensity, but not between thresholds and the other parameters of dance activity, including dance probability. This chapter also contains a description of collaborative work done to look at possible candidate genes that show a correlation with individual variation in the waggle dance activity.

Chapter 5 – Simulations

In the fifth chapter, an agent-based model is described which is used to explore the functional significance of dance probability and dance intensity in maintaining consistent inter-individual differences in recruitment. These models expand on an earlier model with a focus on inter-individual differences. The simulations revealed that dance intensity correlates more with consistent individual differences in recruitment than dance probability. I discuss how these results provide supporting evidence for the results in chapters 1, 2 and 3. I further point out the benefits of agent-based models to these behavioural studies by highlighting future versions of the model which can look at mechanisms and the adaptive benefits of inter-individual differences in the regulation of honey bee foraging.

Chapter A1 – Recruits

The first additional chapter describes experiments in which the forager group size was increased by allowing recruits to join the group. The results show that forager groups can drastically reduce their recruitment activity in a short duration in response to the presence of recruits. They further support results from chapter 2 on the social modulation of the waggle dance activity.

Chapter A2 – Followers

The second additional chapter describes a comparative analysis of the dance follower behaviour among 3 Asian *Apis* species, *A. florea*, *A. dorsata* and *A. cerana*. Comparative studies might provide a hint about the exact mechanism by which dance followers obtain the spatial location of the food source. The results point to interesting differences in the 3 species and are discussed in the context of the current hypotheses regarding mechanisms underlying spatial information transfer in the waggle dance.

References

Anderson, C. (2001). The adaptive value of inactive foragers and the scout-recruit system in honey bee (Apis mellifera) colonies. Behavioral Ecology, 12(1), 111–119.

Anderson, C., & Ratnieks, F. L. W. (1999). Worker allocation in insect societies: Coordination of nectar foragers and nectar receivers in honey bee (Apis mellifera) colonies. Behavioral Ecology and Sociobiology. https://doi.org/10.1007/s002650050595

Andersson, P. L., Rodriguez, A., Brodin, T., Zhang, H., Andersson, M., & Klaminder, J. (2017). ToxTrac : A fast and robust software for tracking organisms. Methods in Ecology and Evolution, 9(3), 460–464. https://doi.org/10.1111/2041-210x.12874

Aplin, L. M., Farine, D. R., Morand-Ferron, J., Cole, E. F., Cockburn, A., & Sheldon, B. C. (2013). Individual personalities predict social behaviour in wild networks of great tits (Parus major). Ecology Letters. https://doi.org/10.1111/ele.12181

Aplin, L M, & Morand-Ferron, J. (2017). Stable producer–scrounger dynamics in wild birds: Sociability and learning speed covary with scrounging behaviour. Proceedings of the Royal Society B: Biological Sciences, 284(1852). https://doi.org/10.1098/rspb.2016.2872

Aplin, Lucy M., Farine, D. R., Morand-Ferron, J., Cockburn, A., Thornton, A., & Sheldon, B. C. (2015). Experimentally induced innovations lead to persistent culture via conformity in wild birds. Nature, 518(7540), 538–541. https://doi.org/10.1038/nature13998

Arenas, A., & Farina, W. M. (2008). Age and rearing environment interact in the retention of early olfactory memories in honeybees. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 194(7), 629–640. https://doi.org/10.1007/s00359-008-0337-z

Arganda, S., Hinz, R. C., de Polavieja, G. G., Pérez-Escudero, A., & Vicente-Page, J. (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. Nature Methods, 11(7), 743–748. https://doi.org/10.1038/nmeth.2994

Aureli, F., Schaffner, C. M., Boesch, C., Bearder, S. K., Call, J., Chapman, C. A., ⋯ Schaik, C. P. van. (2008). Fission‐Fusion Dynamics. Current Anthropology, 49(4), 627–654. https://doi.org/10.1086/586708

Ballerini, M., Cabibbo, N., Candelier, R., Cavagna, A., Cisbani, E., Giardina, I., … Zdravkovic, V. (2008). Interaction ruling animal collective behavior depends on topological rather than metric distance: Evidence from a field study. Proceedings of the National Academy of Sciences, 105(4), 1232–1237. https://doi.org/10.1073/pnas.0711437105

Beauchamp, G., Belisle, M., & Giraldeau, L.-A. (1997). Influence of Conspecific Attraction on the Spatial Distribution of Learning Foragers in a Patchy Habitat. The Journal of Animal Ecology, 66(5), 671. https://doi.org/10.2307/5920

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. Animal Behaviour, 77(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. Annual Review of Entomology, 46, 413–440. https://doi.org/10.1146/annurev.ento.46.1.413

Beshers, S. N., Huang, Z. Y., Oono, Y., & Robinson, G. E. (2001). Social inhibition and the regulation of temporal polyethism in honey bees. Journal of Theoretical Biology, 213(3), 461–479. https://doi.org/10.1006/jtbi.2001.2427

Biesmeijer, J., & Vries, H. de. (2001). Exploration and exploitation of food sources by social insect colonies: a revision of the scout-recruit concept. Behavioral Ecology and Sociobiology, 49(2), 89–99. Retrieved from http://link.springer.com/article/10.1007/s002650000289

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? Trends in Ecology and Evolution, 23(7), 361–368. https://doi.org/10.1016/j.tree.2008.04.003

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends in Ecology and Evolution, 25(11), 653–659. https://doi.org/10.1016/j.tree.2010.08.003

Bradbury, J. W., & Vehrencamp, S. L. (2011). Principles of animal communication (2nd ed.). Sinauer Associates. Retrieved from https://global.oup.com/ushe/product/principles-of-animal-communication-9780878930456?q=Principles of animal communication&lang=en&cc=in

Breed, M. D., Williams, D. B., & Queral, A. (2002). Demand for task performance and workforce replacement: Undertakers in honeybee, Apis mellifera, colonies. Journal of Insect Behavior, 15(3), 319–329. https://doi.org/10.1023/A:1016261008322

Brown, W. D., Smith, A. T., Moskalik, B., & Gabriel, J. (2006). Aggressive contests in house crickets: size, motivation and the information content of aggressive songs. Animal Behaviour, 72(1), 225–233. https://doi.org/10.1016/j.anbehav.2006.01.012

Bult, A., & Lynch, C. B. (2000). Breaking through artificial selection limits of an adaptive behavior in mice and the consequences for correlated responses. Behavior Genetics, 30(3), 193–206. https://doi.org/10.1023/A:1001962124005

Camazine, S., Deneubourg, J.-L., Franks, N. R., Sneyd, J., Thereaulaz, G., & Bonabeau, E. (2001). Self-organization in biological systems. Princeton University Press. Retrieved from https://press.princeton.edu/titles/7104.html

Cantor, M., Wedekin, L. L., Guimarães, P. R., Daura-Jorge, F. G., Rossi-Santos, M. R., & Simões-Lopes, P. C. (2012). Disentangling social networks from spatiotemporal dynamics: the temporal structure of a dolphin society. Animal Behaviour, 84(3), 641–651. https://doi.org/10.1016/j.anbehav.2012.06.019

Cavagna, A., Del Castello, L., Dey, S., Giardina, I., Melillo, S., Parisi, L., & Viale, M. (2015). Short-range interactions versus long-range correlations in bird flocks. Physical Review E - Statistical, Nonlinear, and Soft Matter Physics, 92(1), 1–15. https://doi.org/10.1103/PhysRevE.92.012705

Cavagna, A., Del Castello, L., Giardina, I., Grigera, T., Jelic, A., Melillo, S., … Walczak, A. M. (2014). Flocking and Turning: a New Model for Self-organized Collective Motion. Journal of Statistical Physics, 158(3), 601–627. https://doi.org/10.1007/s10955-014-1119-3

Charbonneau, D., Sasaki, T., & Dornhaus, A. (2017). Who needs 'lazy' workers? Inactive workers act as a 'reserve' labor force replacing active workers, but inactive workers are not replaced when they are removed. PLoS ONE, 12(9), 1–20. https://doi.org/10.1371/journal.pone.0184074

Clark, W. C., & Mangel, M. (1986). The evolutionary avdvantages of group foraging. Theoretical Population Biology, 31(1), 45–75.

Crall, J. D., Gravish, N., Mountcastle, A. M., & Combes, S. A. (2015). BEEtag: A low-cost, image-based tracking system for the study of animal behavior and locomotion. PLoS ONE, 10(9), 1–13. https://doi.org/10.1371/journal.pone.0136487

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Spatial fidelity of workers predicts collective response to disturbance in a social insect. Nature Communications, 9(1), 1–13. https://doi.org/10.1038/s41467-018-03561-w

Crespi, B. J., & Yanega, D. (1994). The definition of eusociality. Behavioral Ecology, 6(1), 109–115.

Curley, E. A. M., Rowley, H. E., & Speed, M. P. (2015). A field demonstration of the costs and benefits of group living to edible and defended prey. Biology Letters, 11(6). https://doi.org/10.1098/rsbl.2015.0152

Czirók, A., Vicsek, M., & Vicsek, T. (1999). Collective motion of organisms in three dimensions. Physica

A: Statistical Mechanics and Its Applications, 264(1–2), 299–304. https://doi.org/10.1016/S0378-4371(98)00468-3

Dall, S. R. X., Bell, A. M., Bolnick, D. I., & Ratnieks, F. L. (2012). An evolutionary ecology of individual differences. Ecology Letters, 15(10), 1189–1198. https://doi.org/10.1111/j.1461-0248.2012.01846.x.An

Daugherty, T. H. F., Toth, A. L., & Robinson, G. E. (2011). Nutrition and division of labor: Effects on foraging and brain gene expression in the paper wasp Polistes metricus. Molecular Ecology, 20(24), 5337–5347. https://doi.org/10.1111/j.1365-294X.2011.05344.x

De Marco, R. J. (2006). How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' `eagerness'. Journal of Experimental Biology, 209(3), 421–432. https://doi.org/10.1242/jeb.02025

De Marco, R. J., & Farina, W. M. (2001). Changes in food source profitability affect the trophallactic and dance behavior of forager honeybees (Apis mellifera L.). Behavioral Ecology and Sociobiology, 50(5), 441–449. https://doi.org/10.1007/s002650100382

Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K., & Van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour, 64(6), 929–938. https://doi.org/10.1006/anbe.2002.2006

Dubois, F., Giraldeau, L., & Grant, J. W. A. (2003). Resource defense in a group-foraging context. Behavioral Ecology, 14(1), 2–9. https://doi.org/10.1093/beheco/14.1.2

Duboscq, J., Romano, V., MacIntosh, A., & Sueur, C. (2016). Social information transmission in animals: Lessons from studies of diffusion. Frontiers in Psychology. https://doi.org/10.3389/fpsyg.2016.01147

Duckworth, R. A. (2008). Adaptive Dispersal Strategies and the Dynamics of a Range Expansion. The American Naturalist, 172(S1), S4–S17. https://doi.org/10.1086/588289

Dyer, F. C. (2002). The biology of the dance language. Annual Review of Entomology, 47(1), 917–949. https://doi.org/10.1146/annurev.ento.47.091201.145306

Farina, W M. (2000). The interplay between dancing and trophallactic behavior in the honey bee Apis mellifera. Journal of Comparative Physiology - A, 186(3), 239–245. https://doi.org/10.1007/s003590050424

Farina, Walter M. (1996). Food-exchange by foragers in the hive – a means of communication among honey bees? Behavioral Ecology, 38, 59–64. https://doi.org/10.1007/s002650050217

Farine, D. R., & Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. Journal of Animal Ecology, 84(5), 1144–1163. https://doi.org/10.1111/1365-2656.12418

Fedigan, L. M. (1983). Dominance and reproductive success in primates. American Journal of Physical Anthropology, 26(1 S), 91–129. https://doi.org/10.1002/ajpa.1330260506

Fewell, J. H. (2003). Social Insect Networks. Science, 301(5639), 1515–1519. https://doi.org/10.1126/science.1088945

Firth, J. A., Voelkl, B., Crates, R. A., Aplin, L. M., Biro, D., Croft, D. P., & Sheldon, B. C. (2017). Wild birds respond to flockmate loss by increasing their social network associations to others. Proceedings. Biological Sciences, 284(1854), 20170299. https://doi.org/10.1098/rspb.2017.0299

Flack, J. C., Girvan, M., De Waal, F. B. M., & Krakauer, D. C. (2006). Policing stabilizes construction of social niches in primates. Nature, 439(7075), 426–429. https://doi.org/10.1038/nature04326

Fletcher Jr., R. J. (2006). Emergent Properties of Conspecific Attraction in Fragmented Landscapes. The American Naturalist, 168(2), 207–219. https://doi.org/10.2307/3844726

Formica, V., Wood, C., Cook, P., & Brodie III, E. (2016). Consistency of animal social networks after disturbance. Behavioral Ecology, 28(1), 85–93. https://doi.org/10.1093/beheco/arw128

Foster, E. A., Franks, D. W., Morrell, L. J., Balcomb, K. C., Parsons, K. M., van Ginneken, A., & Croft, D. P. (2012). Social network correlates of food availability in an endangered population of killer whales, Orcinus orca. Animal Behaviour, 83(3), 731–736. https://doi.org/10.1016/j.anbehav.2011.12.021

Gardner, K. E., Foster, R. L., & O'Donnell, S. (2007). Experimental analysis of worker division of labor in bumblebee nest thermoregulation (Bombus huntii, Hymenoptera: Apidae). Behavioral Ecology and Sociobiology, 61(5), 783–792. https://doi.org/10.1007/s00265-006-0309-7

Gardner, K. E., Seeley, T. D., & Calderone, N. W. (2008). Do honeybees have two discrete dances to advertise food sources? Animal Behaviour, 75(4), 1291–1300. https://doi.org/10.1016/j.anbehav.2007.09.032

Garrison, L. K., Kleineidam, C. J., & Weidenmüller, A. (2018). Behavioral flexibility promotes collective consistency in a social insect. Scientific Reports, 8(1), 1–11. https://doi.org/10.1038/s41598-018-33917-7

George, E. A., & Brockmann, A. (2019). Social modulation of individual differences in dance communication in honey bees. Behavioral Ecology and Sociobiology, 73(4), 41. https://doi.org/10.1007/s00265-019-2649-0

George, E. A., Bröger, A., Thamm, M., Brockmann, A., & Scheiner, R. (2019). Inter ‐ individual variation in honey bee dance intensity correlates with expression of the foraging gene. Genes, Brain and Behavior, (May), gbb.12592. https://doi.org/10.1111/gbb.12592

Giardina, I. (2008). Collective behavior in animal groups: Theoretical models and empirical studies. HFSP Journal, 2(4), 205–219. https://doi.org/10.2976/1.2961038

Giraldeau, L.-A., & Beauchamp, G. (1999). Food exploitation: searching for the optimal joining policy. Trends in Ecology & Evolution, 14(3), 102–106.

Goldenberg, S. Z., Douglas-Hamilton, I., & Wittemyer, G. (2016). Vertical Transmission of Social Roles Drives Resilience to Poaching in Elephant Networks. Current Biology, 26(1), 75–79. https://doi.org/10.1016/j.cub.2015.11.005

Granovskiy, B., Latty, T., Duncan, M., Sumpter, D. J. T., & Beekman, M. (2012). How dancing honey bees keep track of changes: the role of inspector bees. Behavioral Ecology, 23(3), 588–596. https://doi.org/10.1093/beheco/ars002

Grüter, C, & Farina, W. (2009). The honeybee waggle dance: can we follow the steps? Trends in Ecology & Evolution, (March). https://doi.org/10.1016/j.tree.2008.12.007

Grüter, Christoph, & Keller, L. (2016). Inter-caste communication in social insects. Current Opinion in Neurobiology, 38, 6–11. https://doi.org/10.1016/j.conb.2016.01.002

Hart, A. G., & Ratnieks, F. L. W. (2001). Why Do Honey-Bee (Apis mellifera) Foragers Transfer Nectar to Several Receivers ? Information Improvement through Multiple Sampling in a Biological System Author (s): Adam G . Hart and Francis L . W . Ratnieks Published by : Springer Stable URL : Behavioral Ecology and Sociobiology, 49(4), 244–250. Retrieved from https://www.jstor.org/stable/4601883

Hasenjager, M. J., & Dugatkin, L. A. (2017). Fear of predation shapes social network structure and the acquisition of foraging information in guppy shoals. Proceedings of the Royal Society B, 284(1867). https://doi.org/10.1098/rspb.2017.2020

Hepburn, H. R. (2007). Reciprocal interactions between honeybees and combs in the integration of some colony functions in Apis mellifera L. Apidologie, 29(1–2), 47–66. https://doi.org/10.1051/apido:19980103

Hölldobler, B., & Wilson, E. O. (2009). The superorganism. W W Norton (Vol. 456). W.W. Norton &

Company, Inc. https://doi.org/10.1038/456320a

Huang, Z.-Y., & Robinson, G. E. (1996). Regulation of honey bee division of labor by colony age demography. Behavioral Ecology and Sociobiology, 39(3), 147–158. https://doi.org/https://www.jstor.org/stable/4601247

Huang, Z., & Robinson, G. E. (1992). Honeybee colony integration Worker-worker interactions mediate hormonally regulated plasticity in division of labour. Proceedings of the National Academy of Sciences, 89(December), 11726–11729.

Ilany, A., Barocas, A., Koren, L., Kam, M., & Geffen, E. (2013). Structural balance in the social networks of a wild mammal. Animal Behaviour, 85(6), 1397–1405. https://doi.org/10.1016/j.anbehav.2013.03.032

Ilany, A., Booms, A. S., & Holekamp, K. E. (2015). Topological effects of network structure on long-term social network dynamics in a wild mammal. Ecology Letters, 18(7), 687–695. https://doi.org/10.1111/ele.12447

Jarvis, J. (1981). Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. Science, 212(4494), 571–573. https://doi.org/10.1126/science.7209555

Jeanson, R., Fewell, J. H., Gorelick, R., & Bertram, S. M. (2007). Emergence of increased division of labor as a function of group size. Behavioral Ecology and Sociobiology, 62(2), 289–298. https://doi.org/10.1007/s00265-007-0464-5

Jeanson, R., & Weidenmüller, A. (2013). Interindividual variability in social insects - proximate causes and ultimate consequences. Biological Reviews. https://doi.org/10.1111/brv.12074

Johnson, B. R. (2010a). Division of labor in honeybees: Form, function, and proximate mechanisms. Behavioral Ecology and Sociobiology, 64(3), 305–316. https://doi.org/10.1007/s00265-009-0874-7

Johnson, B. R. (2010b). Task partitioning in honey bees: The roles of signals and cues in group-level coordination of action. Behavioral Ecology, 21(6), 1373–1379. https://doi.org/10.1093/beheco/arq138

Johnson, C. A., Grant, J. W. A., & Giraldeau, L. A. (2004). The effect of patch size and competitor number on aggression among foraging house sparrows. Behavioral Ecology, 15(3), 412–418. https://doi.org/10.1093/beheco/arh026

Kelley, J. L., Morrell, L. J., Inskip, C., Krause, J., & Croft, D. P. (2011). Predation risk shapes social networks in fission-fusion populations. PLoS ONE, 6(8). https://doi.org/10.1371/journal.pone.0024280

Khoury, D. S., Barron, A. B., & Myerscough, M. R. (2013). Modelling Food and Population Dynamics in Honey Bee Colonies. PLoS ONE, 8(5). https://doi.org/10.1371/journal.pone.0059084

Kirchner, W. H., & Grasser, A. (1998). The Significance of Odor Cues and Dance Language Information for the Food Search Behavior of Honeybees (Hymenoptera : Apidae). Journal of Insect Behavior, 11(2), 169–178. https://doi.org/10.1023/A:1021098405564

Kirchner, W. H., & Lindauer, M. (1994). The causes of the tremble dance of the honeybee , Apis mellifera. Behavioral Ecology and Sociobiology, 35(5), 303–308. https://doi.org/https://doi.org/10.1007/BF00184419

Kleshchev, M. A., & Osadchuk, L. V. (2014). Social domination and reproductive success in male laboratory mice (Mus musculus). Journal of Evolutionary Biochemistry and Physiology, 50(3), 227–233. https://doi.org/10.1134/s0022093014030053

Kocher, S. D., & Cocroft, R. B. (2019). Signals in Insect Social Organization. Encyclopedia of Animal Behavior, 558–567. https://doi.org/10.1016/B978-0-12-809633-8.90781-7

Krause, J., James, R., & Croft, D. P. (2010). Personality in the context of social networks. Philosophical

Transactions of the Royal Society B: Biological Sciences, 365(1560), 4099–4106. https://doi.org/10.1098/rstb.2010.0216

Lau, C. W., & Nieh, J. C. (2010). Honey bee stop-signal production: temporal distribution and effect of feeder crowding. Apidologie, 41(1), 87–95. https://doi.org/10.1051/apido/2009052

Leonhardt, S. D., Menzel, F., Nehring, V., & Schmitt, T. (2016). Ecology and Evolution of Communication in Social Insects. Cell, 164(6), 1277–1287. https://doi.org/10.1016/j.cell.2016.01.035

Liang, Z. S., Nguyen, T., Mattila, H. R., Rodriguez-Zas, S. L., Seeley, T. D., & Robinson, G. E. (2012). Molecular determinants of scouting behavior in honey bees. Science, 335(6073), 1225–1228. https://doi.org/10.1126/science.1213962

Loehle, C. (1995). Social Barriers to Pathogen Transmission in Wild Animal Populations. Ecology, 76(2), 326–335. https://doi.org/10.2307/1941192

Lusseau, D., Schneider, K., Boisseau, O. J., Haase, P., Slooten, E., & Dawson, S. M. (2003). The bottlenose dolphin community of Doubtful Sound features a large proportion of long-lasting associations: Can geographic isolation explain this unique trait? Behavioral Ecology and Sociobiology, 54(4), 396–405. https://doi.org/10.1007/s00265-003-0651-y

McCann, K. S. (2000). The diversity-stability debate. Nature, 405(May), 228–233. https://doi.org/10.1038/35012234

Michelena, P., Sibbald, A. M., Erhard, H. W., & McLeod, J. E. (2009). Effects of group size and personality on social foraging: The distribution of sheep across patches. Behavioral Ecology, 20(1), 145–152. https://doi.org/10.1093/beheco/arn126

Micheletta, J., Waller, B. M., Panggur, M. R., Neumann, C., Duboscq, J., Agil, M., & Engelhardt, A. (2012). Social bonds affect anti-predator behaviour in a tolerant species of macaque, Macaca nigra. Proceedings of the Royal Society B, 279(1744), 4042–4050. https://doi.org/10.1098/rspb.2012.1470

Moore, A. J., Breed, M. D., & Moor, M. J. (1987). The guard honey bee: ontogeny and behavioural variability of workers performing a specialized task. Animal Behaviour, 35(4), 1159–1167. https://doi.org/10.1016/S0003-3472(87)80172-0

Moore, D., Angel, J. E., Cheeseman, I. M., Fahrbach, S. E., & Robinson, G. E. (1998). Timekeeping in the honey bee colony: Integration of circadian rhythms and division of labor. Behavioral Ecology and Sociobiology, 43(3), 147–160. https://doi.org/10.1007/s002650050476

Moore, D., & Doherty, P. (2009). Acquisition of a time-memory in forager honey bees. Journal of Comparative Physiology A, 195(8), 741–751. https://doi.org/10.1007/s00359-009-0450-7

Moore, D., Siegfried, D., Wilson, R., & Rankin, M. A. (1989). The Influence of Time of Day on the Foraging Behavior of the Honeybee, Apis mellifera. Journal of Biological Rhythms, 4(3), 305–325. https://doi.org/10.1177/074873048900400301

Morand-Ferron, J., Doligez, B., Dall, S. R. X., & Reader, S. M. (2010). Social Information Use. Encyclopedia of Animal Behavior, 394–402. https://doi.org/10.1016/B978-0-12-813251-7.00281-9

Mosqueiro, T., Cook, C., Huerta, R., Gadau, J., Smith, B., & Pinter-Wollman, N. (2017). Task allocation and site fidelity jointly influence foraging regulation in honeybee colonies. Royal Society Open Science, 4(8), 170344. https://doi.org/10.1098/rsos.170344

Mujagic, S., & Erber, J. (2009). Sucrose acceptance, discrimination and proboscis responses of honey bees (Apis mellifera L.) in the field and the laboratory. Journal of Comparative Physiology A, 195(4), 325–339. https://doi.org/10.1007/s00359-008-0409-0

Naug, D., & Gadagkar, R. (1999). Flexible Division of Labor Mediated by Social Interactions in an Insect Colony—a Simulation Model. Journal of Theoretical Biology, 197, 123–133.

https://doi.org/10.1006/jtbi.1998.0862

Nieh, J. C. (1993). The Stop Signal of Honey-bees - Reconsidering Its Message. Behavioral Ecology and Sociobiology, 33(1), 51–56. https://doi.org/10.1007/BF00164346

Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. Current Biology, 20(4), 310–315. https://doi.org/10.1016/j.cub.2009.12.060

Nunn, C. L., Jordan, F., McCabe, C. M., Verdolin, J. L., & Fewell, J. H. (2015). Infectious disease and group size: more than just a numbers game. Philosophical Transactions of the Royal Society B: Biological Sciences, 370(1669). https://doi.org/10.1098/rstb.2014.0111

O'Donnell, S. (1998). Effects of experimental forager removals on division of labour in the primitively eusocial wasp Polistes instabilis (Hymenoptera: Vespidae). Behaviour, 135(2), 173–193. https://doi.org/10.1163/156853998793066348

O'Riain, M. J., Jarvis, J. U. M., Alexander, R., Buffenstein, R., & Peeters, C. (2000). Morphological castes in a vertebrate. Proceedings of the National Academy of Sciences, 97(24), 13194–13197. https://doi.org/10.1073/pnas.97.24.13194

Page, H., Sweeney, A., Pilko, A., & Pinter-Wollman, N. (2018). Underlying mechanisms and ecological context of variation in exploratory behavior of the Argentine ant, Linepithema humile. Journal of Experimental Biology, 221(24), jeb188722. https://doi.org/10.1242/jeb.188722

Pastor, K. A., & Seeley, T. D. (2005). The brief piping signal of the honey bee: Begging call or stop signal? Ethology, 111(8), 775–784. https://doi.org/10.1111/j.1439-0310.2005.01116.x

Pendrel, B. A., & Plowright, R. C. (1981). Larval feeding by adult bumble bee workers (Hymenoptera: Apidae). Behavioral Ecology and Sociobiology, 8(2), 71–76. https://doi.org/10.1007/BF00300817

Pike, T. W., Samanta, M., Lindström, J., & Royle, N. J. (2008). Behavioural phenotype affects social interactions in an animal network. Proceedings of the Royal Society B, 275(1650), 2515–2520. https://doi.org/10.1098/rspb.2008.0744

Pinter-Wollman, N., Hobson, E. a., Smith, J. E., Edelman, a. J., Shizuka, D., de Silva, S., … McDonald, D. B. (2013). The dynamics of animal social networks: analytical, conceptual, and theoretical advances. Behavioral Ecology, 25(2), 242–255. https://doi.org/10.1093/beheco/art047

Pinter-Wollman, Noa, Hubler, J., Holley, J.-A., Franks, N. R., & Dornhaus, A. (2012). How is activity distributed among and within tasks in Temnothorax ants? Behavioral Ecology and Sociobiology, 66(10), 1407–1420. https://doi.org/10.1007/s00265-012-1396-2

Pulliam, H. R., & Caraco, T. (1984). Living in groups: is there an optimal group size? In Behavioural ecology: an evolutionary approach (pp. 122–147). Retrieved from http://www.science.fau.edu/biology/gawliklab/papers/PulliamHRandTCaracobook.pdf

Ramos-Fernández, G., Boyer, D., & Gómez, V. P. (2006). A complex social structure with fission-fusion properties can emerge from a simple foraging model. Behavioral Ecology and Sociobiology, 60(4), 536–549. https://doi.org/10.1007/s00265-006-0197-x

Rivera, M. D., Donaldson-Matasci, M. C., & Dornhaus, A. (2015). Quitting time: When do honey bee foragers decide to stop foraging on natural resources? Frontiers in Ecology and Evolution, 3(May), 1–9. https://doi.org/10.3389/fevo.2015.00050

Robinson, G. E. (1992). Regulation Of Division Of Labor In Insect Societies. Annual Review of Entomology, 37(1), 637–665. https://doi.org/10.1146/annurev.ento.37.1.637

Robinson, G. E., Grozinger, C. M., & Whitfield, C. W. (2005). Sociogenomics: Social life in molecular terms. Nature Reviews Genetics. https://doi.org/10.1038/nrg1575

Robinson, G. E., & Vargo, E. L. (1997). Juvenile hormone in adult eusocial hymenoptera: Gonadotropin and behavioral pacemaker. Archives of Insect Biochemistry and Physiology, 35(4), 559–583. https://doi.org/10.1002/(SICI)1520-6327(1997)35:4<559::AID-ARCH13>3.0.CO;2-9

Rudin, F. S., Tomkins, J. L., & Simmons, L. W. (2016). Changes in dominance status erode personality and behavioral syndromes. Behavioral Ecology, 28(1), 270–279. https://doi.org/10.1093/beheco/arw151

Rudin, F. S., Tomkins, J. L., & Simmons, L. W. (2018). The effects of the social environment and physical disturbance on personality traits. Animal Behaviour, In press, 109–121. https://doi.org/10.1016/j.anbehav.2018.02.013

Rushbrook, B. J., Dingemanse, N. J., & Barber, I. (2008). Repeatability in nest construction by male three-spined sticklebacks. Animal Behaviour, 75(2), 547–553. https://doi.org/10.1016/j.anbehav.2007.06.011

Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., … Thenius, R. (2013). Standard methods for behavioural studies of Apis mellifera. Journal of Apicultural Research, 52(4), 1–58. https://doi.org/10.3896/IBRA.1.52.4.04

Schindler, D. E., Hilborn, R., Chasco, B., Boatright, C. P., Quinn, T. P., Rogers, L. A., & Webster, M. S. (2010). Population diversity and the portfolio effect in an exploited species. Nature, 465(7298), 609–612. https://doi.org/10.1038/nature09060

Seeley, T. D. (1982). Adaptive Significance of the Age Polyethism Schedule in Honeybee Colonies. Behavioral Ecology and Sociobiology, 11, 287–293. https://doi.org/https://doi.org/10.1007/BF00299306

Seeley, T. D. (1983). Division of labor between scouts and recruits in honeybee foraging. Behavioral Ecology and Sociobiology, 12(3), 253–259. https://doi.org/10.1007/BF00290778

Seeley, T. D. (1986). Social foraging by honeybees: how colonies allocate foragers among patches of flowers. Behavioral Ecology and Sociobiology, 19(5), 343–354. https://doi.org/10.1007/BF00295707

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony’s nutritional status. Behavioral Ecology and Sociobiology, 24(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. Behavioral Ecology and Sociobiology, 34, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). The Wisdom of the Hive. Harvard University Press.

Seeley, T. D. (1998). Thoughts on information and integration in honey bee colonies. Apidologie, 29, 67–80. https://doi.org/10.1051/apido:19980104

Seeley, T. D., Mikheyev, A. S., & Pagano, G. J. (2000). Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology, 186(9), 813–819. https://doi.org/10.1007/s003590000134

Shah, A., Jain, R., & Brockmann, A. (2018). Egr-1: A candidate transcription factor involved in molecular processes underlying time-memory. Frontiers in Psychology, 9(JUN), 1–12. https://doi.org/10.3389/fpsyg.2018.00865

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. Trends in Ecology and Evolution, 19(7), 372–378. https://doi.org/10.1016/j.tree.2004.04.009

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Approach. The Quarterly Review of Biology, 79(3), 242–277. https://doi.org/10.1086/516403

Sih, A., Hanser, S. F., & McHugh, K. A. (2009). Social network theory: New insights and issues for behavioral ecologists. Behavioral Ecology and Sociobiology, 63(7), 975–988. https://doi.org/10.1007/s00265-009-0725-6

Silk, M. J., Croft, D. P., Tregenza, T., & Bearhop, S. (2014). The importance of fission–fusion social group dynamics in birds. International Journal of Avian Science, 156, 701–715. https://doi.org/10.1111/ibi.12191

Sober, E., & Wilson, D. S. (2011). Adaptation and Natural Selection revisited. Journal of Evolutionary Biology, 24(2), 462–468. https://doi.org/10.1111/j.1420-9101.2010.02162.x

Sridhar, V. H., Roche, D. G., & Gingins, S. (2019). Tracktor: image-based automated tracking of animal movement and behaviour. Methods in Ecology and Evolution, (January), 1–16. https://doi.org/10.1101/412262

Stamps, J. a. (2015). Individual differences in behavioural plasticities. Biological Reviews, 7, n/a-n/a. https://doi.org/10.1111/brv.12186

Sumpter, D. J. T. (2010). Collective Animal Behavior. Princeton University Press. Retrieved from https://press.princeton.edu/titles/9382.html

Sumpter, T., & Pratt, C. (2003). A Modelling Framework For Understanding social insect foraging. Behavioral Ecology and Sociobiology, 53(3), 131–144. https://doi.org/10.1007/s00265-002-0549-0

Tan, K., Dong, S., Li, X., Liu, X., Wang, C., Li, J., & Nieh, J. C. (2016). Honey Bee Inhibitory Signaling Is Tuned to Threat Severity and Can Act as a Colony Alarm Signal. PLOS Biology, 14(3), e1002423. https://doi.org/10.1371/journal.pbio.1002423

Tan, K., Hu, Z., Chen, W., Wang, Z., Wang, Y., & Nieh, J. C. (2013). Fearful foragers: honey bees tune colony and individual foraging to multi-predator presence and food quality. PloS One, 8(9), e75841. https://doi.org/10.1371/journal.pone.0075841

Tanner, C. J., & Jackson, A. L. (2012). Social structure emerges via the interaction between local ecology and individual behaviour. Journal of Animal Ecology, 81(1), 260–267. https://doi.org/10.1111/j.1365-2656.2011.01879.x

Tarapore, D., Floreano, D., & Keller, L. (2010). Task-dependent influence of genetic architecture and mating frequency on division of labour in social insect societies. Behavioral Ecology and Sociobiology, 64(4), 675–684. https://doi.org/10.1007/s00265-009-0885-4

Tenczar, P., Lutz, C. C., Rao, V. D., Goldenfeld, N., & Robinson, G. E. (2014). Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. Animal Behaviour, 95, 41–48. https://doi.org/10.1016/j.anbehav.2014.06.006

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. Journal of Experimental Biology, 206(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Thom, C, Seeley, T. D., & Tautz, J. (2000). A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. Apidologie, 31(6), 737–738. https://doi.org/10.1051/apido:2000158

Thom, Corinna, Gilley, D. C., & Tautz, J. (2003). Worker piping in honey bees (Apis mellifera): The behavior of piping nectar foragers. Behavioral Ecology and Sociobiology, 53(4), 199–205. https://doi.org/10.1007/s00265-002-0567-y

Tinbergen, N., Impekoven, M., & Franck, D. (1967). An Experiment On Spacing-Out as a Defence Against Predation. Behaviour, 28(3–4), 307–320. https://doi.org/10.1163/156853967X00064

Tinbergen, Niko. (1990). Social behaviour in animals, with special reference to vertebrates. Chapman and Hall.

Treherne, J. E., & Foster, W. A. (1980). The Effects of Group Size on Predator Avoidance in a Marine Insect. Animal Behaviour, 28, 1119–1122. https://doi.org/https://doi.org/10.1016/S0003-3472(80)80100-X

Valone, T. J., & Templeton, J. J. (2002). Public information for the assessment of quality: A widespread social phenomenon. Philosophical Transactions of the Royal Society B: Biological Sciences, 357(1427), 1549–1557. https://doi.org/10.1098/rstb.2002.1064

Van Nest, B. N., Otto, M. W., & Moore, D. (2018). High experience levels delay recruitment but promote simultaneous time-memories in honey bee foragers. The Journal of Experimental Biology, 221(23), jeb187336. https://doi.org/10.1242/jeb.187336

Van Oers, K., De Jong, G., Drent, P. J., & Van Noordwijk, A. J. (2004). A genetic analysis of avian personality traits: Correlated, response to artificial selection. Behavior Genetics, 34(6), 611–619. https://doi.org/10.1007/s10519-004-5588-z

Visscher, P. K., & Seeley, T. D. (1982). Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest. Ecology, 63(6), 1790–1801. https://doi.org/10.2307/1940121

von Frisch, K. (1967). The dance language and orientation of bees. Harvard University Press. Harvard University Press.

von Rueden, C. R., & Jaeggi, A. V. (2016). Men's status and reproductive success in 33 nonindustrial societies: Effects of subsistence, marriage system, and reproductive strategy. Proceedings of the National Academy of Sciences, 113(39), 10824–10829. https://doi.org/10.1073/pnas.1606800113

Wagner, A. E., Van Nest, B. N., Hobbs, C. N., & Moore, D. (2013). Persistence, reticence and the management of multiple time memories by forager honey bees. Journal of Experimental Biology, 216(7), 1131–1141. https://doi.org/10.1242/jeb.064881

Ward, A. J. W., Herbert-Read, J. E., Sumpter, D. J. T., & Krause, J. (2011). Fast and accurate decisions through collective vigilance in fish shoals. Proceedings of the National Academy of Sciences, 108(6), 2312–2315. https://doi.org/10.1073/pnas.1007102108

Ward, A., & Webster, M. (2016). Sociality: The behaviour of group-living animals. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-28585-6

Ward, P., & Zahavi, A. (1973). The Importance of Certain Assemblages of Birds As "Information-Centres" for Food-Finding. Ibis, 115, 517–534. https://doi.org/10.1111/j.1474-919X.1973.tb01990.x

Waters, J. S., & Fewell, J. H. (2012). Information processing in social insect networks. PLoS ONE, 7(7). https://doi.org/10.1371/journal.pone.0040337

Webb, C. E., Romero, T., Franks, B., & De Waal, F. B. M. (2017). Long-term consistency in chimpanzee consolation behaviour reflects empathetic personalities. Nature Communications, 8(1), 1–8. https://doi.org/10.1038/s41467-017-00360-7

Webster, M. M., & Ward, A. J. W. (2011). Personality and social context. Biological Reviews, 86(4), 759–773. https://doi.org/10.1111/j.1469-185X.2010.00169.x

Weidenmüller, A. (2004). The control of nest climate in bumblebee (Bombus terrestris) colonies: interindividual variability and self reinforcement in fanning response. Behavioral Ecology, 15(1), 120–128. https://doi.org/10.1093/beheco/arg101

Wild, B., Rosemann, B., Landgraf, T., Wario, F., Dormagen, D., & Boenisch, F. (2018). Tracking All Members of a Honey Bee Colony Over Their Lifetime Using Learned Models of Correspondence. Frontiers in Robotics and AI, 5(April), 1–10. https://doi.org/10.3389/frobt.2018.00035

Williams, R., & Lusseau, D. (2006). A killer whale social network is vulnerable to targeted removals. Biology Letters, 2(4), 497–500. https://doi.org/10.1098/rsbl.2006.0510

Wilson, E O. (1971). The Insect Societies. Cambridge, MA, 548. Retrieved from https://www.cabdirect.org/cabdirect/abstract/19720503745

Wilson, Edward O., & Hölldobler, B. (2005). Eusociality: Origin and Consequences. Proceedings of the National Academy of Sciences, 102(38), 13367–13371. https://doi.org/10.1073/pnas.0505858102

Wittwer, B., Hefetz, A., Simon, T., Murphy, L. E. K., Elgar, M. A., Pierce, N. E., & Kocher, S. D. (2017). Solitary bees reduce investment in communication compared with their social relatives.

Proceedings of the National Academy of Sciences, 114(25), 6569–6574. https://doi.org/10.1073/pnas.1620780114

Wolf, M., & Krause, J. (2014). Why personality differences matter for social functioning and social structure. Trends in Ecology and Evolution, 29(6), 306–308. https://doi.org/10.1016/j.tree.2014.03.008

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. Trends in Ecology and Evolution, 27(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Wrangham, R. W., Gittleman, J. L., & Chapman, C. A. (1993). Constraints on group size in primates and carnivores: population density and day-range as assays of exploitation competition. Behavioral Ecology and Sociobiology, 32(3), 199–209. https://doi.org/10.1007/BF00173778

Wrona, F. J., & Dixon, R. W. J. (1991). Group size and predation risk: a field analysis of encounter and dilution effects. The American Naturalist, 137(2), 186–201. https://doi.org/https://doi.org/10.1086/285153

Chapter

II

Consistency

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Chapter 2 – Consistency

Introduction

Animals must necessarily respond to external stimulus in an appropriate manner to survive under natural conditions. However, individuals from the same species, and even from the same population can respond differently to the same stimulus. This difference can be in terms of either another behavioural response or even a change in intensity of the same behavioural response (Ryan, 2012). Moreover, these differences can be consistent, i.e., individuals repeatedly show the same differences in their behavioural responses to the same stimuli (Bell, Hankison, & Laskowski, 2009). Behavioural syndromes or 'personalities' refer to the phenomenon of having correlated consistent inter-individual differences across behaviours (Sih, Bell, & Johnson, 2004). These syndromes incorporate inter-individual variation along with some inflexibility amongst individuals while responding to different stimuli (Dall, Bell, Bolnick, & Ratnieks, 2012).

There is a growing interest in the field of animal behaviour on these behavioural syndromes and their importance with respect to individual and group fitness (Wolf & Weissing, 2012). This interest has been driven by recent advances in the field in terms of both analytical frameworks and data collection methods which have enabled scientists to look at the causes and consequences of inter-individual variation (Araújo et al., 2008; Nakagawa & Schielzeth, 2010). Consistent inter-individual differences in behavioural responses is observed across the animal kingdom (Bell et al., 2009). These differences have been seen in a variety of behaviours including aggression (Brown, Smith, Moskalik, & Gabriel, 2006), boldness (Mazue, Dechaume-Moncharmont, & Godin, 2015), courtship (Rushbrook, Dingemanse, & Barber, 2008), exploration (Dingemanse, Both, Drent, Van Oers, & Van Noordwijk, 2002), foraging (Missoweit, Engels, & Sauer, 2007) and migration (Battley, 2006). Interestingly, in social insects, studies on inter-individual variation have historically focussed on behavioural specialisation with respect to division of labour (Dall et al., 2012).

Inter-individual variation in behavioural responses can arise from interaction between multiple sources in any species (Dall et al., 2012; Jeanson & Weidenmüller, 2013).

Although it is difficult to pinpoint the exact contribution of various sources of inter individual variation, different studies have tackled this problem by focussing on one of these sources and trying to elucidate how it leads to individual differences. The most predominantly explored source of variation is genetic diversity (Bult & Lynch, 2000; Dingemanse et al., 2002; Van Oers, De Jong, Drent, & Van Noordwijk, 2004). Other factors which affect behavioural differences include natural variation in developmental pathways between individuals, differences in environmental stimuli leading to diverging behavioural responses and epigenetic effects (Dall et al., 2012). In eusocial insects, the preimaginal environment, as well as stimuli faced during the early life stages can influence adult behavioural responses (Ament et al., 2011; Arenas & Farina, 2008; Daugherty, Toth, & Robinson, 2011; Judd, Magnus, & Fasnacht, 2010). Finally, in social groups and eusocial insects, the social environment and the responses of other group members can also affect an individual's behavioural response (Krause, James, & Croft, 2010; Webster & Ward, 2011).

There is growing evidence about how inter-individual differences can be beneficial both for populations and smaller groups (Wolf & Weissing, 2012). Inter-individual variation in a population can have a wide range of ecological effects on its dynamics, including effects on life history traits (Biro & Stamps, 2008), population production (Duckworth, 2008) and flexibility in responding to perturbations (McCann, 2000). From an evolutionary perspective, these individual differences can have an important effect on behavioural specialisation (Dall et al., 2012) and increase the diversity of the population's behavioural repertoire (Seid & Traniello, 2006), thus directly influencing speciation. In eusocial insects, theoretical studies have shown that individual variation is an essential component of division of labour (Jeanson, Fewell, Gorelick, & Bertram, 2007; Tarapore, Floreano, & Keller, 2010), leading to more efficient utilisation of the colony's task force (Bertram, Gorelick, & Fewell, 2003; Gove, Hayworth, Chhetri, & Rueppell, 2009). Manipulative experiments have also indicated that these inter-individual behavioural variations play a vital role in how resilient the colony is to perturbations (Crall et al., 2018; Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012; Weidenmüller, 2004).

Inter-individual differences in group living species can affect the group structure and dynamics (Krause et al., 2010). Thus, understanding these differences are essential to understanding how such groups are formed and maintained. The influence of this variation can also depend on the type of social group. For example, vertebrate social networks and eusocial insect networks are known to differ in their group structure and the type of interactions present within them (Fewell, 2003; Waters & Fewell, 2012). Moreover, while selection within social groups generally tends to act on individual traits, in eusocial insects, selection will act prominently at the colony level, since each colony is a single unit of reproduction (Sober & Wilson, 2011; Waters & Fewell, 2012). As a result, the mechanisms underlying these differences and the benefits of these differences for the group is likely to be different. Although behavioural syndromes have been well studied in vertebrates, there are fewer studies that have observed them within the context of social groups. The literature on consistent inter-individual variation in eusocial insects is even more scarce. However, recent studies have started bringing together the concept of behavioural syndromes and within worker variation in eusocial insect colonies (Crall et al., 2018; Garrison, Kleineidam, & Weidenmüller, 2018; Muller, Grossmann, & Chittka, 2010).

In this chapter, I will focus on consistency in inter-individual differences in waggle dance activity shown by individuals from the same forager group in honey bees. In honey bees, previous studies have shown some consistency in the behavioural responses like trophallaxis, aggression (Walton & Toth, 2016) and trap lining behaviour of foragers (Buatois & Lihoreau, 2016). Importantly, there have been no studies which have looked at whether consistent inter-individual differences can exist in the recruitment behaviour of any social insect. Understanding the degree of consistency and behavioural variation present amongst foragers will improve our understanding of the regulation of foraging behaviour in honey bees and can give deeper insights into how social insect colonies are structured in general.

Materials and Methods

Apis mellifera colonies

All experiments were done on the campus of the National Centre for Biological Sciences, Bangalore.

Apis mellifera colonies were obtained from a commercial beekeeper (Spruce Impex Ltd.). Colonies were usually composed of 4 frames with one or two frames of brood. Experiments were done inside a flight cage (20 m length x 4 m height x 4 m width). The flight cage was completely covered with a mesh structure, thus preventing any bees inside the cage from moving out. Each colony was moved into the flight cage 4-6 days before the start of the experiments. Two feeders, containing sucrose solution and pollen respectively, were provided inside the flight cage. The colony did not have access to any other food source inside the flight cage. This ensured that most of the nectar and pollen foragers in the colony were active at these two food sources respectively.

Forager training

Foragers were trained to the feeder containing 1M sucrose solution over 2-3 days. The feeder consisted of a brightly coloured stand and a plastic plate (Seeley, 1995; von Frisch, 1967). The plate was filled with sucrose solution for the same 3 hours on each training day. Foragers were trained by first placing the feeder near the hive and then by manually transporting foragers that were coming outside the hive to the feeder using a stick smeared with sucrose solution. Foragers were repeatedly transported from the hive entrance to the feeder till at least one forager started coming on its own. Once this happened, the foragers were allowed to recruit their nest mates to the feeder and no more transporting was done. When there were around 10 foragers at the feeder, the feeder was slowly shifted away from the hive (Scheiner et al., 2013). The shift was done in small steps of about 1 metre. The feeder was kept at each location for 30 minutes or so, to ensure that enough foragers were still coming to it. The feeder was shifted to a final distance of 13 m from the hive. During the training phase, the pollen feeder was only provided when the sucrose feeder was not active. Once the feeder was shifted to the final distance, the colony was transferred into an observation hive within a small enclosure at one end of the flight cage. The observation hive was able to accommodate 3 frames of the colony, placed vertically. The walls on both sides of the observation hive were made of glass, allowing easy visual access to in-hive activities without disturbing the colony. A wedge was also placed at the bottom of the observation hive (Visscher & Seeley, 1982). This directed incoming foragers to one side of the observation hive, ensuring that all the waggle dance activity of the foragers happened on one side of the frame.

Individual Identification

Foragers which were active at the sucrose feeder were caught and kept on ice for a short duration till they became inactive. Identification tags (small coloured plastic tags with numbers on them) were then affixed to their backs using glue, based on established bee tagging protocols (Scheiner et al., 2013). Around 20-30 foragers were randomly selected from the group of foragers visiting the feeder for tagging. This ensured that the forager groups were made of individuals which were representative of the inter-individual variation in activity present in the colony. However, no other specific information regarding the demographics of the group (like the age of the individual foragers) could be obtained in our experimental protocol. The marked foragers were released back to the hive and were then monitored for 2 days before the experiments were started. Sometimes, the plastic tags got removed from the foragers due to the general activity of the hive etc. If less than 12 individually marked foragers were coming to the feeder after 2 days of marking, the whole marking process was repeated.

Experimental Protocol

The experiments were started 2 days after the tagging process and consisted of 3 consecutive days of observation of the individually marked foragers (except in the case of 2 experimental repeats in which observations could only be done for 2 days, see Table 2.1). All experiments were started when there were at least 12 foragers coming to the feeder. After this, any other marked individual forager was caught and put on ice for the duration of the observations on that day. All unmarked recruits that were coming to the feeder during this time were also caught and put on ice till the end of the observation period on each day.

On each day, the observations lasted for 3 hours (Fig. 2.1). The feeder was filled with 1M sucrose during the first hour, 2M during the second hour and 1M again during the third hour, following an established experimental protocol (Seeley, 1994). During these 3 hours, the foraging and dance activity of the individual foragers were observed. The colony had access to only the sucrose feeder and not the pollen feeder during the experimental time. None of the pollen foragers were active during this time, and the behavioural observations were limited to the individually marked sucrose foragers.

Two observers were present at the feeder throughout the 3 hours. One of them noted the time (with a precision of one minute) when each forager landed at the feeder. The other caught all the recruits that were coming to the feeder to keep the individually marked foragers motivated to dance throughout the experiments (Seeley, 1995). Near the observation hive, there was another observer who would video record the dances of the individually marked foragers. Recordings were made using a Sony Handycam (HDR CX260/HDR CX240) at 1080p and 25/50 frames per second. The recordings were started when any of the marked individuals started dancing and were stopped when none of the marked individuals were dancing. A total of 117 foragers, from 12 forager group and 8 different colonies were observed (Table 2.1).

Table 2.1

Forager Group Name	**Month**	**Year**	**Experimental Days**	**Forager Group Size**	**Colony**
H1*	January	2014	2	8	1
H2*	January	2014	2	11	1
H3	February	2014	3	7	1
H4	February	2014	3	10	1
H5	April	2014	3	9	2
H6	February	2016	3	12	3
H7	April	2016	3	8	4
H8	September	2016	3	8	5

L1	October	2016	3	10	6
L2	November	2016	3	12	7
L3	November	2016	3	11	7
L4	January	2017	3	11	8

Table 2.1: The identity of the forager group, the month and year when the group was observed, the number of experimental days, the forager group size and the colony to which the forager group belonged to in the consistency experiments. Forager groups marked with an * had a 2-day observation phase. The different letters in the forager group name correspond to the types of removal done (see chapter 3 for details).

Video Analysis

The recorded dances of the foragers were then analysed manually using the open source software VLC Media Player. Since the feeder location was very close to the hive, most foragers did dances with very short waggle phases (Gardner, Seeley, & Calderone, 2008). The total number of circuits performed by each forager during each dance was obtained from the videos. Each dance circuit involved the forager walking in a circular path with a short waggling motion of her abdomen at the end of the path (Sen Sarma, Esch, & Tautz, 2004).

Statistical Analysis

The statistical analysis focussed on 6 different behavioural parameters estimated for each bee for each day. They were: 1) *total number of dance circuits*, 2) *total number of dances*, 3) *total number of foraging trips*, 4) *the probability of dancing* (the ratio of the total number of dances to the total number of trips), 5) the ratio of the total number of circuits to the total number of dances (referred to as *Circuits/Dances*) and 6) the ratio of the total number of circuits to the total number of trips (referred to as *Circuits/Trips*). The last two ratios are different methods of calculating the intensity of the dances made by the foragers. Circuits/dances gives an estimate of how much a forager dances on average and is a good proxy for how rewarding the food source is (Seeley, 1994). On the other hand, the circuits/trips parameter provides an estimate for the intensity of the dances normalised to differences in the number of foraging trips made by different foragers over the 3 days. All 6 parameters were calculated from the total activity of each forager over 3 hours.

Figure 2.1

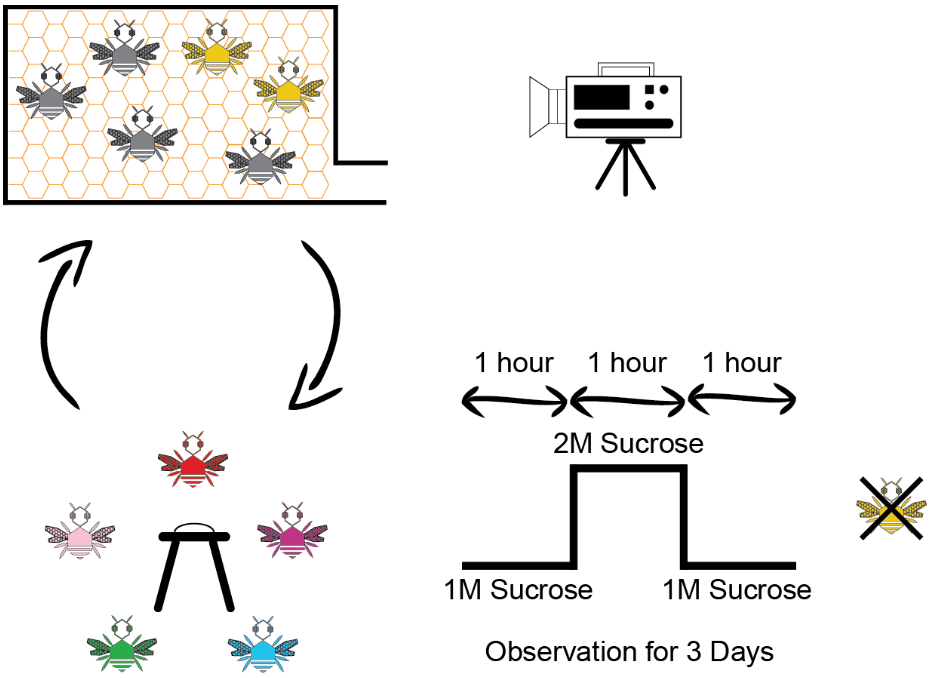

Figure 2.1: Experimental protocol for the observations of consistency in dance activity of honey bee foragers. Individually marked foragers were provided with a feeder containing 1M, 2M and 1M sucrose consecutively for one hour each. The dance activity of these foragers was then videotaped at the observation hive. Observations lasted for 3 consecutive days, and all unmarked recruits coming to the feeder were caught and kept on ice during the observation time.

Individual level consistency in activity in each of the 6 parameters was obtained using repeatability estimates (Nakagawa & Schielzeth, 2010). Repeatability estimates compare the variation within individuals to the variation across individuals in the specific parameter of interest. They are obtained using generalized linear mixed-effects models (GLMMs) fitted by restricted maximum likelihood. These estimates thus are better than other methods of comparing individual consistency as they can be used to account for various random effects structures in the experimental design and can be used to model different distributions like the Poisson, Binomial etc. [7]. The estimate has a range from 0 to 1, with a value close to 1 indicating highly consistent inter-individual differences in activity in that parameter. Confidence intervals around the repeatability estimate were obtained using parametric bootstrapping (1,000 iterations). Permutation tests (1,000 permutations) were used to test the null hypothesis that the repeatability estimate is significantly different from zero.

Repeatability estimates were obtained for each of the 3 hours separately as well as the combined data from all 3 hours for each of the parameters. A different model was built to obtain the repeatability estimate for each of the parameter of interest. The parameter of interest was the response variable and the individual identity was the random effect in these models. The error distribution of the model was decided based on the parameter. For parameters based on count data (total number of dance circuits, total number of dances and total number of trips), a Poisson error distribution was used. A Gaussian error distribution was used for parameters that were ratios (probability of dancing, circuits/dances and circuits/trips). The rptR package (Stoffel, Nakagawa, & Schielzeth, 2017) in R was used to build the GLMMs, bootstrap the model, run the permutation tests and obtain the repeatability estimates.

Visualisation of the raw data, the mean and standard deviation of each individual for each parameter and the repeatability estimates was done in R (R Core Team, 2018) using the ggplot2 (Wickham, 2016) and cowplot (Claus O. Wilke, 2018) package.

Results

Dance Circuits

Foragers showed large inter-individual differences in their dance circuits for the same feeder reward (Fig. 2.2). The most active forager (in this parameter) amongst all 117 foragers observed made an average 643.667 ± 262.709 number of circuits over 3 days, while the least active forager made an average 4.5 ± 6.364 number of circuits (Fig. 2.8 *a*). Even within forager groups, there were inter-individual differences in the total dance circuits. The ratio of the most active to the least active forager was on average 13.978 ± 11.911 (n = 12, range = 2.24 – 47.704). That is, on average, there was a 13-fold difference between the most active and least active forager within a forager group active at the same food reward in the total number of dance circuits that they performed. The coefficient of variation in dance circuits within forager groups was on average 0.587 ± 0.162 (n = 12, range = 0.289 – 0.851).

These inter-individual differences in dance circuits were consistent (Fig. 2.10 *a*). The repeatability estimate for the combined dance circuits (from all 3 hours) was 0.5263 (CI = 0.4295 – 0.6002, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.5519, CI = 0.4365 – 0.6471, $p < 0.001$), 2M (0.5637, CI = 0.4570 – 0.6551, $p < 0.001$) and the second 1M (0.6690, CI = 0.5540 – 0.7528, $p < 0.001$).

Number of Dances

Individuals showed large differences in their number of dances for the same feeder reward (Fig. 2.3). The most active forager (in this parameter) amongst all 117 foragers observed made an average 43.667 ± 12.342 number of dances over 3 days, while the least active forager made an average 1 ± 1.414 number of dances (Fig. 2.8 *b*). Even within forager groups, there were inter-individual differences in the total number of dances. The ratio of the most active to the least active forager was on average 7.136 ± 4.863 (n = 12, range = 1.605 – 20.333). That is, on average, there was a 7-fold difference between the most active and least active forager within a forager group active at the same food reward in the number of dances that they did. The coefficient of variation in the number of dances within forager groups was on average 0.444 ± 0.140 (n = 12, range = 0.173 – 0.683).

These inter-individual differences in the number of dances were highly consistent (Fig. 2.10 *b*). The repeatability estimate for number of dances was 0.6479 (CI = 0.5493 – 0.7319, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.5804, CI = 0.4396 – 0.6843, $p < 0.001$), 2M (0.5883, CI = 0.4648 – 0.6708, $p < 0.001$) and the second 1M (0.7598, CI = 0.6709 – 0.8849, $p < 0.001$).

Foraging Trips

Foragers showed inter-individual differences in their number of foraging trips for the same feeder reward (Fig. 2.4). The most active forager (in this parameter) amongst all 117 foragers observed made an average 79 ± 1 foraging trips over 3 days, while the least active forager made an average 24 ± 8.485 foraging trips (Fig. 2.8 *c*).

Figure 2.2

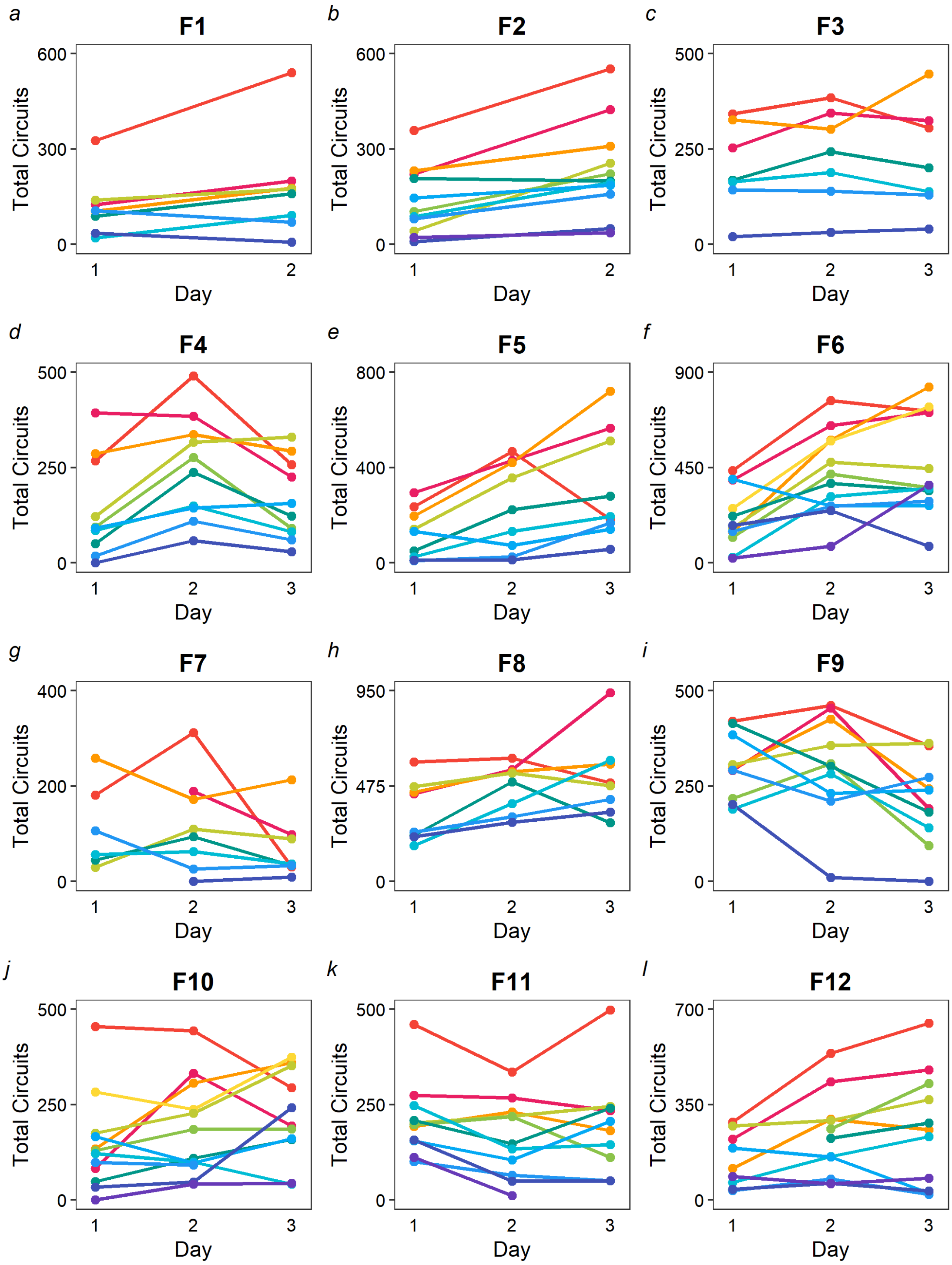

Figure 2.2: The total dance circuits made by all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

Figure 2.3

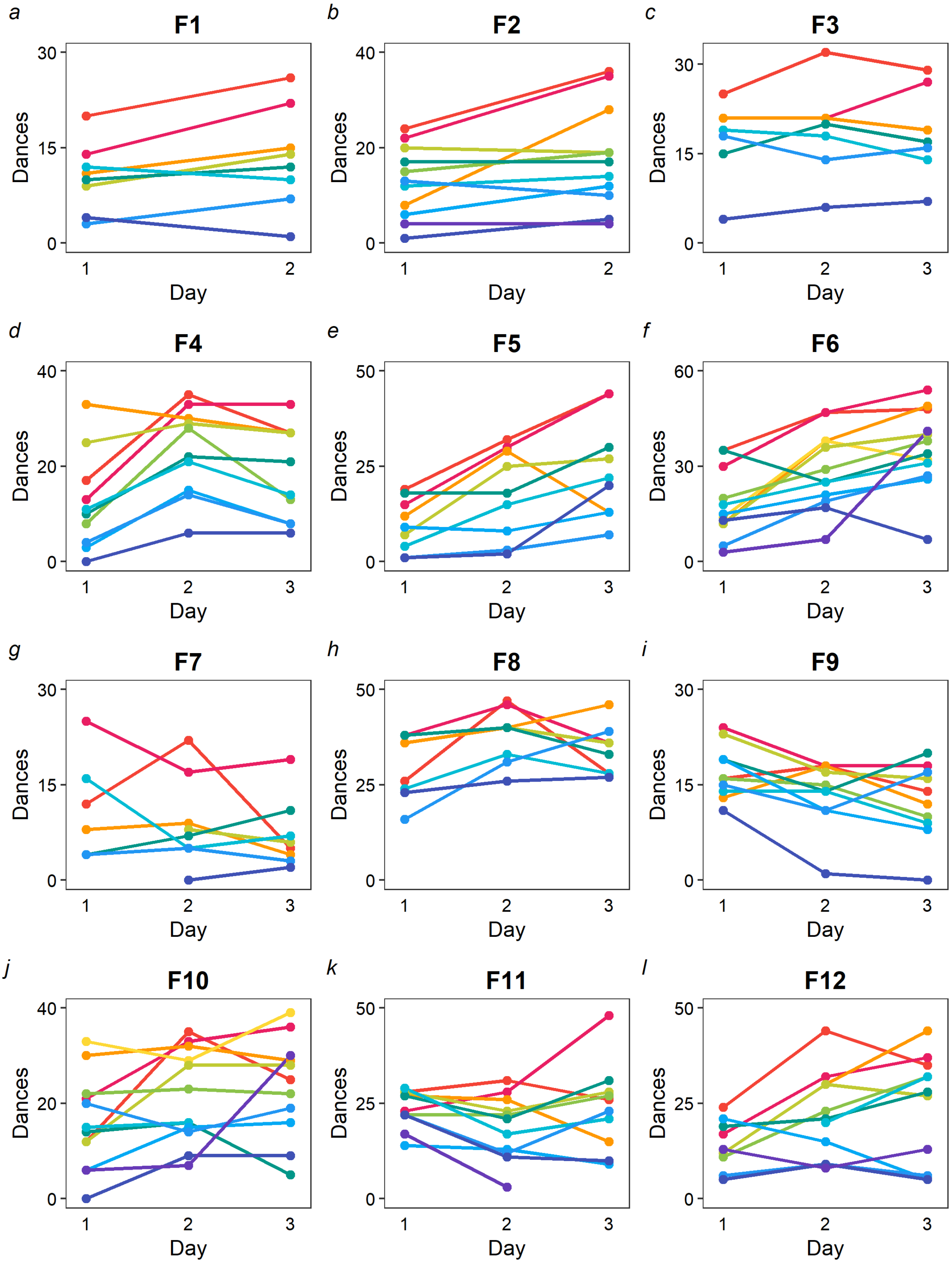

Figure 2.3: The number of dances made by all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

However, within forager groups, there were much smaller inter-individual differences in the foraging trips performed. The ratio of the most active to the least active forager was on average 1.676 ± 0.269 (n = 12, range = 1.333 – 2.25). That is, most foragers performed similar number of foraging trips in all the forager groups. The coefficient of variation in the number of trips within forager groups was on average 0.156 ± 0.042 (n = 12, range = 0.088 – 0.242).

These inter-individual differences in foraging trips showed some degree of consistency (Fig. 2.10 *c*). The repeatability estimate for number of foraging trips were 0.4644 (CI = 0.3372 – 0.5700, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.3136, CI = 0.1692 – 0.3979, $p < 0.001$), 2M (0.3043, CI = 0.1663 – 0.3854, $p < 0.001$) and the second 1M (0.4377, CI = 0.3009 – 0.5397, $p < 0.001$).

Probability of Dancing

There were large inter-individual differences in the probability of dancing of the 117 foragers observed (Fig. 2.5). The most active forager (in this parameter) had a probability of dancing of 0.904 ± 0.084 on average, whereas the least active forager had a probability of dancing of 0.013 ± 0.019 on average (Fig. 2.9 *a*). This inter-individual variation in the probability of dancing could be observed even within forager groups. The ratio of the most active to the least active forager was on average 6.589 ± 4.896 (n = 12, range = 1.568 – 20.681). On average, there was a 6-fold variation between the most active and least active forager within a forager group active at the same food reward. The coefficient of variation in the probability of dancing within forager groups was on average 0.425 ± 0.131 (n = 12, range = 0.171 – 0.621).

Inter-individual differences in the probability of dancing were highly consistent (Fig. 2.10 *d*). The repeatability estimate was 0.7014 (CI = 0.6148 – 0.7702, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.5439, CI = 0.4375 – 0.6330, $p < 0.001$), 2M (0.6501, CI = 0.5565 – 0.7253, $p < 0.001$) and the second 1M (0.6361, CI = 0.5368 – 0.7189, $p < 0.001$).

Circuits/Dances

Individual foragers showed large differences in their circuits/dances for the same feeder reward conditions (Fig. 2.6). Of the 117 foragers observed, the most active forager (in this parameter) had an average of 24.016 ± 3.013 circuits/dances while the least active forager had an average of 2.25 ± 3.182 circuits/dances (Fig. 2.9 *b*). Within forager groups, there was on average a 3-fold difference between the most and least active forager (n = 12, mean ± standard deviation = 3.134 ± 1.923, range = 1.555 – 8.879). The coefficient of variation in the circuits/dances of foragers within forager groups was on average 0.307 ± 0.097 (n = 12, range = 0.140 – 0.518).

These inter-individual differences in circuits/dances were highly consistent (Fig. 2.10 *e*). The repeatability estimate was 0.6845 (CI = 0.5925 – 0.7552, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.4927, CI = 0.3709 – 0.5936, $p < 0.001$), 2M (0.6343, CI = 0.5288 – 0.7176, $p < 0.001$) and the second 1M (0.5207, CI = 0.4006 – 0.6196, $p < 0.001$).

Circuits/Trips

Foragers showed large inter-individual differences in their circuits/trips for the same feeder reward (Fig. 2.7). The most active forager (in this parameter) amongst all 117 foragers observed made an average 13.305 ± 3.381 circuits/trips over 3 days, while the least active forager made an average 0.061 ± 0.084 circuits/trips (Fig. 2.9 *c*). Even within forager groups, there were inter-individual differences in their circuits/trips. The ratio of the most active to the least active forager was on average 15.122 ± 15.06 (n = 12, range = 2.167 – 59.881). That is, on average, there was a 15-fold difference between the most active and least active forager within a forager group active at the same food reward in their circuits/trips. The coefficient of variation in the circuits/trips of foragers within forager groups was on average 0.597 ± 0.165 (n = 12, range = 0.294 – 0.808).

Figure 2.4

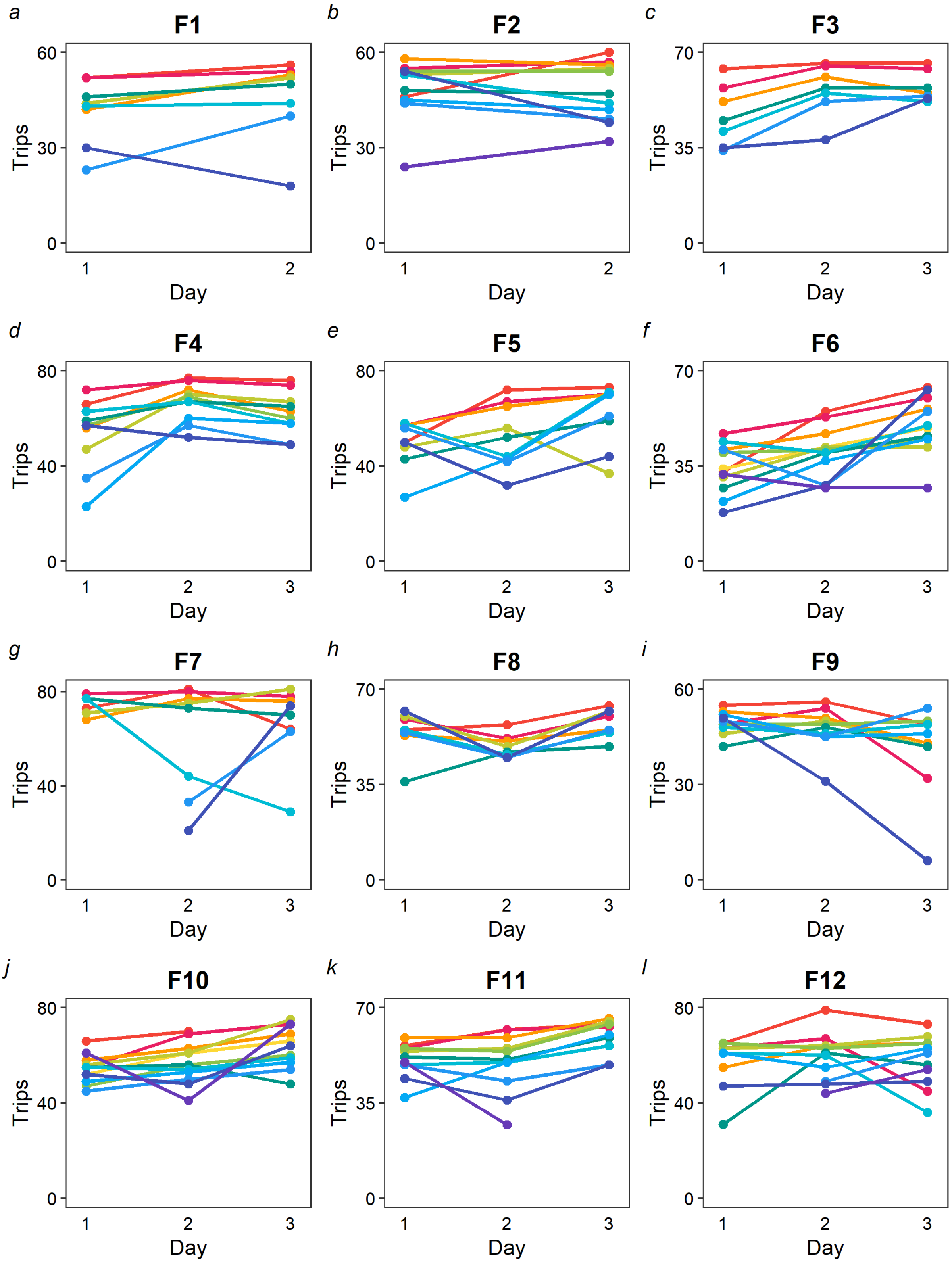

Figure 2.4: The number of foraging trips made by all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

Figure 2.5

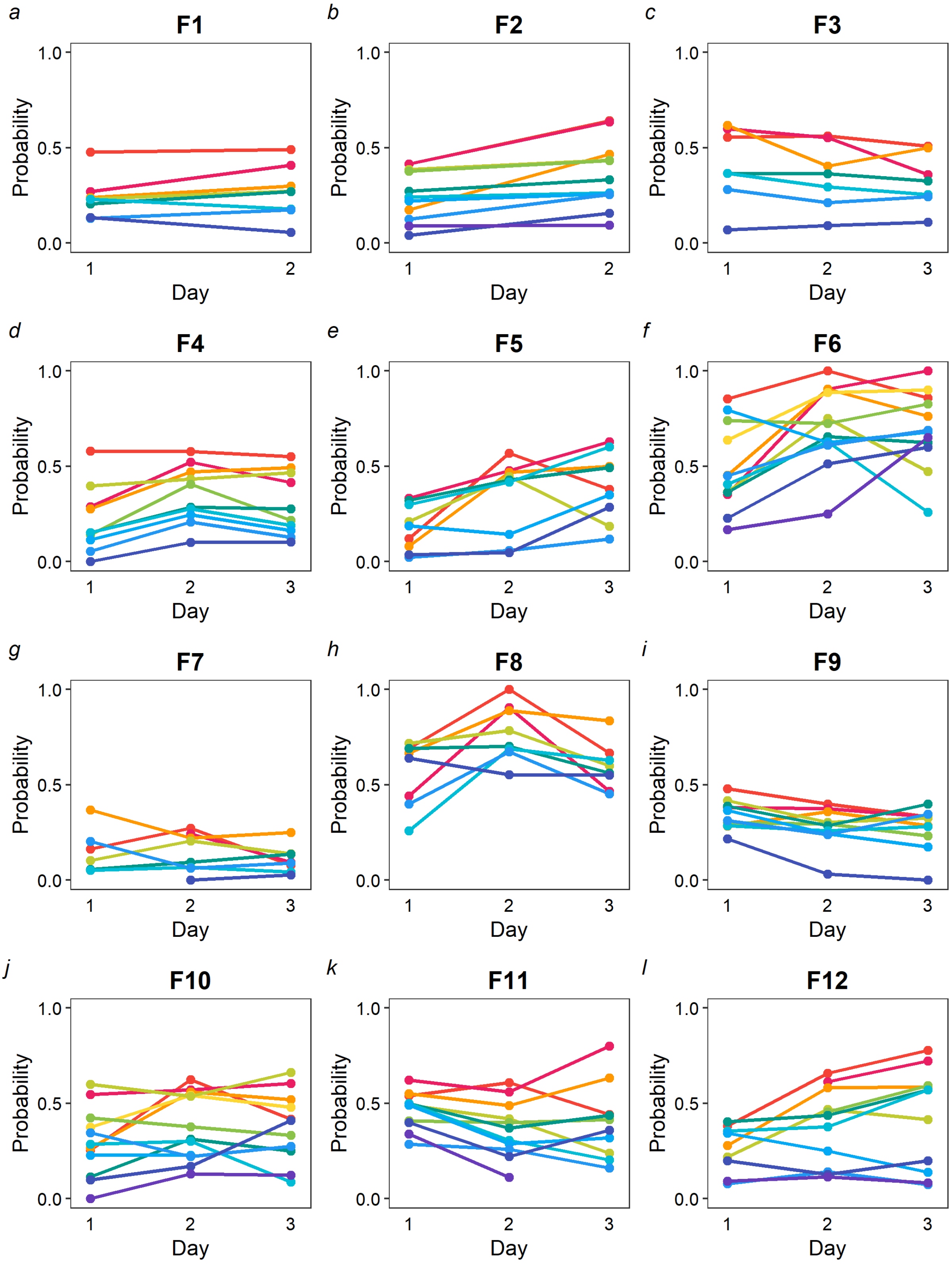

Figure 2.5: The probability of dancing of all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

Figure 2.6

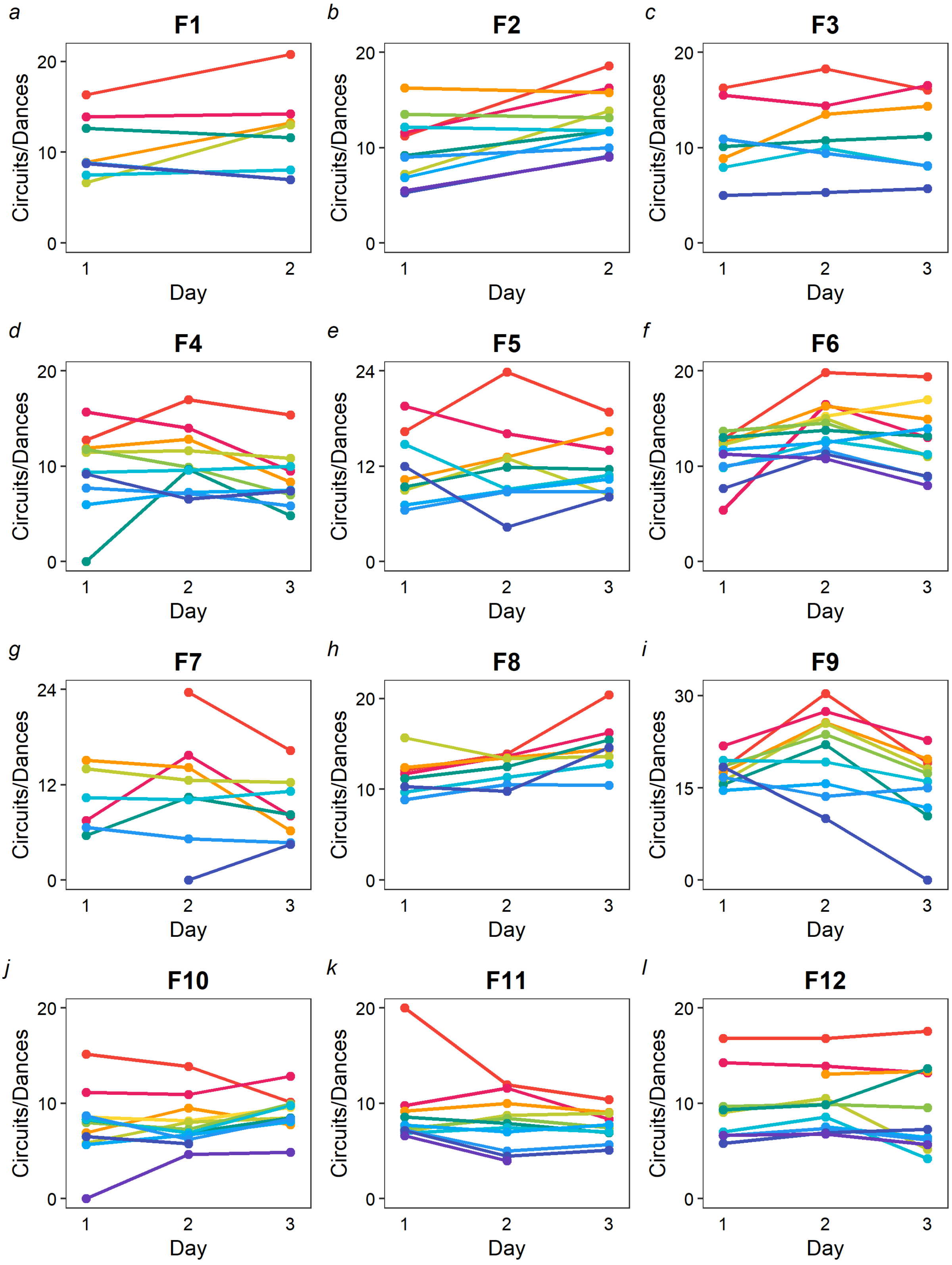

Figure 2.6: The circuits/dances of all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

Figure 2.7

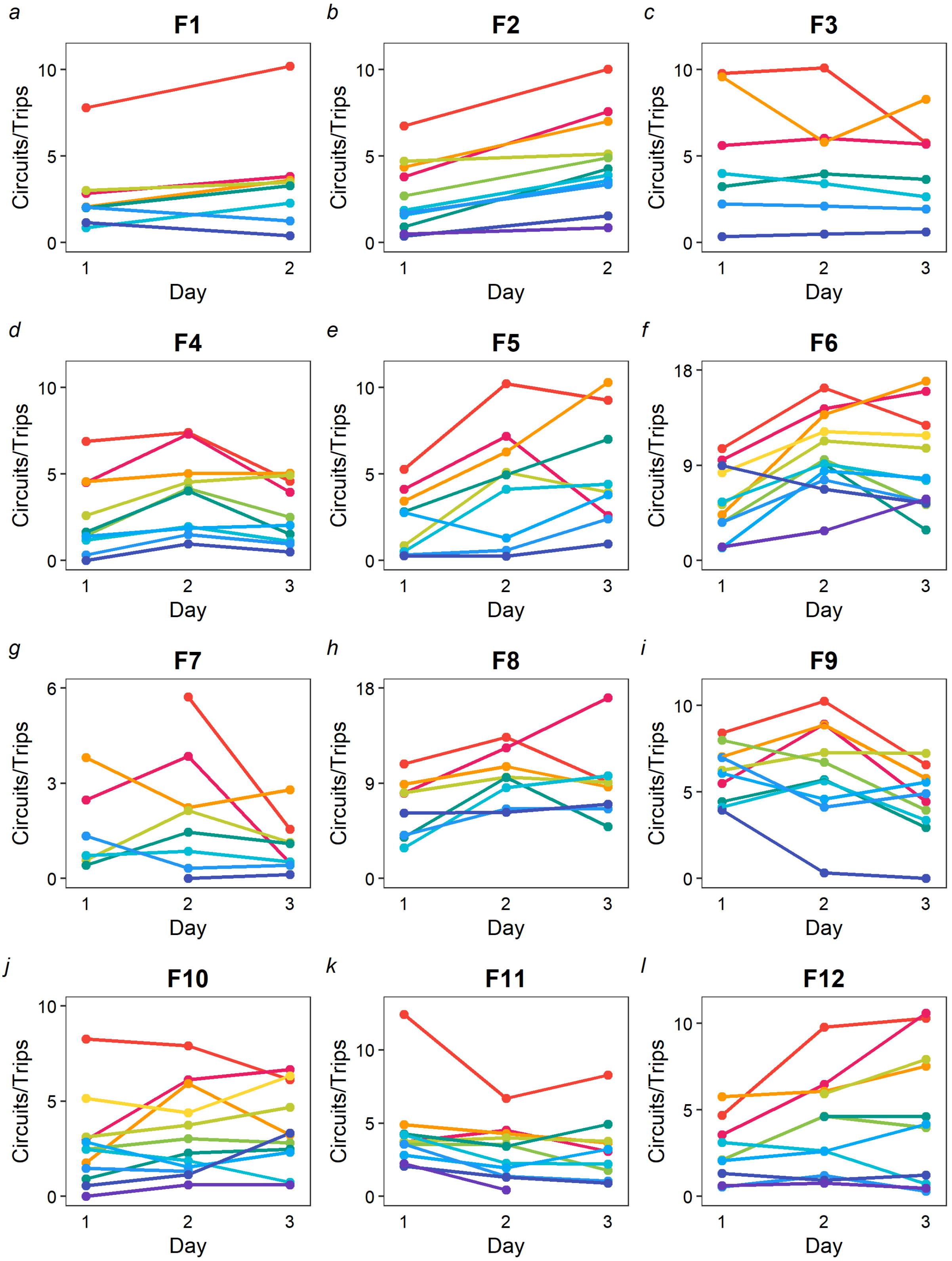

Figure 2.7: The circuits/trips of all individuals observed from each of the 12 forager groups over 3 days (2 days in the case of forager groups F1 and F2). Each forager within one forager group is represented by a different colour.

These inter-individual differences were highly consistent (Fig. 2.10 *f*). The repeatability estimate for circuits/trips was 0.7006 (CI = 0.6138 – 0.7692, $p < 0.001$). This was similar to the repeatability estimate for the first 1M (0.5454, CI = 0.4362 – 0.6401, $p < 0.001$), 2M (0.6983, CI = 0.6136 – 0.7664, $p < 0.001$) and the second 1M (0.6309, CI = 0.5317 – 0.7066, $p < 0.001$).

Discussion

Foragers showed large inter-individual differences in their dance activity in all the parameters measured. These differences could be seen even in individuals from the same forager groups, active at the same feeder at the same time. Moreover, the inter-individual differences in dance activity were highly consistent over the 3 days of observation. Individuals showed the greatest repeatability in their probability of dancing (repeatability estimate = 0.7014) and circuits/trips (repeatability estimate = 0.7006), followed by circuits/dances (repeatability estimate = 0.6845) and the number of dances (repeatability estimate = 0.6479). Individuals also showed consistent inter-individual differences in the total number of dance circuits performed (repeatability estimate = 0.5263). Finally, even though significantly consistent inter-individual differences could be seen in the foraging trips done by the foragers, the repeatability estimate (0.4644) was lower than in the other parameters. This indicates that inter-individual differences in foraging trips were not as strong in comparison with the other dance activity parameters.

Consistent inter-individual differences have been observed across the animal kingdom (Bell et al., 2009). In vertebrates, such differences have been seen in behaviours like anti-predatory behaviour (Kralj-Fišer, Scheiber, Blejec, Moestl, & Kotrschal, 2007), habitat selection (Kamel & Mrosovsky, 2005) and mate choice (Hoysak & Godin, 2007). In group living animals also, consistent behavioural differences have been shown in risk taking (Ioannou & Dall, 2016) and various social behaviours (Aplin et al., 2013, 2015). However, such differences have not been well studied in insects, especially in eusocial insects (Bell et al., 2009). In honey bees, repeatable behavioural differences are present in some behaviours like trophallaxis and trapline foraging (Buatois & Lihoreau, 2016; Walton & Toth, 2016). The results of the consistency experiments show that consistent inter-individual differences exist even in the recruitment behaviour of eusocial insects. Interestingly, the repeatability estimates obtained in these experiments are at the higher end of estimates obtained from earlier studies on other behavioural responses in insects and specifically honey bees (Bell et al., 2009; Walton & Toth, 2016). This suggests that consistent inter-individual variation in dance activity may have an adaptive function with respect to the colony's regulation of foraging (Jeanson & Weidenmüller, 2013).

Interestingly, foragers showed relatively low consistency in the foraging trips that they made to the same feeder. Inter-individual differences in foraging trips were also not as varied as for the other parameters. The most active and least active forager in foraging trips within forager groups showed a 1.6-fold difference on average. In contrast, in all the other parameters, the difference between the most active and least active forager (in that particular parameter) within forager groups was much higher (14-fold in number of dance circuits, 7-fold in number of dances, 6.5-fold in probability of dancing, 3.1-fold in circuits/dances and 15-fold in circuits/trips). The low variation in foraging trips could be an artefact of the experimental set-up. In our experiments, the foragers only had to fly 13 m to reach the feeder from the hive. It would have taken most foragers a few seconds to cover this distance (Esch & Burns, 1996). As a result, most foragers ended up making similar number of foraging trips during the observation time.

Our results on the consistency in dance activity should hold true under natural conditions also, wherein foraging dynamics might be different than this set up. In this experimental set up, foragers had access to an unlimited food source at a very short distance from the hive. In nature, most foragers must fly for much longer to obtain profitable food sources (Dukas & Visscher, 1994; Dyer & Seeley, 1991; Tenczar, Lutz, Rao, Goldenfeld, & Robinson, 2014; Thom, Seeley, & Tautz, 2000). These food sources would also have a limited amount of nectar at varying flow rates (Núñez, 1982). Thus, foragers would end up visiting multiple flowers, possibly within the same patch, before returning to the hive (Degen et al., 2015; S. Wolf et al., 2014). Under such conditions, foragers would have to decide the time they spend in foraging and in communicating about the food source, likely leading to stronger inter-individual differences, particularly in the number of foraging trips made by individuals.

Figure 2.8

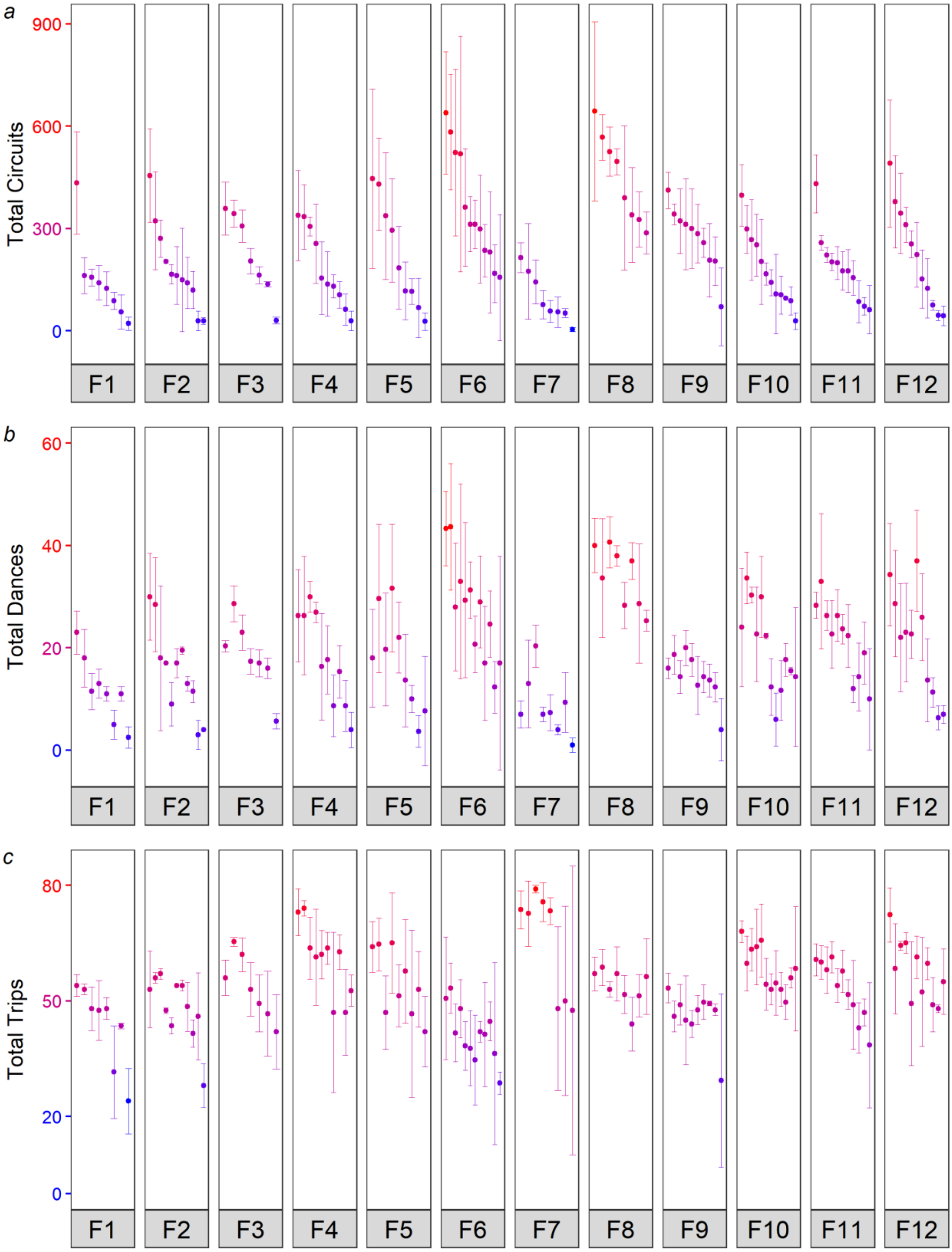

Figure 2.8: Mean (circles) and standard deviation (error bars) of *(a)* total dance circuits, *(b)* number of dances and *(c)* number of foraging trips of all 117 foragers from 12 forager groups. The colour of the

circles represents the value of the parameter, ranging from blue for low values to red for high values. Circles in the same column in *(a)*, *(b)* and *(c)* represent the same individual.

Figure 2.9

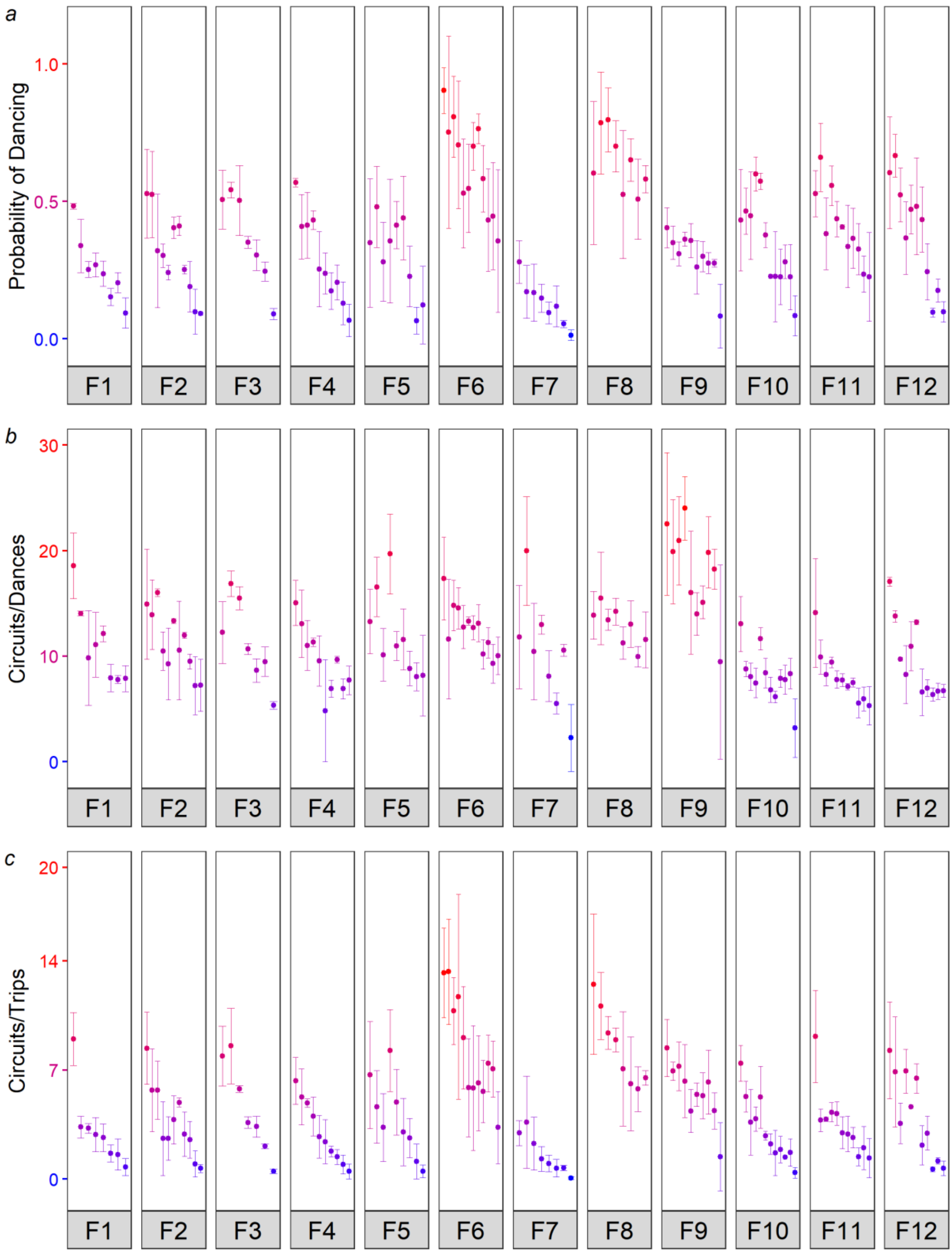

Figure 2.9: Mean (circles) and standard deviation (error bars) of *(a)* probability of dancing, *(b)* circuits/dances and *(c)* circuits/trips of all 117 foragers from 12 forager groups. The colour of the circles represents the value of the parameter, ranging from blue for low values to red for high values. Circles in the same column in *(a)*, *(b)* and *(c)* represent the same individual.

Figure 2.10

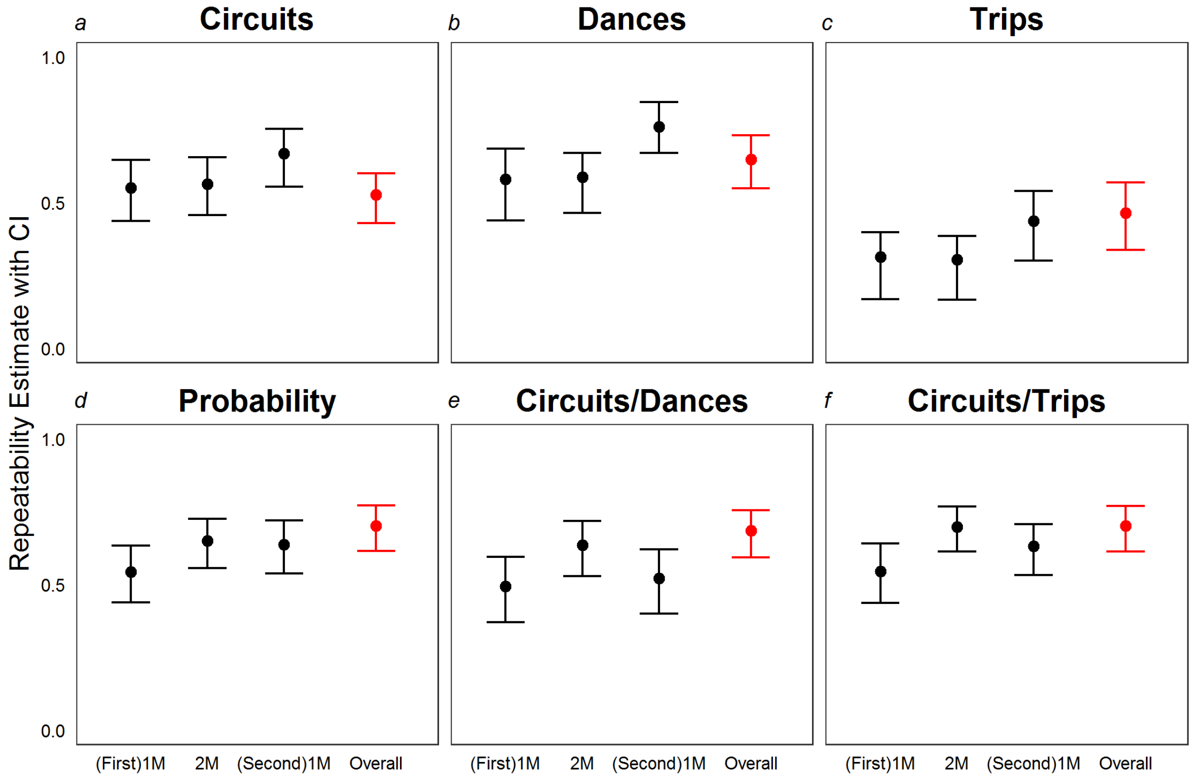

Figure 2.10: Repeatability estimate and 95% confidence intervals (circles and error bars) of all 117 foragers for *(a)* total number of dance circuits, *(b)* number of dances, *(c)* number of foraging trips, *(d)* probability of dancing, *(e)* circuits/dances and *(f)* circuits/trips. The repeatability estimates for each of the three hours (first 1M, 2M and second 1M) were similar to the overall repeatability estimate obtained from pooling the data for all three hours (red colour).

The dance activity of honey bee foragers is linked to the perception of the food reward. Previous work showed that there are inter-individual differences in the perception of the food reward (Seeley, 1994). This perception is tied to the energy dynamics of each individual forager (Seeley, 1994). The results of the consistency experiments show that these differences in perception are consistent. Each forager group observed was composed of foragers which perceived the same reward differently. More active dancers perceived the food as being more rewarding whereas less active dancers perceived the food as being less rewarding. The differences in perception of the food reward could be representative of differences in response thresholds amongst the foragers.

Sucrose responsiveness, based on gustatory response thresholds, strongly correlates with division of labour in eusocial insects (Beshers, Robinson, & Mittenthal, 1999; Page, Scheiner, Erber, & Amdam, 2006; Perez, Rolland, Giurfa, & D'Ettorre, 2013). In honey bees, nurses and foragers differ in their sucrose responsiveness (Pankiw & Page, 1999). Even within foragers, pollen foragers have a lower threshold as compared to nectar foragers (Scheiner, Page, & Erber, 2001). The consistency experiments show that within forager groups, there is partitioning of the task of recruitment amongst the individual foragers. Sucrose responsiveness could be correlated with this fine scale task partitioning also. In chapter 4, this correlation will be explored in detail.

Inter-individual differences in dance activity has important implications for the regulation of foraging at the colony level. The partitioning of the task of recruitment amongst the foragers within a forager group would help the colony control the amount of recruitment to each food source. Each forager group acts as the information source for one particular food source that they are active at (Seeley, 1994; Seeley & Towne, 1992). If all foragers performed the same amount of recruitment to a food source, then the recruitment to that food source would either increase very drastically in a short duration or would proceed at a very slow pace. This would potentially lead to overcrowding at the food source (Kirchner & Lindauer, 1994; C. Thom, 2003; Corinna Thom, Gilley, & Tautz, 2003) or under exploitation of the food source.

In addition to differences in dance activity, differences in perception of the food reward could also help the colony regulate foraging. The perception of the food reward correlates with the persistence at a food source (Al Toufailia, Grüter, & Ratnieks, 2013; Townsend-Mehler, Dyer, & Maida, 2010). Foragers which perceived the food as being more rewarding would be more likely to continue at that source. In contrast, foragers which perceived the food as being less rewarding would be more likely to abandon the food source. Thus, inter-individual differences in perception of the food reward would also help the colony to regulate the number of foragers active at any food source.

The interplay between inter-individual differences in the perception of the food reward, the information communication about the food reward and the persistence at a food source should play a major role in the regulation of foraging at the colony level. Thus, consistent inter-individual differences in dance activity can have a major functional role in the colony. It would help the colony to efficiently utilise its entire foraging force to effectively exploit the food available to it.

Future Directions

Future experiments could focus on quantifying consistent inter-individual differences in other aspects of the foraging behaviour under more natural conditions. It would be useful to measure repeatability estimates for forager groups active at food sources much further away from the colony to confirm the results in this chapter. This can also be used to explore whether longer flight distances would reveal more consistent inter-individual differences in foraging trips. Other aspects of foraging also merit further exploration to understand the extent to which consistent inter-individual differences are present in various contexts associated with foraging. For example, the nectar load that individual foragers bring in and the time spent interacting with receivers and other nest mates in the hive after a foraging trip are two aspects that can be explored. This can provide a clearer picture of the presence of behavioural syndromes or ‘personalities’ amongst honey bee foragers.

Another interesting aspect that can be explored in a future experiment would be the effect of different food reward conditions on the consistency in dance activity of foragers from the same forager group. A higher food reward might lead to a greater variation in the dance activity amongst individuals, leading to a higher repeatability. To quantify this effect, longer experimental observations of individual foragers would be needed with a single feeder reward offered for multiple days. Although in this chapter, foragers were offered multiple feeder rewards (1M and 2M), since they were offered one after the other, it would be difficult to dissociate any effect of the preceding reward on the dance activity for the current reward condition. This could explain the similarity in the repeatability estimates for the different food rewards in these experiments.

References

Al Toufailia, H., Grüter, C., & Ratnieks, F. L. W. (2013). Persistence to Unrewarding Feeding Locations by Honeybee Foragers (Apis mellifera): the Effects of Experience, Resource Profitability and Season. Ethology, 119(12), 1096–1106. https://doi.org/10.1111/eth.12170

Ament, S. A., Chan, Q. W., Wheeler, M. M., Nixon, S. E., Johnson, S. P., Rodriguez-Zas, S. L., … Robinson, G. E. (2011). Mechanisms of stable lipid loss in a social insect. Journal of Experimental Biology, 214(22), 3808–3821. https://doi.org/10.1242/jeb.060244

Aplin, L. M., Farine, D. R., Morand-Ferron, J., Cole, E. F., Cockburn, A., & Sheldon, B. C. (2013). Individual personalities predict social behaviour in wild networks of great tits (Parus major). Ecology Letters. https://doi.org/10.1111/ele.12181

Aplin, L. M., Firth, J. A., Farine, D. R., Voelkl, B., Crates, R. A., Culina, A., … Sheldon, B. C. (2015). Consistent individual differences in the social phenotypes of wild great tits, Parus major. Animal Behaviour, 108, 117–127. https://doi.org/10.1016/j.anbehav.2015.07.016

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guuimarães, P., Dos Reis, S. F., & Bolnick, D. I. (2008). Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. Ecology, 89(7), 1981–1993. https://doi.org/10.1890/07-0630.1

Arenas, A., & Farina, W. M. (2008). Age and rearing environment interact in the retention of early olfactory memories in honeybees. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 194(7), 629–640. https://doi.org/10.1007/s00359-008-0337-z

Battley, P. F. (2006). Consistent annual schedules in a migratory shorebird. Biology Letters, 2(4), 517–520. https://doi.org/10.1098/rsbl.2006.0535

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. Animal Behaviour, 77(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Bertram, S. M., Gorelick, R., & Fewell, J. H. (2003). Colony response to graded resource changes: An analytical model of the influence of genotype, environment, and dominance. Theoretical Population Biology, 64(2), 151–162. https://doi.org/10.1016/S0040-5809(03)00064-9

Beshers, S. N., Robinson, G. E., & Mittenthal, J. E. (1999). Response thresholds and division of labor in insect colonies. In Information Processing in Social Insects (pp. 115–139). Basel: Birkhäuser Basel. https://doi.org/10.1007/978-3-0348-8739-7_7

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? Trends in Ecology and Evolution, 23(7), 361–368. https://doi.org/10.1016/j.tree.2008.04.003

Brown, W. D., Smith, A. T., Moskalik, B., & Gabriel, J. (2006). Aggressive contests in house crickets: size, motivation and the information content of aggressive songs. Animal Behaviour, 72(1), 225–233. https://doi.org/10.1016/j.anbehav.2006.01.012

Buatois, A., & Lihoreau, M. (2016). Evidence of trapline foraging in honeybees. The Journal of Experimental Biology, 219(16), 2426–2429. https://doi.org/10.1242/jeb.143214

Bult, A., & Lynch, C. B. (2000). Breaking through artificial selection limits of an adaptive behavior in mice and the consequences for correlated responses. Behavior Genetics, 30(3), 193–206. https://doi.org/10.1023/A:1001962124005

Claus O. Wilke. (2018). cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2.” R Package Version 0.9.3. Retrieved from https://cran.r-project.org/package=cowplot

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Spatial fidelity of workers predicts collective response to disturbance in a social insect. Nature Communications, 9(1), 1–13. https://doi.org/10.1038/s41467-018-03561-w

Dall, S. R. X., Bell, A. M., Bolnick, D. I., & Ratnieks, F. L. (2012). An evolutionary ecology of individual differences. Ecology Letters, 15(10), 1189–1198. https://doi.org/10.1111/j.1461-0248.2012.01846.x.An

Daugherty, T. H. F., Toth, A. L., & Robinson, G. E. (2011). Nutrition and division of labor: Effects on foraging and brain gene expression in the paper wasp Polistes metricus. Molecular Ecology, 20(24), 5337–5347. https://doi.org/10.1111/j.1365-294X.2011.05344.x

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., … Menzel, R. (2015). Exploratory behaviour of honeybees during orientation flights. Animal Behaviour, 102, 45–57. https://doi.org/10.1016/j.anbehav.2014.12.030

Dingemanse, N. J., Both, C., Drent, P. J., Van Oers, K., & Van Noordwijk, A. J. (2002). Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour, 64(6), 929–938. https://doi.org/10.1006/anbe.2002.2006

Duckworth, R. A. (2008). Adaptive Dispersal Strategies and the Dynamics of a Range Expansion. The American Naturalist, 172(S1), S4–S17. https://doi.org/10.1086/588289

Dukas, R., & Visscher, P. K. (1994). Lifetime Learning by Foraging Honey bees. Animal Behaviour, 48, 1007–1012. https://doi.org/DOI 10.1006/anbe.1994.1333

Dyer, F. C., & Seeley, T. D. (1991). Dance Dialects and Foraging Range in 3 Asian Honey-Bee Species. Behavioral Ecology and Sociobiology, 28(4), 227–233. https://doi.org/10.1007/BF00175094

Esch, H. E., & Burns, J. E. (1996). Distance Estimation by Foraging Honeybees, 162, 155–162.

Fewell, J. H. (2003). Social Insect Networks. Science, 301(5639), 1515–1519. https://doi.org/10.1126/science.1088945

Gardner, K. E., Seeley, T. D., & Calderone, N. W. (2008). Do honeybees have two discrete dances to advertise food sources? Animal Behaviour, 75(4), 1291–1300. https://doi.org/10.1016/j.anbehav.2007.09.032

Garrison, L. K., Kleineidam, C. J., & Weidenmüller, A. (2018). Behavioral flexibility promotes collective consistency in a social insect. Scientific Reports, 8(1), 1–11. https://doi.org/10.1038/s41598-018-33917-7

Gove, R., Hayworth, M., Chhetri, M., & Rueppell, O. (2009). Division of labour and social insect colony performance in relation to task and mating number under two alternative response threshold models. Insectes Sociaux, 56(3), 319–331. https://doi.org/10.1007/s00040-009-0028-y

Hoysak, D. J., & Godin, J. G. J. (2007). Repeatability of male mate choice in the mosquitofish, Gambusia holbrooki. Ethology, 113(10), 1007–1018. https://doi.org/10.1111/j.1439-0310.2007.01413.x

Ioannou, C. C., & Dall, S. R. X. (2016). Individuals that are consistent in risk-taking benefit during collective foraging. Scientific Reports, 6(September), 1–9. https://doi.org/10.1038/srep33991

Jeanson, R., Fewell, J. H., Gorelick, R., & Bertram, S. M. (2007). Emergence of increased division of labor as a function of group size. Behavioral Ecology and Sociobiology, 62(2), 289–298. https://doi.org/10.1007/s00265-007-0464-5

Jeanson, R., & Weidenmüller, A. (2013). Interindividual variability in social insects - proximate causes and ultimate consequences. Biological Reviews. https://doi.org/10.1111/brv.12074

Judd, T. M., Magnus, R. M., & Fasnacht, M. P. (2010). A nutritional profile of the social wasp Polistes metricus: Differences in nutrient levels between castes and changes within castes during the annual life cycle. Journal of Insect Physiology, 56(1), 42–56. https://doi.org/10.1016/j.jinsphys.2009.09.002

Kamel, S. J., & Mrosovsky, N. (2005). Repeatability of nesting preferences in the hawksbill sea turtle, Eretmochelys imbricata, and their fitness consequences. Animal Behaviour, 70(4), 819–828. https://doi.org/10.1016/j.anbehav.2005.01.006

Kirchner, W. H., & Lindauer, M. (1994). The causes of the tremble dance of the honeybee , Apis mellifera. Behavioral Ecology and Sociobiology, 35(5), 303–308.

https://doi.org/https://doi.org/10.1007/BF00184419

Kralj-Fišer, S., Scheiber, I. B. R., Blejec, A., Moestl, E., & Kotrschal, K. (2007). Individualities in a flock of free-roaming greylag geese: Behavioral and physiological consistency over time and across situations. Hormones and Behavior, 51(2), 239–248. https://doi.org/10.1016/j.yhbeh.2006.10.006

Krause, J., James, R., & Croft, D. P. (2010). Personality in the context of social networks. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), 4099–4106. https://doi.org/10.1098/rstb.2010.0216

Mazue, G. P. F., Dechaume-Moncharmont, F.-X., & Godin, J.-G. J. (2015). Boldness-exploration behavioral syndrome: interfamily variability and repeatability of personality traits in the young of the convict cichlid (Amatitlania siquia). Behavioral Ecology, 00, arv030-. https://doi.org/10.1093/beheco/arv030

McCann, K. S. (2000). The diversity-stability debate. Nature, 405(May), 228–233. https://doi.org/10.1038/35012234

Missoweit, M., Engels, S., & Sauer, K. P. (2007). Foraging Ability in the Scorpionfly Panorpa vulgaris: Individual Differences and Heritability. Behavioral Ecology and Sociobiology, 61(3), 487–492.

Muller, H., Grossmann, H., & Chittka, L. (2010). ‘Personality’ in bumblebees: individual consistency in responses to novel colours? Animal Behaviour, 80(6), 1065–1074. https://doi.org/10.1016/j.anbehav.2010.09.016

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. Biological Reviews, 85(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Núñez, J. (1982). Honeybee foraging strategies at a food source in relation to its distance from the hive and the rate of sugar flow. Journal of Apicultural Research, 21(3), 139–150. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=GB19840006503

Page, R. E., Scheiner, R., Erber, J., & Amdam, G. V. (2006). The Development and Evolution of Division of Labor and Foraging Specialization in a Social Insect (Apis mellifera L.). Current Topics in Developmental Biology, 74, 253–286. https://doi.org/10.1016/S0070-2153(06)74008-X

Pankiw, T., & Page, R. E. (1999). The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (Apis mellifera L.). J Comp Physiol A, 185, 207–213. https://doi.org/10.1007/s003590050196

Perez, M., Rolland, U., Giurfa, M., & D’Ettorre, P. (2013). Sucrose responsiveness, learning success, and task specialization in ants. Learning and Memory, 20(8), 417–420. https://doi.org/10.1101/lm.031427.113

Pinter-Wollman, N., Hubler, J., Holley, J.-A., Franks, N. R., & Dornhaus, A. (2012). How is activity distributed among and within tasks in Temnothorax ants? Behavioral Ecology and Sociobiology, 66(10), 1407–1420. https://doi.org/10.1007/s00265-012-1396-2

R Core Team. (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rushbrook, B. J., Dingemanse, N. J., & Barber, I. (2008). Repeatability in nest construction by male three-spined sticklebacks. Animal Behaviour, 75(2), 547–553. https://doi.org/10.1016/j.anbehav.2007.06.011

Ryan, M. J. (2012). An Introduction to Animal Behaviour: An Integrative Approach. Animal Behaviour (Vol. 84). https://doi.org/10.1016/j.anbehav.2012.09.004

Scheiner, R., Abramson, C. I., Brodschneider, R., Crailsheim, K., Farina, W. M., Fuchs, S., … Thenius, R. (2013). Standard methods for behavioural studies of Apis mellifera. Journal of Apicultural Research, 52(4), 1–58. https://doi.org/10.3896/IBRA.1.52.4.04

Scheiner, R., Page, R. E., & Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (Apis mellifera L.). Neurobiology of Learning and Memory, 76(2), 138–150. https://doi.org/10.1006/nlme.2000.3996

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. Behavioral Ecology and Sociobiology, 34, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). The Wisdom of the Hive. Harvard University Press.

Seeley, T. D., & Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? Behavioral Ecology and Sociobiology, 30(1), 59–69. https://doi.org/10.1007/BF00168595

Seid, M. A., & Traniello, J. F. A. (2006). Age-related repertoire expansion and division of labor in Pheidole dentata (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. Behavioral Ecology and Sociobiology, 60, 631–644. https://doi.org/10.1007/s00265-006-0207-z

Sen Sarma, M., Esch, H. E., & Tautz, J. (2004). A comparison of the dance language in Apis mellifera carnica and Apis florea reveals striking similarities. Journal of Comparative Physiology A, 190(1), 49–53. https://doi.org/10.1007/s00359-003-0470-7

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. Trends in Ecology and Evolution, 19(7), 372–378. https://doi.org/10.1016/j.tree.2004.04.009

Sober, E., & Wilson, D. S. (2011). Adaptation and Natural Selection revisited. Journal of Evolutionary Biology, 24(2), 462–468. https://doi.org/10.1111/j.1420-9101.2010.02162.x

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods in Ecology and Evolution. https://doi.org/10.1111/2041-210X.12797

Tarapore, D., Floreano, D., & Keller, L. (2010). Task-dependent influence of genetic architecture and mating frequency on division of labour in social insect societies. Behavioral Ecology and Sociobiology, 64(4), 675–684. https://doi.org/10.1007/s00265-009-0885-4

Tenczar, P., Lutz, C. C., Rao, V. D., Goldenfeld, N., & Robinson, G. E. (2014). Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. Animal Behaviour, 95, 41–48. https://doi.org/10.1016/j.anbehav.2014.06.006

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. Journal of Experimental Biology, 206(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Thom, C, Seeley, T. D., & Tautz, J. (2000). A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. Apidologie, 31(6), 737–738. https://doi.org/10.1051/apido:2000158

Thom, Corinna, Gilley, D. C., & Tautz, J. (2003). Worker piping in honey bees (Apis mellifera): The behavior of piping nectar foragers. Behavioral Ecology and Sociobiology, 53(4), 199–205. https://doi.org/10.1007/s00265-002-0567-y

Townsend-Mehler, J. M., Dyer, F. C., & Maida, K. (2010). Deciding when to explore and when to persist: a comparison of honeybees and bumblebees in their response to downshifts in reward. Behavioral Ecology and Sociobiology, 65(2), 305–312. https://doi.org/10.1007/s00265-010-1047-4

Van Oers, K., De Jong, G., Drent, P. J., & Van Noordwijk, A. J. (2004). A genetic analysis of avian personality traits: Correlated, response to artificial selection. Behavior Genetics, 34(6), 611–619. https://doi.org/10.1007/s10519-004-5588-z

Visscher, P. K., & Seeley, T. D. (1982). Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest. Ecology, 63(6), 1790–1801. https://doi.org/10.2307/1940121

von Frisch, K. (1967). The dance language and orientation of bees. Harvard University Press. Harvard University Press.

Walton, A., & Toth, A. L. (2016). Variation in individual worker honey bee behavior shows hallmarks of personality. Behavioral Ecology and Sociobiology, 70(7), 999–1010. https://doi.org/10.1007/s00265-016-2084-4

Waters, J. S., & Fewell, J. H. (2012). Information processing in social insect networks. PLoS ONE, 7(7). https://doi.org/10.1371/journal.pone.0040337

Webster, M. M., & Ward, A. J. W. (2011). Personality and social context. Biological Reviews, 86(4), 759–773. https://doi.org/10.1111/j.1469-185X.2010.00169.x

Weidenmüller, A. (2004). The control of nest climate in bumblebee (Bombus terrestris) colonies: interindividual variability and self reinforcement in fanning response. Behavioral Ecology, 15(1), 120–128. https://doi.org/10.1093/beheco/arg101

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing (2nd ed.). Springer. https://doi.org/10.1007/978-3-319-24277-4

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. Trends in Ecology and Evolution, 27(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Wolf, S., McMahon, D. P., Lim, K. S., Pull, C. D., Clark, S. J., Paxton, R. J., & Osborne, J. L. (2014). So near and yet so far: Harmonic radar reveals reduced homing ability of nosema infected honeybees. PLoS ONE, 9(8). https://doi.org/10.1371/journal.pone.0103989

Chapter

III

Removal

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Chapter 3 – Removal

Introduction

Social cues and signals play a major role in driving individual behavioural responses in group living animals (Morand-Ferron, Doligez, Dall, & Reader, 2010). Valuable information about conditions outside the group, like the presence of food and predators are encoded in these cues and signals. They can also be associated with within-group properties, like dominance hierarchies and infections (Fernald, 2014; Kavaliers & Choleris, 2018). Social interactions can thus play a major role in the formation and maintenance of such groups through their effects on individual behaviour (Conradt & Roper, 2003). The structure and dynamics of groups can be drastically affected by changes in environmental conditions and other natural processes (Pruitt & Goodnight, 2014). Group responses to such perturbations relies on social interactions within members of the group (Chate et al., 2015; Giardina et al., 2010; Sinhuber, van der Vaart, & Ouellette, 2019).

Eusocial insect colonies can contain hundreds if not thousands of individual workers and multiple levels of social interactions, across various modalities of communication, are present within these colonies (Hölldobler & Wilson, 2009). Usually, multiple groups of workers are engaged in various tasks at the same time in these colonies (Traniello & Rosengaus, 1997). Interactions within and across these groups are essential to maintain the efficient functioning of the colony (Leonhardt, Menzel, Nehring, & Schmitt, 2016). Selection in such colonies tends to act at the colony level (Fewell, 2003). Thus, social cues and signals present in these groups are mostly associated with information regarding the state of the colony and the state of the environment as pertaining to its effect on the colony. Social cues and signals play a particularly important role in how eusocial colonies respond to perturbations (Crall et al., 2018; Garrison, Kleineidam, & Weidenmüller, 2018).

Individuals in social groups, particularly in eusocial groups, can respond to social cues and signals that originate from other individuals within the group as well as from individuals in other groups (Grüter & Keller, 2016; Kocher & Cocroft, 2019). Intra-group interactions are particularly important when groups contain 'key' individuals

(Keiser, Pruitt, Sih, Watters, & Modlmeier, 2014; King & Cowlishaw, 2009). These individuals can have a disproportionate effect on the behavioural responses of other group members. For example, they can act as leaders, driving movement and foraging patterns of the group (Brown & Irving, 2014; Couzin, Krause, Franks, & Levin, 2005; Strandburg-Peshkin, Papageorgiou, Margaret, & Farine, 2018; Sumana & Sona, 2013). The cues and signals they produce can also have inhibitory effects on the activity of other group members (Clarke & Faulkes, 1997). Inter-group interactions can also have stimulatory or inhibitory effects on individuals within social insect groups. In this case, most individuals would experience the same stimuli, although there would still be inter-individual differences in the response to these cues (Beshers & Fewell, 2001).

In honey bee forager groups, both intra- and inter-group interactions affect the foraging and dance behaviour (Walter M Farina, 1996; Seeley, 1989, 1995). Intra-group interactions include stimulatory signals like the waggle dance and inhibitory signals like the stop signal (Nieh, 2010; von Frisch, 1967). When foragers are trained to a food source at a particular time, they tend to follow dances from other foragers in the forager group exclusively during that time (Moore, Siegfried, Wilson, & Rankin, 1989). Foragers, which have experienced predation or overcrowding at a food source, target other foragers active at the same food source with stop signals (Kietzman & Visscher, 2015; Lau & Nieh, 2010; Tan et al., 2016; Thom, 2003). These signals serve to reduce the motivation of these foragers to recruit to and forage at that food source. Previous work has also shown that individuals differ in their dance activity within forager groups (Seeley, 1994). However, no studies have looked at whether 'key' individuals may be present within these groups.

Inter-group interactions are also present between foragers and other nest mates engaged in various tasks in the same spatial location within the hive. Returning nectar foragers unload the food they have brought in onto receiver bees (Seeley, 1989, 1995). Multiple aspects of their interactions with these receivers influences the motivation of foragers to recruit to the food source. The wait time that foragers face before unloading has been shown to correlate with the nectar influx into the colony as well as the colony food stores (Seeley, 1989). Trophallactic interactions with receivers also modulate the dance activity of nectar foragers (De Marco & Farina, 2001; W M Farina, 2000). Foragers also receive inhibitory stop signals from guard bees or other foragers in case of predation close to the colony entrance (Tan et al., 2016). In such a situation these signals can drastically reduce the motivation of foragers to fly out to gather food for the colony.

In this chapter, I manipulate the social environment experienced by individuals in a forager group by removing some of the foragers. I remove either the foragers most active in their recruitment behaviour (High Removal, HR) or the foragers least active in their recruitment behaviour (Low Removal, LR). If group members had disproportionate effects on the activity of others in the forager group, then the HR and the LR experiments would have different effects. Contrarily, if all individual foragers respond similarly to the changing group composition, then the HR and the LR experiments would have similar effects on the dance activity. Finally, if individual variation in dance activity is not modulated by the changing social environment, then neither the HR nor the LR would have an effect on their dance activity. I also compare the changes at the group level and at the individual forager level in these experiments. Individual level observations of behavioural responses to social cues and signals in honey bee forager groups are vital to understand how these groups responds to perturbations like changing environmental conditions.

Materials and Methods

Apis mellifera colonies

The maintenance of the colonies used in these experiments followed the same protocol as in chapter 2.

Forager training

The protocol for training the foragers in these experiments were the same as described earlier in chapter 2.

Individual Identification

The protocol for tagging and identifying individuals in these experiments were the same as described earlier in chapter 2.

Experimental Protocol

The experiments were started 2 days after the tagging process and consisted of 6 consecutive days of observation of the individually marked foragers (except in the case of 3 experimental repeats, where observations could only be done for 5 days, see Table 3.1). All experiments were started when there were at least 12 foragers coming to the feeder. After this, any other marked individual forager was caught and put on ice for the duration of the observations on that day. All unmarked recruits that were coming to the feeder during this time were also caught and put on ice till the end of the observation period on each day.

On each day, the observations lasted for 3 hours. The feeder was filled with 1M sucrose during the first hour, 2M during the second hour and 1M again during the third hour, following an established experimental protocol (Seeley, 1994). During these 3 hours, the foraging and dance activity of the individual foragers were observed.

Table 3.1

Forager group Name	**Month**	**Year**	**Number of Experimental Days**	**Forager group Size**	**Number of Foragers Removed**	**Colony**
H1*	January	2014	5	8	2	1
H2*	January	2014	5	11	4	1
H3	February	2014	6	7	3	1
H4	February	2014	6	10	4	1
H5	April	2014	6	9	2	2
H6	February	2016	6	12	4	3
H7	April	2016	6	8	3	4
H8	September	2016	6	8	4	5
L1^{+}	October	2016	5	10	3	6
L2	November	2016	6	12	4	7
L3	November	2016	6	11	4	7
L4	January	2017	6	11	4	8

Table 3.1: The identity of the forager group, the month and year when the group was observed, the number of experimental days, the forager group size and the colony to which the forager group belonged to in the removal experiments. Forager groups starting with the 'H' represent groups in which the High Removal (HR) experiments were done, whereas groups starting with the 'L' represent groups in which the Low Removal (LR) experiments were done. Forager groups marked with an * had a 2-day pre-removal observation phase and groups marked with a + had a 2-day post-removal observation phase.

Two observers were present at the feeder throughout the 3 hours. One of them noted the time when each forager landed at the feeder with a resolution of one minute. The other caught all the recruits that were coming to the feeder to keep the individually marked foragers motivated to dance throughout the experiments (Seeley, 1995). Near the observation hive, there was another observer who would video record the dances of the individually marked foragers. Recordings were made using a Sony Handycam (HDR CX260/HDR CX240) at 1080p and 25/50 frames per second. The recordings were started when any of the marked individuals started dancing and were stopped when none of the marked individuals were dancing.

The removal experiments consisted of 2 3-day phases; a Pre and a Post-removal phase (Fig. 3.1). The Pre-removal phase was the same as the consistency experiments described previously in chapter 2. In two experiments, this phase consisted only of observations on 2 consecutive days. On the 4th day, shortly before the observation time, 2-4 foragers from the forager group were removed. The Post-removal phase then consisted of 3 consecutive days of observation, except in the case of one experiment, where it only lasted for 2 days. A total of 76 individuals from 12 different forager groups from 8 different colonies were observed during both the Pre and Post-removal phases (Table 3.1).

Figure 3.1

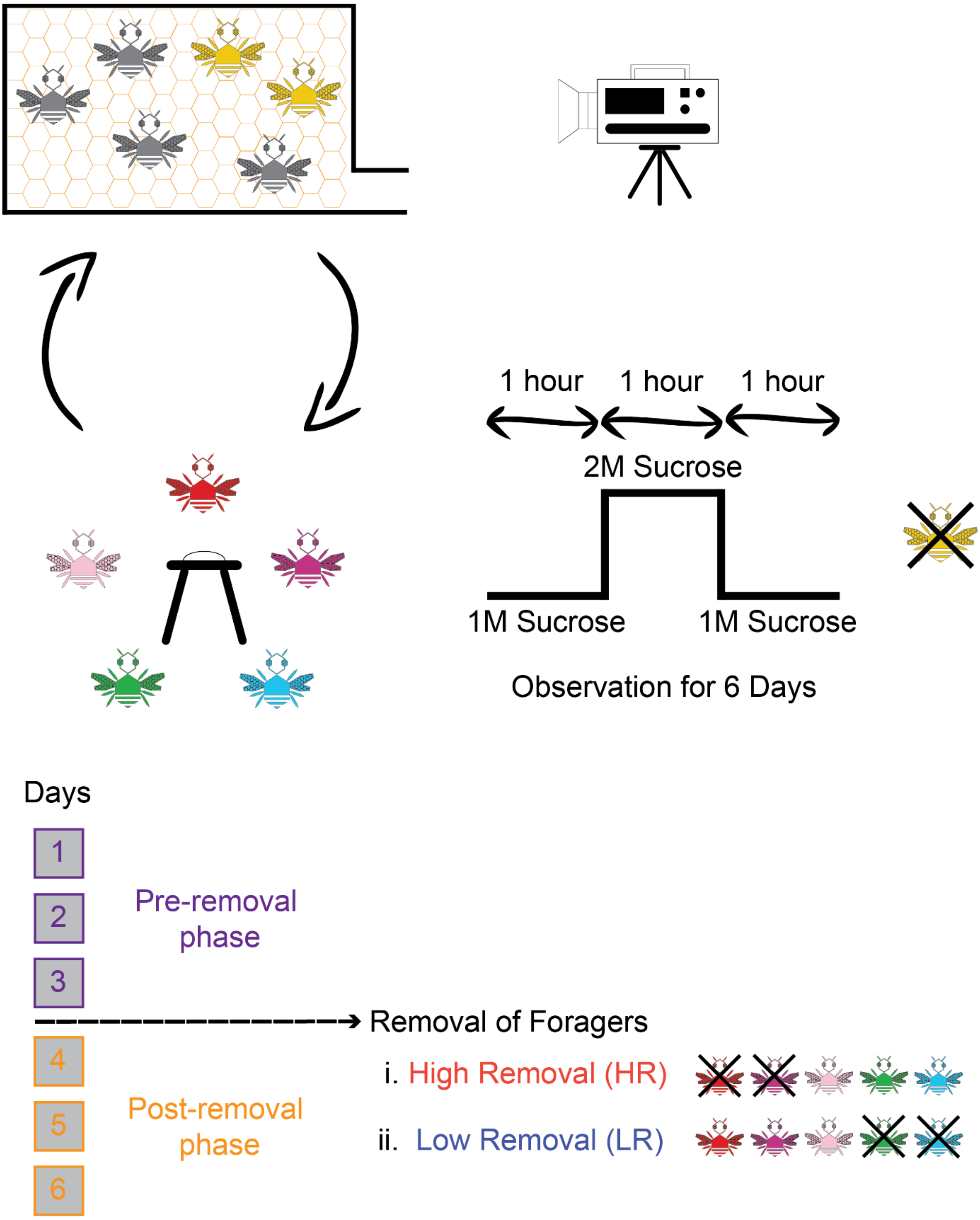

Figure 3.1: Experimental protocol for the removal experiments. The basic experimental protocol is the same as the consistency experiments with 3-hour observations per day. The removal experiments were done over 6 days and consisted of 2 phases, a pre-removal phase (days 1-3) and a post-removal phase (days 4-6). Some of the foragers from the observed group were removed before the start of observations on day 4. Two types of removal experiments were done, high removal (HR) in which the foragers most

active in the recruitment process were removed and low removal (LR) in which the least active foragers were removed (see Table 3.2).

Removal of Foragers

Two types of removal experiments were done; one in which the most active foragers were removed (high removal (HR), 8 replicates, H1 to H8) and another in which the least active foragers were removed (low removal (LR), 4 replicates, L1 to L4). This was to understand whether forager groups were made up of 'key' individuals, who would have a disproportionate effect on the other foragers. If the HR and LR experiments affected foragers differently, then it is likely that either the high dance activity foragers or the low dance activity foragers are key individuals in the forager group, based on whether the HR or the LR had the greater effect on the activity of the remaining foragers. In contrast, if both HR and LR affected the remaining individuals similarly, then foragers in the group are likely responding to the loss of foragers from the group itself and not to the identity of the individuals removed. Finally, if neither HR or LR had an effect on the remaining foragers, then the dance activity of foragers within these groups only depends on environmental conditions (like food reward) and not on social conditions (like the composition of the forager group).

In the HR replicates, only those foragers were removed who were more active than the average pre-removal activity level of the whole forager group (Table 3.2). A maximum of 4 most active foragers which had contributed to at least 50% of the total dance activity of the group before the removal (except in one forager group H5) were removed. In the LR replicates, only those foragers were removed whose activity was less than the average activity of the whole forager group (Table 3.2). A maximum of 4 least active foragers who had contributed to at most 20% of the total dance activity of the group before the removal were removed. There was no significant difference (two-tailed t-test, $t = 0.53$, $df = 9.38$, $p = 0.61$) in the percentage of foragers removed from both sets of replicates (HR, $n = 8$, mean ± standard deviation = 35.91% ± 9.09%; LR, $n = 4$, mean ± standard deviation = 34.01% ± 3.03%).

Table 3.2

Forager Group Name	Forager Group Size	Number of Foragers Removed	(Average Individual Activity/Average Group Activity) of Removed Foragers	Percentage Contribution of Removed Foragers to Total Pre-Removal Activity
H1	8	2	2.94, 1.10	50.40
H2	11	4	2.44,1.73,1.45,1.09	61.12
H3	7	3	1.62,1.56,1.39	65.34
H4	10	4	1.82,1.80,1.65,1.38	66.60
H5	9	2	1.99,1.91	43.35
H6	12	4	1.77,1.61,1.45,1.44	52.19
H7	8	3	2.21,1.80,1.48	66.56
H8	8	4	1.44,1.27,1.18,1.11	62.44
L1	10	3	0.26,0.75,0.76	17.77
L2	12	4	0.16,0.36,0.50,0.60	13.46
L3	11	4	0.19,0.36,0.42,0.50	10.80
L4	11	4	0.20,0.20,0.34,0.56	12.88

Table 3.2: The parameters by which the foragers were removed in the removal experiments. The initial size of the forager group, the number of foragers removed for each group, along with the ratio of the average activity of the removed foragers to the average activity of the whole group, and the percentage contribution of the removed foragers to the total activity of the whole group before the removal is given. The ratio of the average individual activity to the average group activity was used to decide which forgers were removed. Foragers which were more active than the group average (ratio > 1) were removed in the High Removal (HR) and foragers which were less active than the group average (ratio < 1) were removed in the Low Removal (LR). The percentage contribution of the foragers to the total pre-removal activity of each forager group is also provided. In HR, up to the 4 most active foragers were removed which had contributed to at least 50% of the total dance activity (except in H5). In LR, up to the 4 least active foragers were removed which had contributed to at most 20% of the total dance activity.

Video Analysis

The recorded dances of the foragers were then analysed manually using the open source VLC Media Player. Since the feeder location was very close to the hive, most foragers did dances with very short waggle phases (Gardner, Seeley, & Calderone, 2008). The total number of circuits performed by each forager during each dance was obtained from the videos. Each dance circuit involved the forager walking in a circular path with a short waggling motion of her abdomen at the end of the path (Sen Sarma, Esch, & Tautz, 2004).

Statistical Analysis

The statistical analysis focussed on 6 different behavioural parameters estimated for each bee for each day. They were: 1) *total number of dance circuits*, 2) *total number of dances*, 3) *total number of foraging trips*, 4) *the probability of dancing* (the ratio of the total number of dances to the total number of trips), 5) the ratio of the total number of circuits to the total number of dances (referred to as *Circuits/Dances*) and 6) the ratio of the total number of circuits to the total number of trips (referred to as *Circuits/Trips*). The last two ratios are different methods of calculating the intensity of the dances made by the foragers. Circuits/dances gives an estimate of how much a forager dances on average and is a good proxy for how rewarding the food source is (Seeley, 1994). On the other hand, the circuits/trips parameter provides an estimate for the intensity of the dances normalised to differences in the number of foraging trips made by different foragers over the 6 days. All 6 parameters were calculated from the total activity of each forager over 3 hours.

The analysis of the removal experiments was done at the level of the forager group and at the level of the individual forager to compare the responses at the two different levels. Only data from those experimental foragers that were remaining after the removal were used in this analysis (76 individuals). Further, at each level, the analysis was done to address two specific questions: 1) which forager groups/individual foragers showed a change in activity due to the removal and 2) which predictors correlated with this change?

Linear mixed effects models (LMMs) were built to compare the Pre and Post-removal activity of each forager group/individual forager. The 6 parameters were the response variables, an interaction term between the variable of interest (forager groups/individual foragers; both categorical variables) and the removal (a categorical variable of 2 levels, Pre and Post) was the predictor and the observation number was a random effect in the models. The parameters were scaled to normalise them with a mean of zero and a standard deviation of one. For the forager group level analysis, the scaling was done over each individual forager's activity throughout the experiment. This allowed comparisons of the average group activity without the confounding of individual differences in activity. For the individual forager level analysis, the scaling was done over each forager group's activity throughout the experiment to compare across parameters and forager groups. Data from each forager group was analysed separately at the level of the individual forager.

To identify which predictors correlated with the change in activity shown by forager groups, model comparisons were done (Table 3.3). Sets of generalized linear models (GLMs) were built with the difference in mean activity between Post and Pre-removal condition in the parameters as the response variables (obtained from the previous analysis), different predictors in each model and a Gaussian error distribution. A cut-off value of 95% Akaike weights was used to shortlist models (Wagenmakers & Farrell, 2004). Only those predictors in these models with a large model averaged effect size and confidence intervals not overlapping zero at the 95% confidence level were considered to be significantly correlated with the difference in activity in the forager groups.

LMMs were built to test if individual differences in activity after the removal correlated with the Pre-removal ranking (in the 3 parameters and in circuits, dances and trips) amongst the remaining foragers. The response variable was the difference in mean activity between Post and Pre-removal condition of individuals in the 6 parameters. Separate models were built for each parameter with the same set of six predictors and with the forager group as a random effect. For each of the parameters, data from only those forager groups in which at least one forager showed a significant change were used in this analysis. The effect sizes and bootstrapped confidence intervals of each of the predictors were used to determine which predictor had an important effect on the response variable.

Table 3.3

Predictor	**Number of Models Present In**	**Model Numbers**
Colony Experience	5	1,7,8(*),9(*),10(*), 11(*)
Year	1	2
Month	3	2,7,8(*)
Forager group Size	5	3,9(*),12(*),14,15
Number of Foragers Removed	5	3,10(*),13(*),16,17
Proportion of Foragers Removed	6	4,11(*),12(*),13(*),18,19
Average Circuits (Pre-Removal)	5	5,14,16,18,20
Average Intensity (Pre-Removal)	5	5,14,16,18,20
Average Trips (Pre-Removal)	5	5,14,16,18,20
Average Circuits (Post-Removal)	5	6,15,17,19,20
Average Circuits/Dances (Post-Removal)	5	6,15,17,19,20
Average Trips (Post-Removal)	5	6,15,17,19,20

Table 3.3: Predictors used for model comparisons along with the number of models they are present in and the model numbers used for the analysis of the removal experiments at the forager group level. Model numbers with (*) indicate that an interaction term is present in the model between the two predictors with the same model number.

LMMs were built using the nlme (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2018) and lme4 package (Bates, Mächler, Bolker, & Walker, 2015) and multiple comparisons and p value adjustments were done using the glht function in the multcomp package (Hothorn, Bretz, & Westfall, 2008). Generalized linear models were built using the base stats package and model selection and averaging was done using the MuMIn package (Barton, 2017) in R (R Core Team, 2018). All the graphs were made in R using the ggplot2 (Wickham, 2016) and cowplot (Claus O. Wilke, 2018) package.

Results

Dance Circuits

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the dance circuits at the forager group level (Fig. 3.2 *a*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = 0.6065 ± 0.4181 vs -0.0157 ± 0.6360, difference estimate = -0.6221, CI = -1.2957 – 0.0515, p = 0.0666). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *a*; HR vs LR: n = 47 vs 29, scaled mean ± standard deviation = 0.5178 ± 0.7654 vs -0.0015 ± 0.8539, difference estimate = -0.5589, CI = -1.1830 – 0.0651, *p* = 0.0739).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Forager group level analysis

The removal of foragers led to a significant change in the average dance circuits of 2 out of 12 forager groups observed (Fig. 3.4 *a*). Both these forager groups (H4 and H7) showed an increase in their average dance circuits.

The model comparisons showed that the important predictors for the change in average dance circuits of the forager group were the proportion of foragers removed, the forager group size, the number of foragers removed and the colony experience (number of experiments done with the colony) (Table 3.4). Of these, only the proportion of foragers removed had a large effect size and it had a significant positive correlation with the change in activity (effect size = 4.497, CI = 0.266 – 8.728, *p* = 0.037). Forager group size (effect size = -0.268, CI = -0.502 – -0.034, *p* = 0.025) had a smaller effect size with a significant negative correlation. The number of foragers removed (effect size = 0.491, CI = -0.014 – 0.997, *p* = 0.057) and the colony experience (effect size = 0.203, CI = -0.167 – 0.574, *p* = 0.282) both had small non-significant positive correlations with the change in the average probability of dancing of forager groups.

Individual forager level analysis

The removal of foragers led to a significant change in the dance circuits of 12 out of 76 foragers from 8 out of 12 forager groups observed (Fig. 3.5, 3.6 and 3.17 *a*). 9 foragers from 7 different forager groups (2 foragers from H1, 1 from H3, 2 from H4, 1 from H7, 1 from L2, 1 from L3, 1 from L4) showed an increase in their dance circuits. 3 foragers from 3 different forager groups (1 forager each from H1, l1 and L3) showed a decrease in their dance circuits.

To identify the correlation between the change in dance circuits and the activity level of individual foragers before the removal, only data from the 8 forager groups in which at least one forager showed a significant change in its dance circuits were considered. The change in dance circuits at the individual forager level did not correlate with the pre-removal ranking in any of the 6 parameters (Fig. 3.19 *a*).

Comparison of group level and individual level responses

In the total dance circuits, two forager groups (H4 and H7) showed a change after the removal, whereas some individual foragers in 8 forager groups (H1, H3, H4, H7, L1, L2, L3 and L4) showed a significant change in their activity after the removal. Thus, in the case of 6 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity.

Number of Dances

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the number of dances at the forager group level (Fig. 3.2 *b*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = 0.8142 ± 0.5218 vs 0.2359 ± 0.4576, difference estimate = -0.5783, CI = -1.2652 – 0.1085, p = 0.0901). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *b*; HR vs LR: n =

47 vs 29, scaled mean ± standard deviation = 0.7176 ± 0.7135 vs 0.2074 ± 0.9519, difference estimate = -0.5445, CI = -1.1196 – 0.0305, *p* = 0.0611).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Figure 3.2

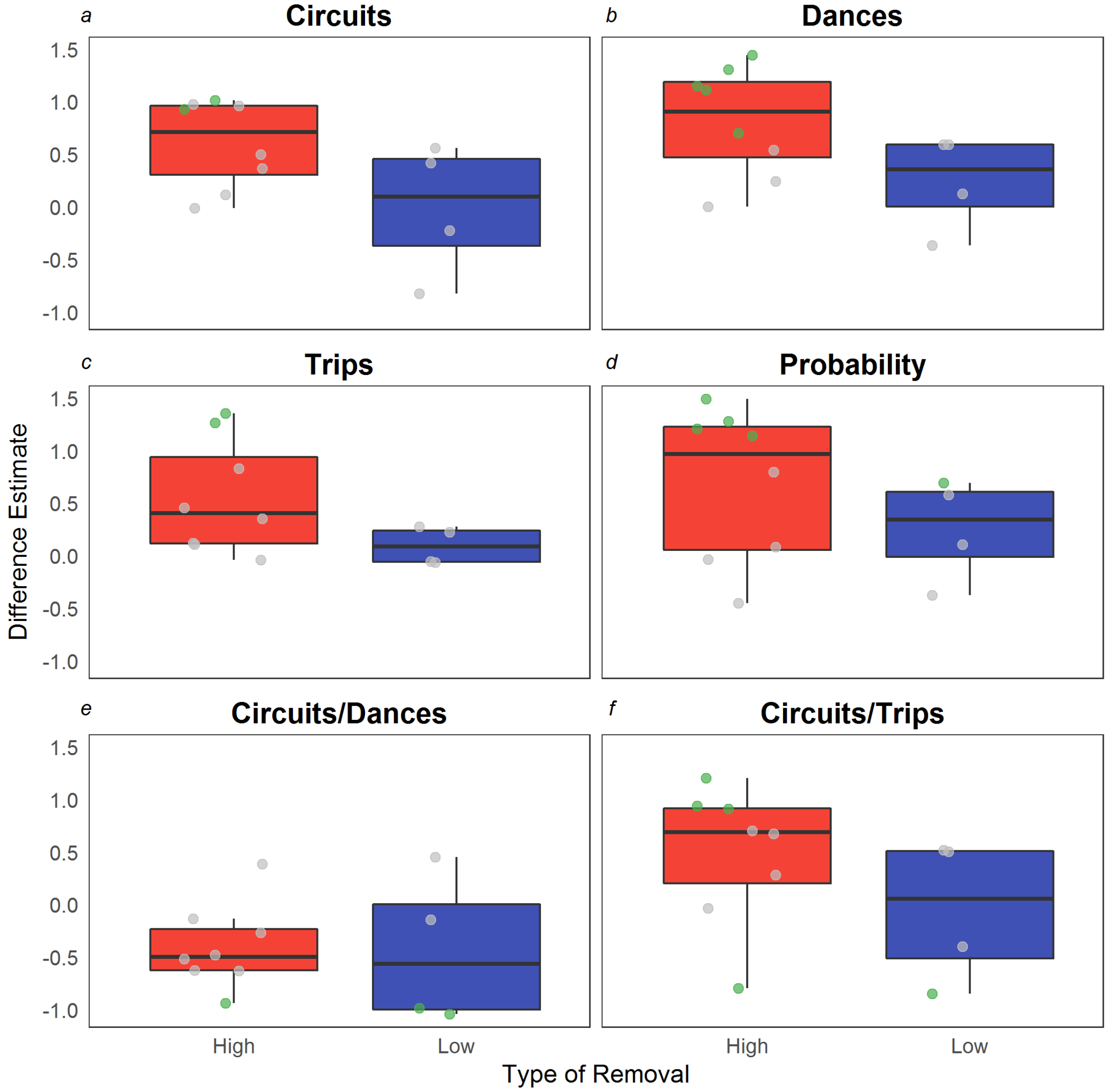

Figure 3.2: Boxplots of the change in activity in all 6 parameters after the High Removal (HR) and Low Removal (LR) experiments. Each circle represents the change in scaled activity shown by a single forager group (n = 12). Green circles represent groups which showed a significant change in activity while grey circles represent groups which did not show any significant change in activity. There were no

significant differences in the change in activity shown by forager groups between the two types of removals (HR vs LR) in any of the 6 parameters.

Figure 3.3

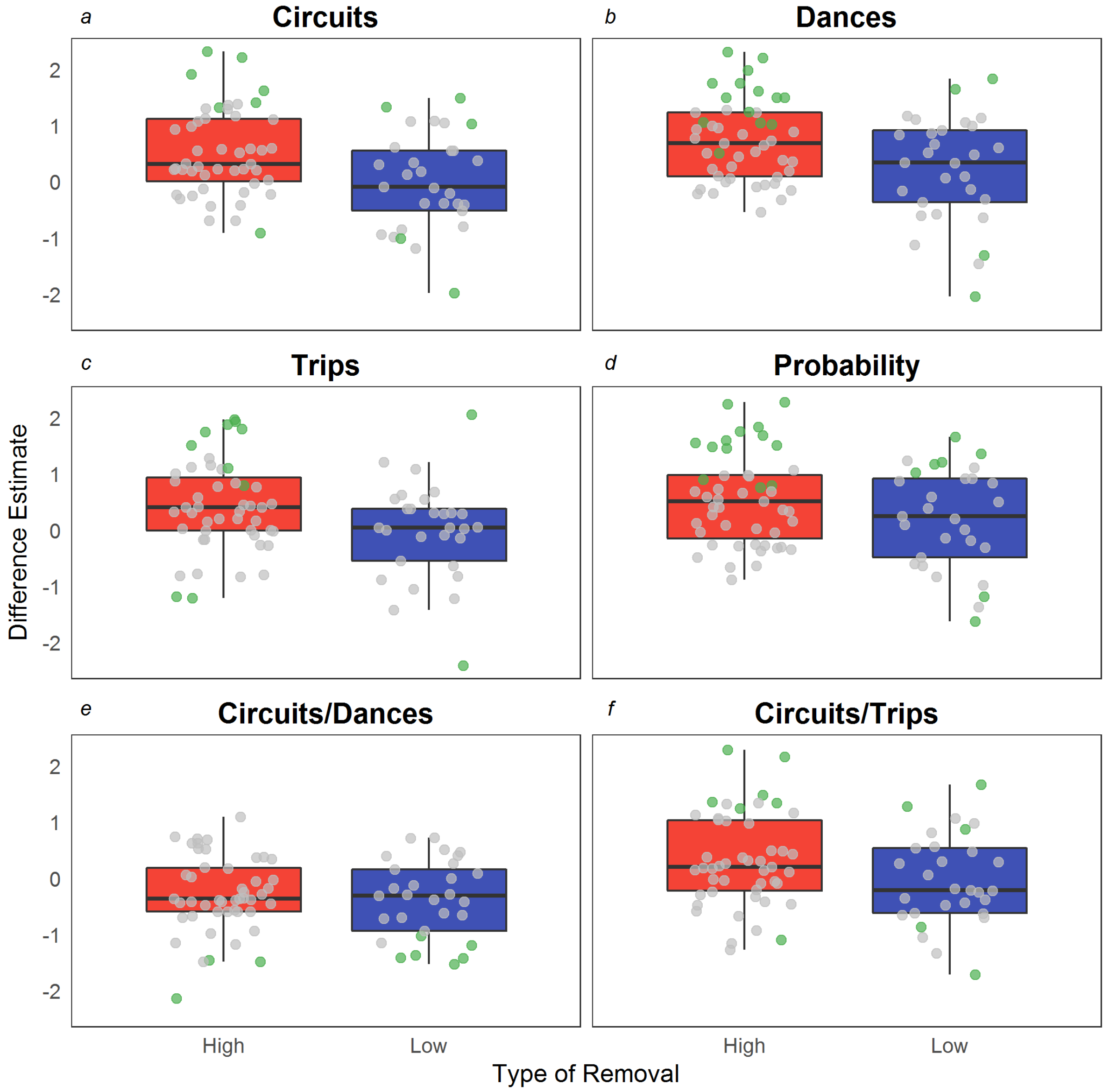

Figure 3.3: Boxplots of the change in activity in all 6 parameters after the High Removal (HR) and Low Removal (LR) experiments. Each circle represents the change in scaled activity shown by a single forager (n = 76). Green circles represent individuals which showed a significant change in activity while grey circles represent individuals which did not show a significant change in activity. There were no significant differences in the change in activity shown by foragers between the two types of removals (HR vs LR) in any of the 6 parameters.

Figure 3.4

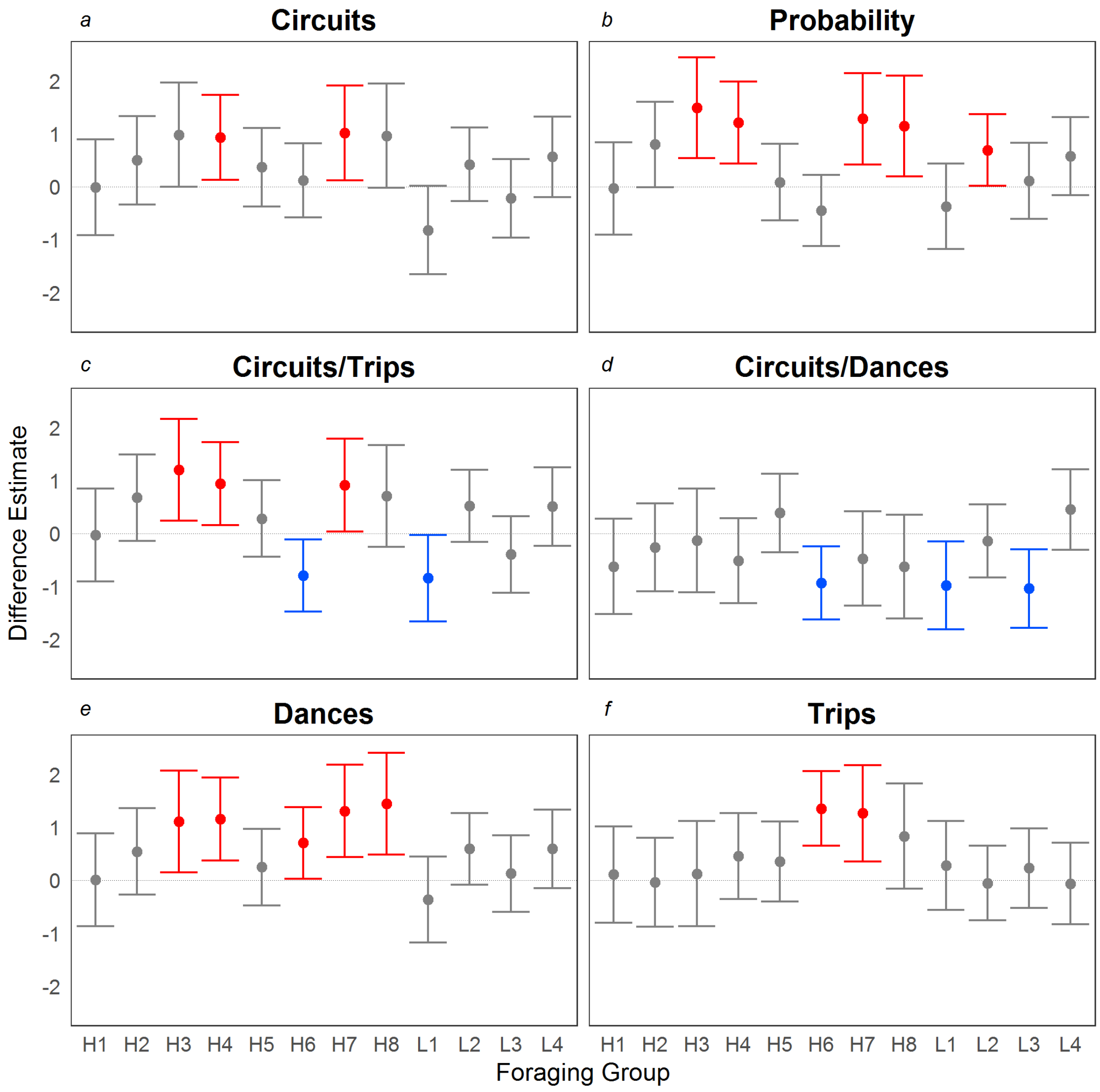

Figure 3.4: The difference in the average scaled activity between post-removal and pre-removal phase of 12 forager groups in all 6 parameters. Circles represent the difference estimate obtained from linear mixed effects models and the error bars represent the 95% confidence intervals around this estimate. Circles and error bars are colour coded based on the significance and the direction of change. Red represents groups which showed a significant increase, blue represents groups which showed a significant decrease and grey represents groups which showed no significant change.

Table 3.4

Parameter	**Predictor**	**Effect Size (Confidence Interval)**	**Relative Importance of Predictor (p Value)**	**dAIC of Model containing Predictor (Cumulative Weight)**
Dance Circuits	*Proportion of foragers removed*	*4.497* *(0.266 – 0.728)*	*0.72* *(0.037)*	*0.00* *(0.681)*
	Forager group size	-0.268 (-0.502 – -0.034)	0.17 (0.025)	2.94 (0.157)
	Number of foragers removed	0.491 (-0.014 – 0.997)	0.17 (0.057)	2.94 (0.157)
	Colony experience	0.203 (-0.167 – 0.574)	0.11 (0.282)	3.67 (0.109)
Number of Dances	*Proportion of foragers removed*	*5.765* *(2.435 – 9.095)*	*0.87* *(0.001)*	*0.00* *(0.836)*
	Forager group size	-0.290 (-0.482 – -0.099)	0.13 (0.003)	3.86 (0.122)
	Number of foragers removed	0.648 (0.234 – 1.061)	0.13 (0.002)	3.86 (0.122)
Number of Foraging Trips	Proportion of foragers removed	1.388 (-3.138 – 5.914)	0.46 (0.548)	0.00 (0.436)
	Colony experience	-0.093 (-0.435 – 0.248)	0.44 (0.593)	0.11 (0.413)
	Average circuits (Post-Removal)	-0.001 (-0.003 – 0.002)	0.10 (0.818)	3.17 (0.090)
	Average circuits/dances (Post-removal)	0.016 (-0.107 – 0.138)	0.10 (0.800)	3.17 (0.090)
	Average trips (Post-removal)	0.037 (0.006 – 0.071)	0.10 (0.019)	3.17 (0.090)

Probability	*Proportion of foragers removed*	*6.331* *(1.918 – 10.744)*	*0.62* *(0.005)*	*0.00* *(0.591)*
	Forager group size	-0.377 (-0.605 – -0.149)	0.32 (0.002)	1.31 (0.307)
	Number of foragers removed	0.692 (0.199 – 1.185)	0.32 (0.006)	1.31 (0.307)
	Colony experience	0.339 (-0.067 – 0.745)	0.05 (0.101)	4.87 (0.052)
Circuits/ Dances	Proportion of foragers removed	-0.981 (-5.560 – 3.589)	0.53 (0.675)	0.00 (0.492)
	Colony experience	-0.023 (-0.371 – 0.325)	0.47 (0.897)	0.24 (0.435)
Circuits/ Trips	Proportion of foragers removed	4.615 (-0.905 – 10.135)	0.49 (0.101)	0.00 (0.469)
	Colony experience	0.307 (-0.123 – 0.737)	0.32 (0.161)	0.86 (0.305)
	Forager group size	-0.322 (-0.616 – -0.029)	0.18 (0.032)	1.98 (0.174)
	Number of foragers removed	0.485 (0.149 – 1.119)	0.18 (0.134)	1.98 (0.174)

Table 3.4: Results of model comparisons done to look at what predictors correlated with the change in activity of forager groups in each of the 6 parameters. The important predictors and their effect sizes, confidence intervals, *p* values, relative importance, the number of models the predictor is present in, dAIC and cumulative weight of the corresponding model is provided for all parameters. Predictors are represented with numbers, with 1 for *Proportion of Foragers Removed*, 2 for *Forager Group Size*, 3 for *Number of Foragers Removed*, 4 for *Colony Experience*, 5 for *Average Circuits (Post-Removal)*, 6 for *Average Circuits/Dances (Post-Removal)*, and 7 for *Average Trips (Post-Removal)*. Predictors highlighted in italics in the table were considered to show strong correlations with the parameter of interest as they had large effect sizes with confidence intervals not overlapping zero.

Figure 3.5

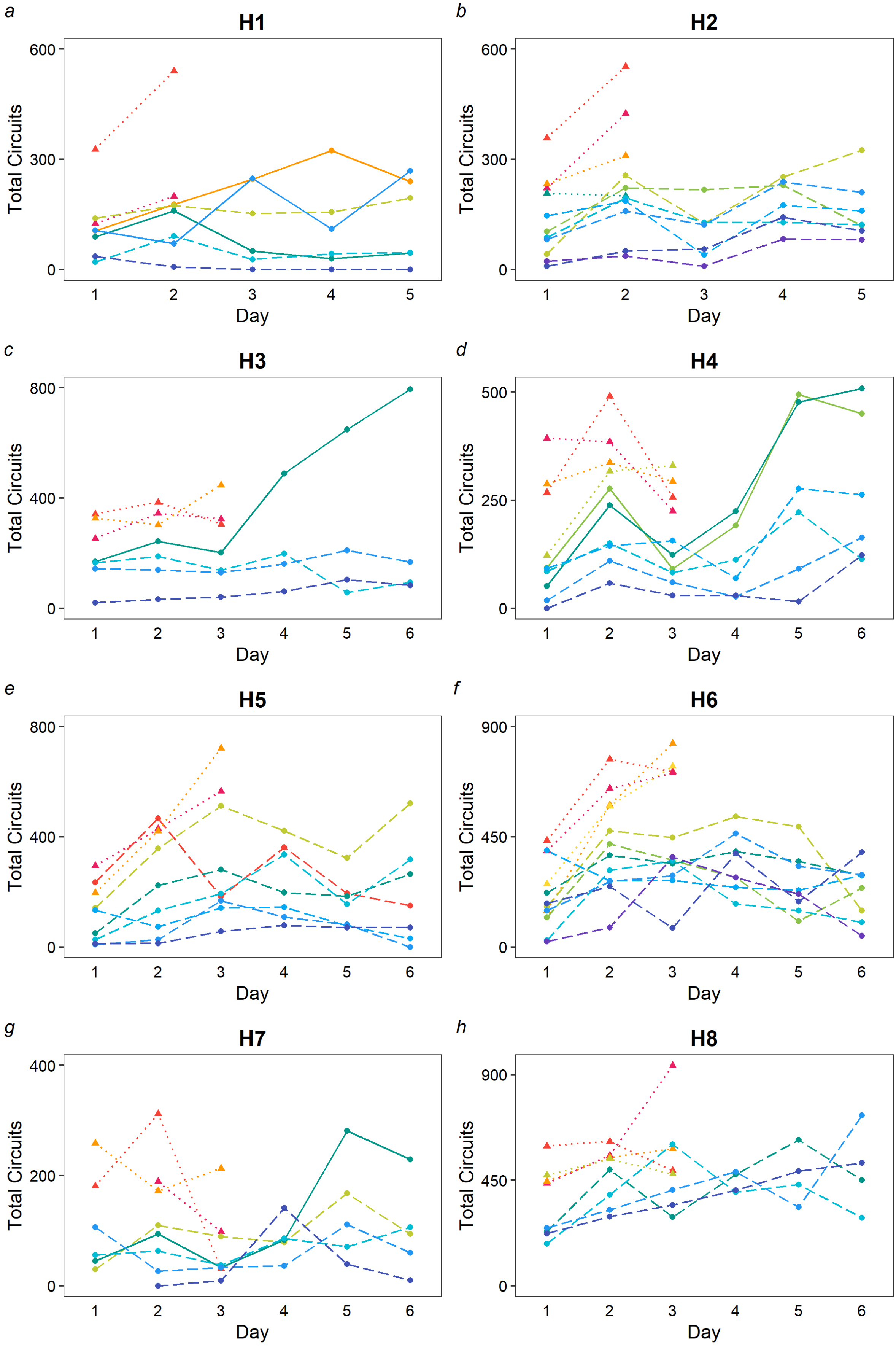
a
H1
Total Circuits
600
300
0
1
2
3
4
5
Day
b
H2
Total Circuits
600
300
0
1
2
3
4
5
Day
c
H3
Total Circuits
800
400
0
1
2
3
4
5
6
Day
d
H4
Total Circuits
500
250
0
1
2
3
4
5
6
Day
e
H5
Total Circuits
800
400
0
1
2
3
4
5
6
Day
f
H6
Total Circuits
900
450
0
1
2
3
4
5
6
Day
g
H7
Total Circuits
400
200
0
1
2
3
4
5
6
Day
h
H8
Total Circuits
900
450
0
1
2
3
4
5
6
Day

Figure 3.5: The total dance circuits made by all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.6

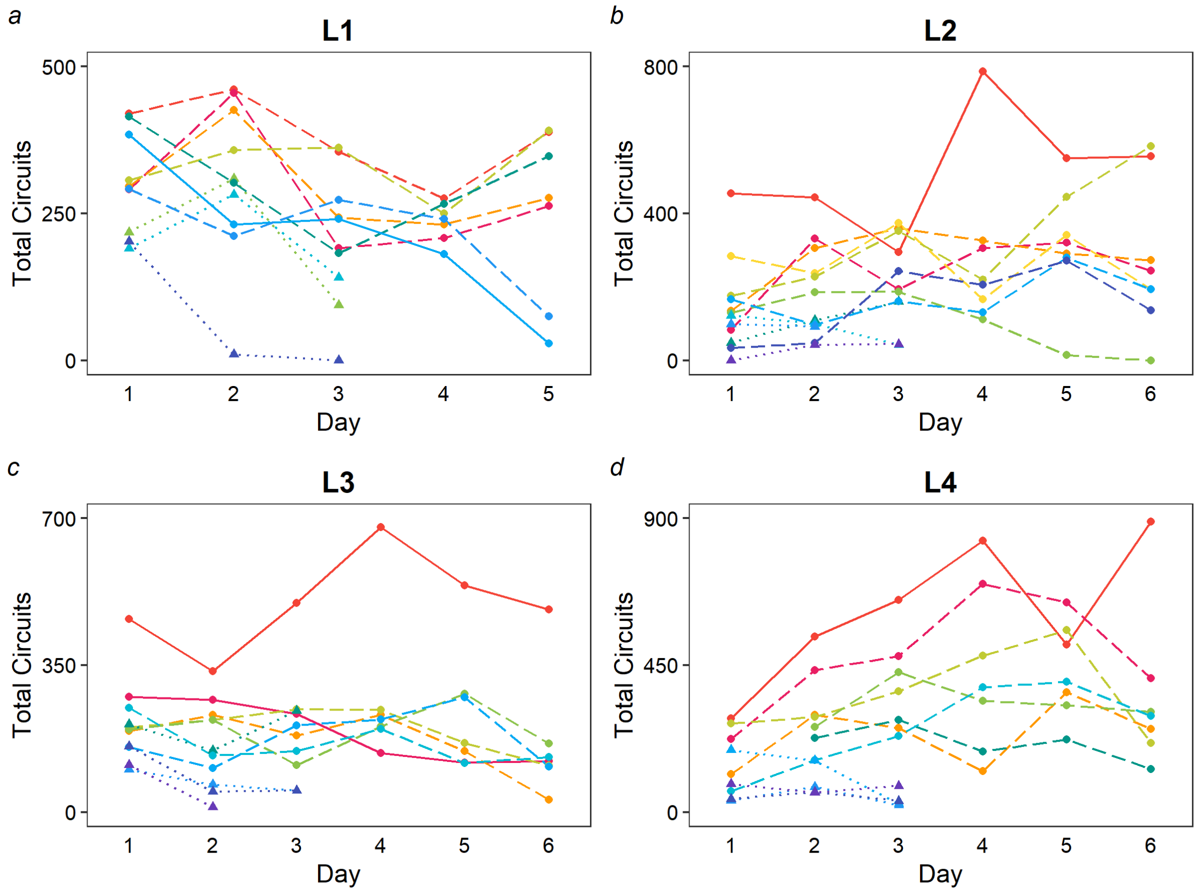

Figure 3.6: The total dance circuits made by all individuals observed from each of the 4 forager groups in the Low Removal (LR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Forager group level analysis

The removal of foragers led to a significant change in the average number of dances of 5 out of 12 forager groups observed (Fig. 3.4 *b*). All 5 forager groups (H3, H4, H6, H7 and H8) showed an increase in their average number of dances.

The model comparisons showed that the important predictors for the change in average number of dances of the forager group were the proportion of foragers removed, the forager group size and the number of foragers removed (Table 3.4). Of these, only the proportion of foragers removed had a large effect size and it had a significant positive correlation with the change in activity (effect size = 5.765, CI = 2.435 – 9.095, p = 0.001). Forager group size (effect size = -0.290, CI = -0.482 – -0.099, p = 0.003) and the number of foragers removed (effect size = 0.648, CI = 0.234 – 1.061, p = 0.002) had smaller effect sizes with a significant negative and positive correlation respectively.

Individual forager level analysis

The removal of foragers led to a significant change in the number of dances of 18 out of 76 foragers from 10 out of 12 forager groups observed (Fig. 3.7, 3.8 and 3.17 *b*). 16 foragers from 8 different forager groups (2 foragers from H1, 1 from H2, 3 from H3, 4 from H4, 1 from H7, 3 from H8, 1 from L3 and 1 from L4) showed an increase in their number of dances. 2 foragers from 2 different forager groups (1 forager each from L1 and L2) showed a decrease in their number of dances.

To identify the correlation between the change in number of dances and the activity level of individual foragers before the removal, only data from the 10 forager groups in which at least one forager showed a significant change in its number of dances were considered. The change in the number of dances at the individual forager level did not correlate with the pre-removal ranking in any of the 6 parameters (Fig. 3.19 *b*).

Figure 3.7

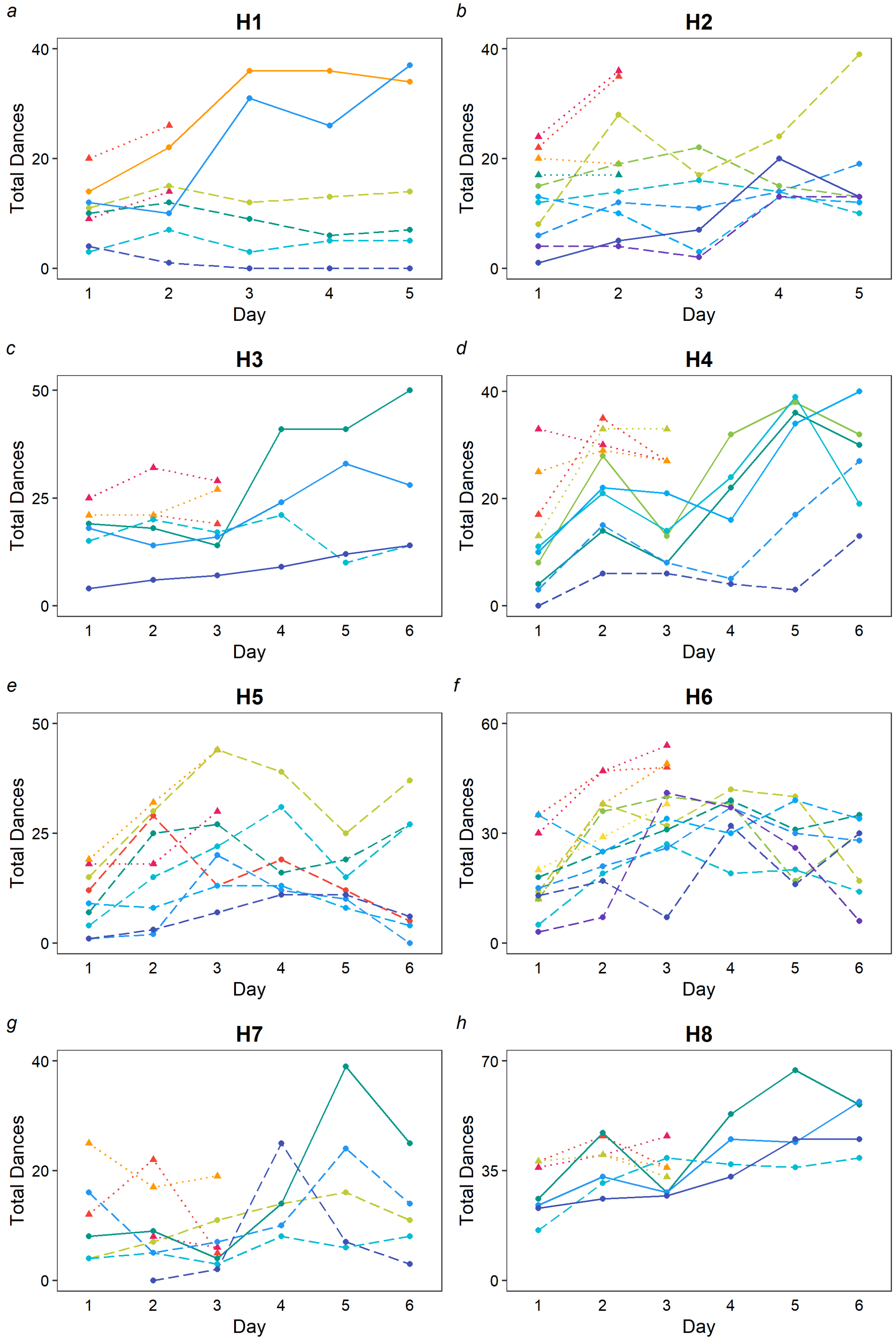
a
H1
Total Dances
0
20
40
1
2
3
4
5
Day
b
H2
Total Dances
0
20
40
1
2
3
4
5
Day
c
H3
Total Dances
0
25
50
1
2
3
4
5
6
Day
d
H4
Total Dances
0
20
40
1
2
3
4
5
6
Day
e
H5
Total Dances
0
25
50
1
2
3
4
5
6
Day
f
H6
Total Dances
0
30
60
1
2
3
4
5
6
Day
g
H7
Total Dances
0
20
40
1
2
3
4
5
6
Day
h
H8
Total Dances
0
35
70
1
2
3
4
5
6
Day

Figure 3.7: The number of dances made by all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.8

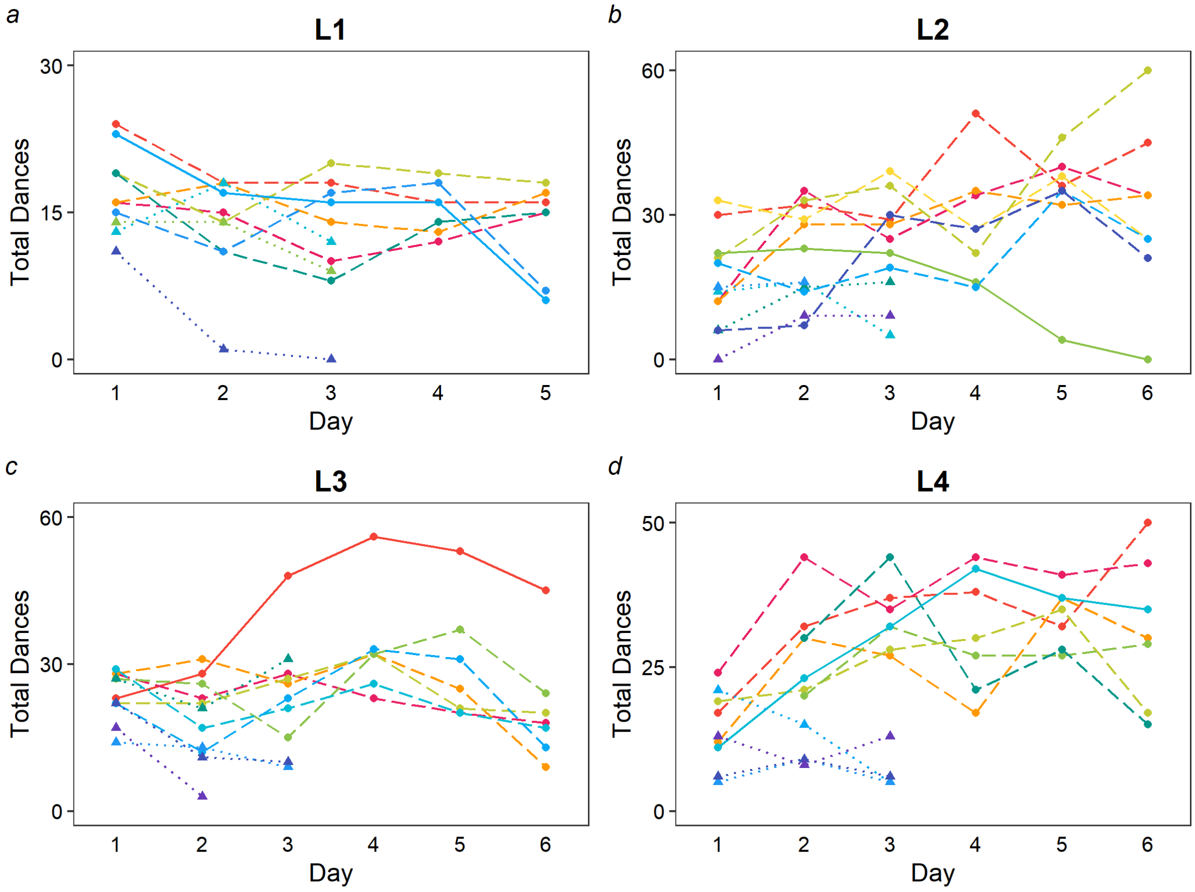

Figure 3.8: The number of dances made by all individuals observed from each of the 4 forager groups in the Low Removal (LR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Comparison of group level and individual level responses

In case of number of dances, only 5 forager groups (H3, H4, H6, H7 and H8) showed a change after the removal. Whereas some individual foragers in 10 forager groups (H1, H2, H3, H4, H7, H8, L1, L2, L3 and L4) showed a significant change in their activity after the removal. Thus, in the case of 5 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity. Interestingly, even though one forager group (H6) showed a change at the group level, there were no individual foragers which showed a significant change in activity in this group.

Foraging Trips

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the number of foraging trips at the forager group level (Fig. 3.2 *c*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = 0.5562 ± 0.5349 vs 0.0972 ± 0.1805, difference estimate = -0.4591, CI = -1.0844 – 0.1662, p = 0.1329). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *c*; HR vs LR: n = 47 vs 29, scaled mean ± standard deviation = 0.4249 ± 0.8049 vs -0.0147 ± 0.8784, difference estimate = -0.4197, CI = -1.0077 – 0.1682, *p* = 0.1428).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Forager group level analysis

The removal of foragers led to a significant change in the average number of foraging trips of 2 out of 12 forager groups observed (Fig. 3.4 *c*). Both these forager groups (H6 and H7) showed an increase in their average number of foraging trips.

The model comparisons showed that the important predictors for the change in average foraging trips of the forager group were the proportion of foragers removed, the colony experience (number of experiments done with the colony), the average Post-removal circuits done by the forager group, the average Post-removal circuits/dances of the forager group and the average Post-removal number of foraging trips done by the forager group (Table 3.4). Of these, only the average Post-removal number of foraging trips showed a significant correlation, but with a small positive effect size (effect size = 0.037, CI = 0.006 – 0.071, *p* = 0.019). The proportion of foragers removed (effect size = 1.388, CI = -3.138 – 5.914, *p* = 0.548) had a relatively larger effect size but the correlation was not significant. Colony experience (effect size = -0.093, CI = -0.435 – 0.248, *p* = 0.593) and the average Post-removal circuits (effect size = -0.001, CI = -0.003 – 0.002, *p* = 0.818) both had small negative effect sizes which were not significantly correlated. The average post-removal circuits/dances (effect size = 0.016, CI = -0.107 – 0.138, *p* = 0.800) had a small positive effect size which was not significantly correlated.

Individual forager level analysis

The removal of foragers led to a significant change in the number of foraging trips of 12 out of 76 foragers from 8 out of 12 forager groups observed (Fig. 3.9, 3.10 and 3.17 *c*). 9 foragers from 6 different forager groups (1 forager from H1, 1 from H2, 3 from H6, 1 from H7, 2 from H8 and 1 from L3) showed an increase in their number of foraging trips. 3 foragers from 3 different forager groups (1 forager each from H1, H3 and L2) showed a decrease in their number of foraging trips.

To identify the correlation between the change in number of foraging trips and the activity level of individual foragers before the removal, only data from the 8 forager groups in which at least one forager showed a significant change in its number of foraging trips were considered. The change in the number of foraging trips at the individual forager level did not correlate with the pre-removal ranking in any of the 6 parameters (Fig. 3.19 *c*).

Comparison of group level and individual level responses

In the number of foraging trips made, only 2 forager groups (H6 and H7) showed a change after the removal. Whereas some individual foragers in 8 forager groups (H1, H2, H3, H6, H7, H8, L2 and L3) showed a significant change in their activity after the removal. Thus, in the case of 6 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity.

Figure 3.9

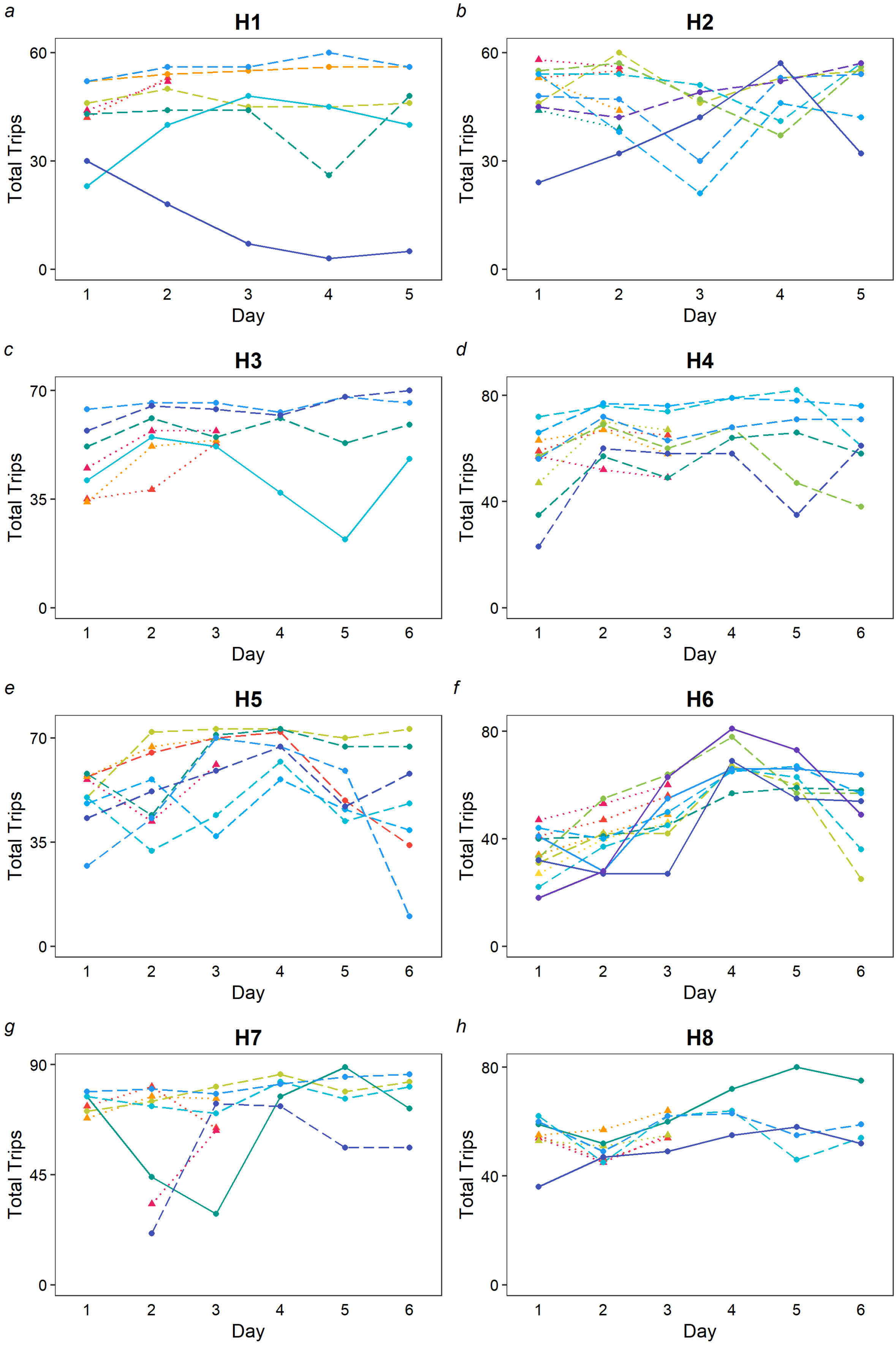
a
H1
Total Trips
60
30
0
1
2
3
4
5
Day
b
H2
Total Trips
60
30
0
1
2
3
4
5
Day
c
H3
Total Trips
70
35
0
1
2
3
4
5
6
Day
d
H4
Total Trips
80
40
0
1
2
3
4
5
6
Day
e
H5
Total Trips
70
35
0
1
2
3
4
5
6
Day
f
H6
Total Trips
80
40
0
1
2
3
4
5
6
Day
g
H7
Total Trips
90
45
0
1
2
3
4
5
6
Day
h
H8
Total Trips
80
40
0
1
2
3
4
5
6
Day

Figure 3.9: The number of foraging trips made by all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.10

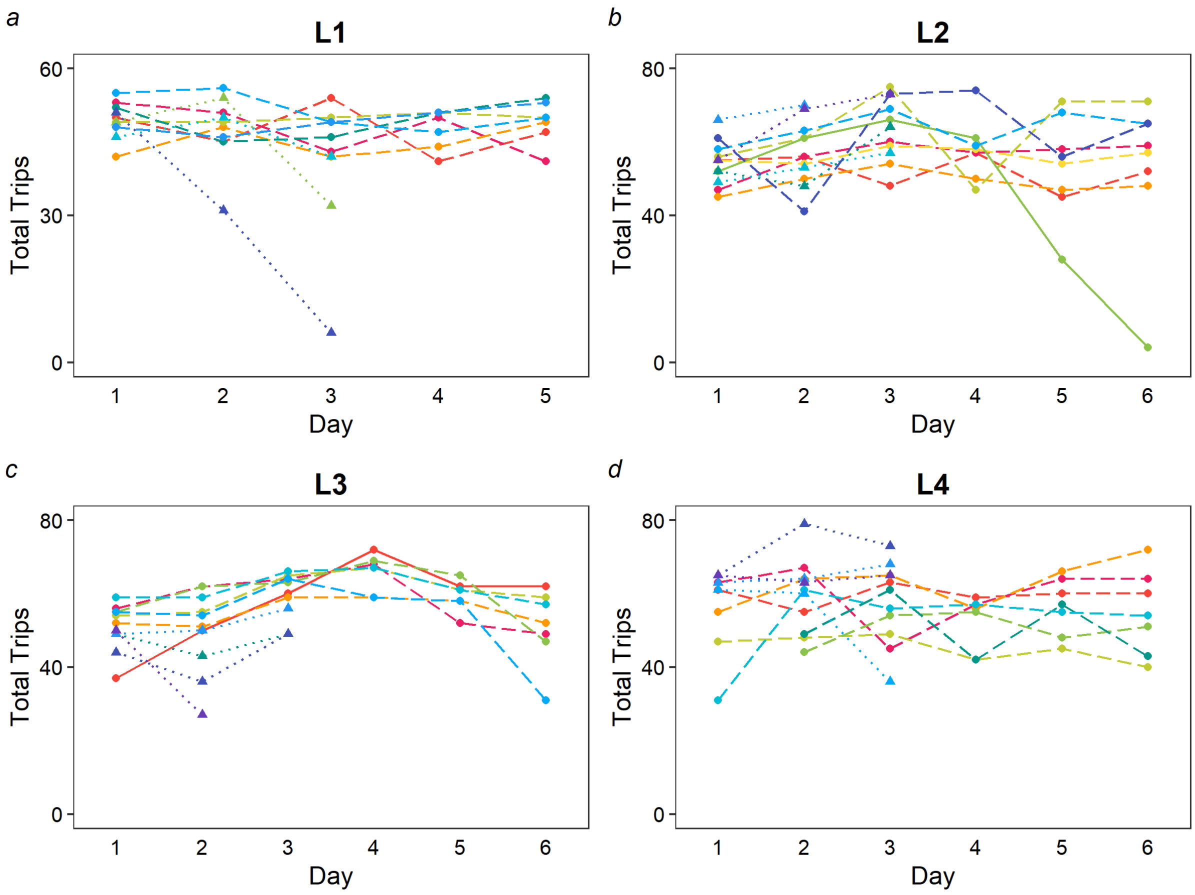

Figure 3.10: The number of foraging trips made by all individuals observed from each of the 4 forager groups in the Low Removal (LR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Probability of Dancing

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the probability of dancing at the forager group level (Fig. 3.2 *d*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = 0.6904 ± 0.7236 vs 0.2509 ± 0.4860, difference estimate = -0.4395, CI = -1.3419 – 0.4628, *p* = 0.3033). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *d*; HR vs LR: n = 47 vs 29, scaled mean ± standard deviation = 0.5388 ± 0.8025 vs 0.2088 ± 0.9007, difference estimate = -0.4064, CI = -1.1358 – 0.3229, *p* = 0.2427).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Forager group level analysis

The removal of foragers led to a significant change in the average probability of dancing of 5 out of 12 forager groups observed (Fig. 3.4 *d*). All 5 forager groups (H3, H4, H7, H8 and L2) showed a significant increase in their average probability of dancing.

The model comparisons showed that the important predictors for the change in average probability of dancing of the forager group were the proportion of foragers removed, the forager group size, the number of foragers removed and the colony experience (number of experiments done with the colony) (Table 3.4). Of these, only the proportion of foragers removed had a large effect size and it had a significant positive correlation with the change in activity (effect size = 6.331, CI = 1.918 – 10.744, *p* = 0.005). Forager group size (effect size = -0.377, CI = -0.605 – -0.149, *p* = 0.002) and the number of foragers removed (effect size = 0.692, CI = 0.199 – 1.185, *p* = 0.006) had smaller effect sizes with a significant negative and positive correlation respectively. The colony experience had a small non-significant correlation with the change in the average probability of dancing of forager groups (effect size = 0.339, CI = -0.067 – 0.745, *p* = 0.101).

Figure 3.11

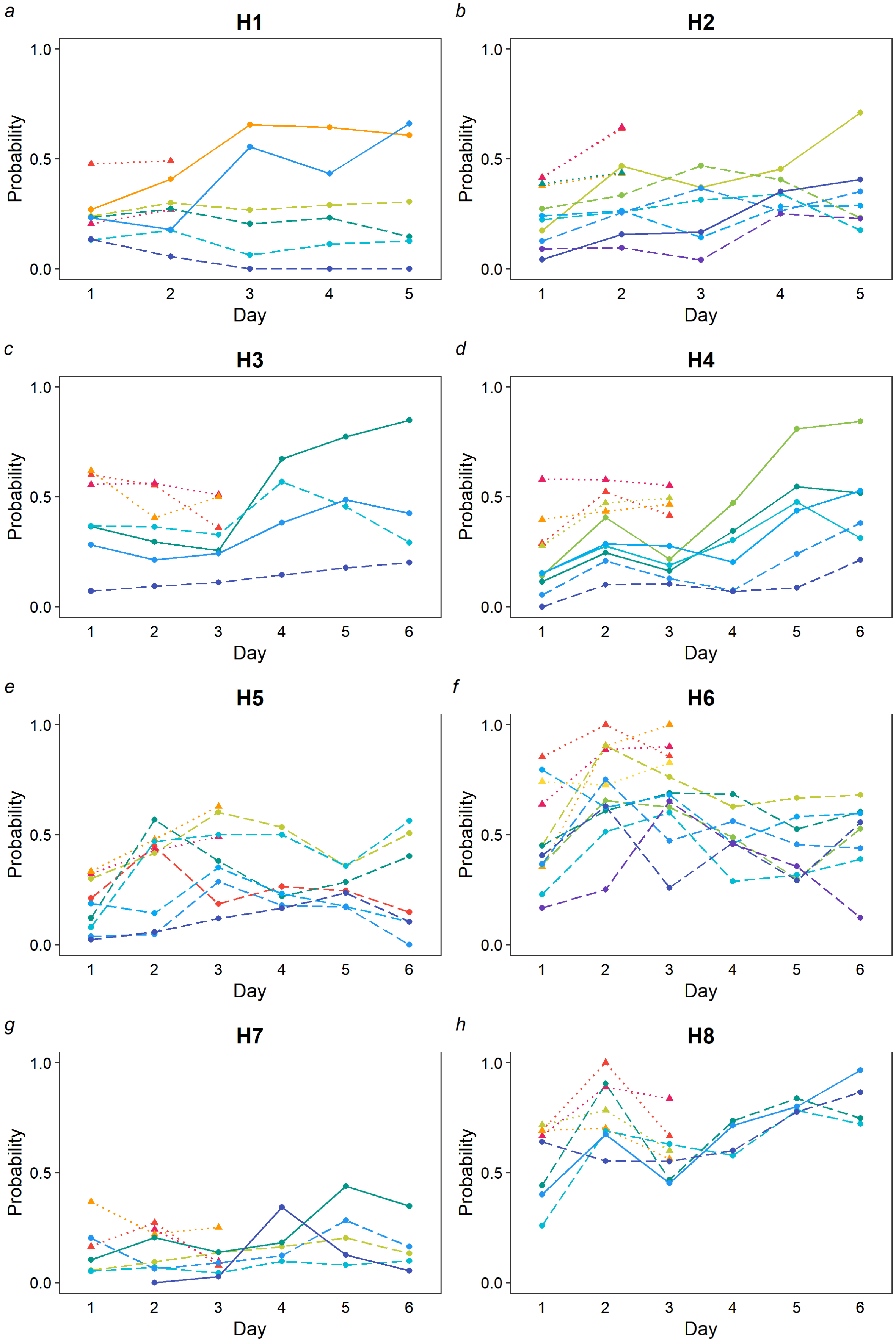
a
H1
b
H2
c
H3
d
H4
e
H5
f
H6
g
H7
h
H8
Probability
0.0
0.5
1.0
Day
1
2
3
4
5
6

Figure 3.11: The probability of dancing of all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.12

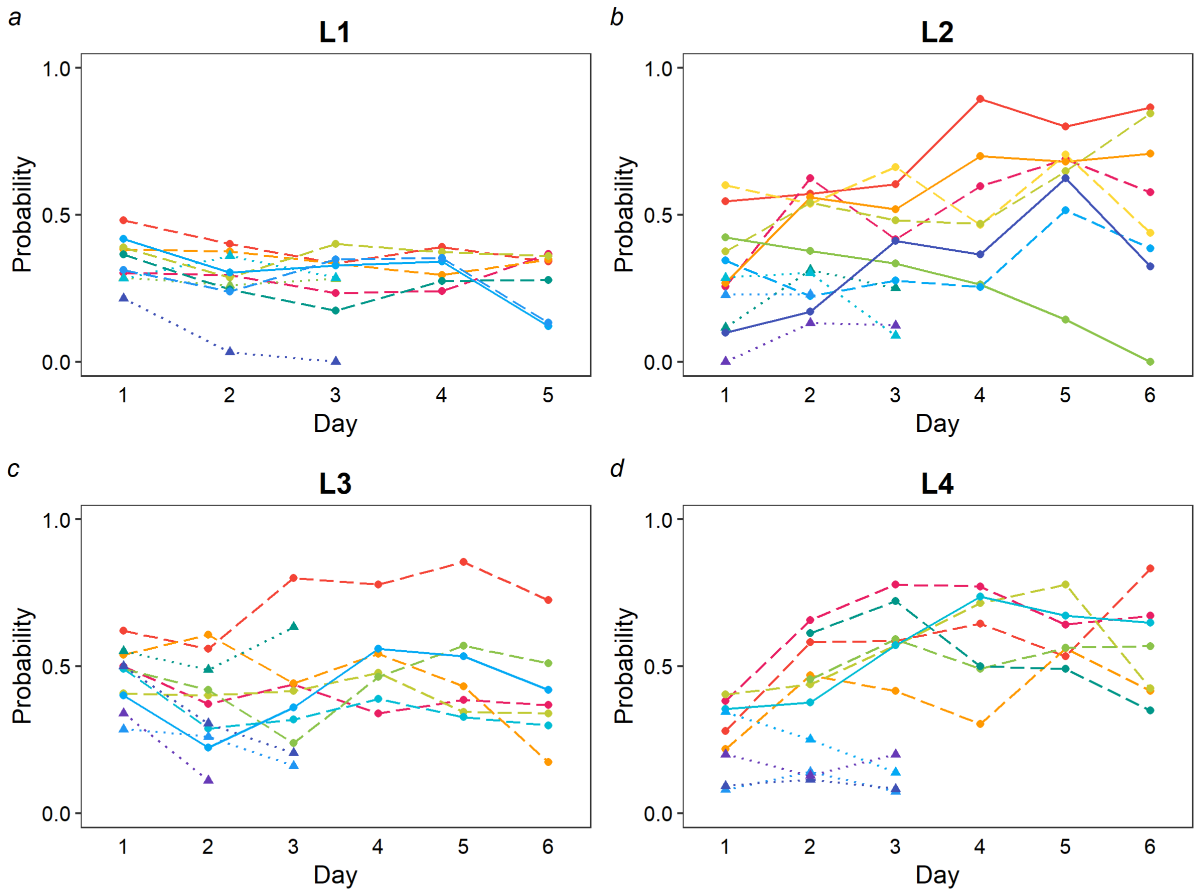

Figure 3.12: The probability of dancing of all individuals observed from each of the 4 forager groups in the Low Removal (LR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Individual forager level analysis

The removal of foragers led to a significant change in the dance probability of 20 out of 76 foragers from 10 out of 12 forager groups observed (Fig. 3.11, 3.12 and 3.18 *a*). 18 foragers from 9 different forager groups (2 foragers from H1, 2 from H2, 2 from H3, 4 from H4, 2 from H7, 1 from H8, 3 from L2, 1 from L3 and 1 from L4) showed an increase in their dance probability. 2 foragers from 2 different forager groups (1 forager from L1 and 1 forager from L2) showed a decrease in their dance probability.

To identify the correlation between the change in probability of dancing and the activity level of individual foragers before the removal, only data from the 10 forager groups in which at least one forager showed a significant change in its probability of dancing were considered. The change in probability of dancing strongly correlated with the ranking in pre-removal dance circuits (Fig. 3.19 *d*; effect size = -0.453, CI = -0.858 – -0.049, *p* = 0.029). The pre-removal ranking in the other parameters showed no correlation with the change in probability of dancing at the individual forager level.

Comparison of group level and individual level responses

In the probability of dancing, only 5 forager groups (H3, H4, H7, H8 and L2) showed a change after the removal. Whereas some individual foragers in 10 forager groups (H1, H2, H3, H4, H7, H8, L1, L2, L3 and L4) showed a significant change in their activity after the removal. Thus, in the case of 5 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity.

Circuits/Dances

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the circuits/dances at the forager group level (Fig. 3.2 *e*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = -0.3985 ± 0.4002 vs -0.4281 ± 0.7165, difference estimate = -0.0296, CI = -0.7334 – 0.6742, *p* = 0.9272). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *e*; HR vs LR: n =

47 vs 29, scaled mean ± standard deviation = -0.2838 ± 0.6755 vs -0.3768 ± 0.6840, difference estimate = -0.1026, CI = -0.6743 – 0.4690, *p* = 0.6975).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Forager group level analysis

The removal of foragers led to a significant change in the average circuits/dances of 3 out of 12 forager groups observed (Fig. 3.4 *e*). All 3 forager groups (H6, L1 and L3) showed a significant decrease in their average circuits/dances.

The model comparisons showed that the important predictors for the change in average circuits/dances of the forager group were the proportion of foragers removed and the colony experience (number of experiments done with the colony) (Table 3.4). Both proportion of foragers removed (effect size = -0.981, CI = -5.560 – 3.589, *p* = 0.675) and the colony experience (effect size = -0.023, CI = -0.371 – 0.325, *p* = 0.897) had small negative effect sizes which did not show a significant correlation with the change in average circuits/dances after the removal.

Individual forager level analysis

The removal of foragers led to a significant change in the circuits/dances of 9 out of 76 foragers from 5 forager groups observed (Fig. 3.13, 3.14 and 3.18 *b*). All 9 foragers from 5 different forager groups (2 foragers from H1, 1 from H6, 2 from L1, 1 from L2 and 2 from L3) showed a decrease in their circuits/dances.

To identify the correlation between the change in circuits/dances and the activity level of individual foragers before the removal, only data from the 5 forager groups in which at least one forager showed a significant change in its circuits/dances were considered. The pre-removal ranking in the 6 parameters showed no correlation with the change in circuits/dances at the individual forager level (Fig. 3.19 *e*).

Figure 3.13

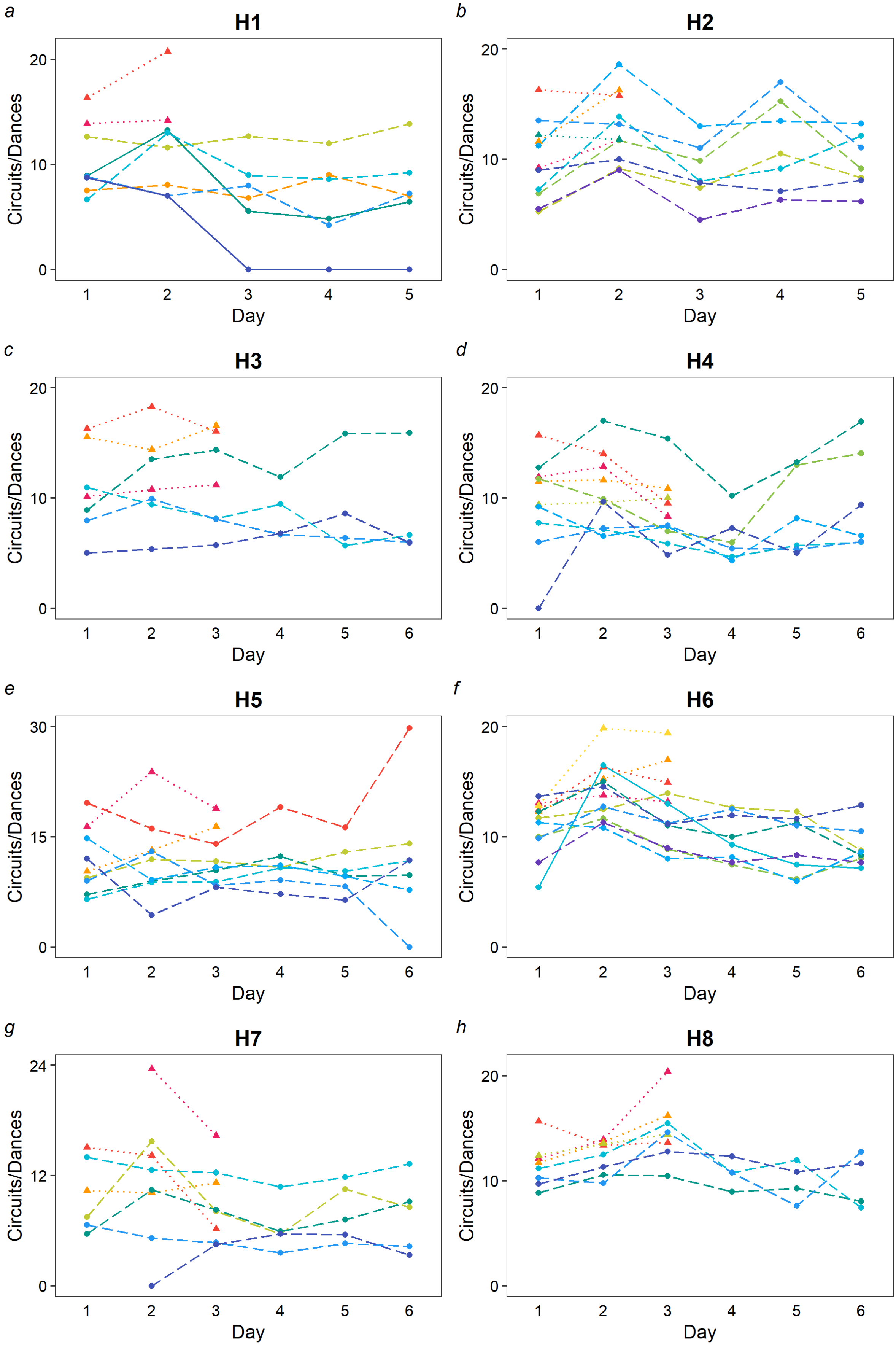
a
H1
b
H2
c
H3
d
H4
e
H5
f
H6
g
H7
h
H8
Circuits/Dances
Day
0
10
20
15
30
12
24
1
2
3
4
5
6

Figure 3.13: The circuits/dances of all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.14

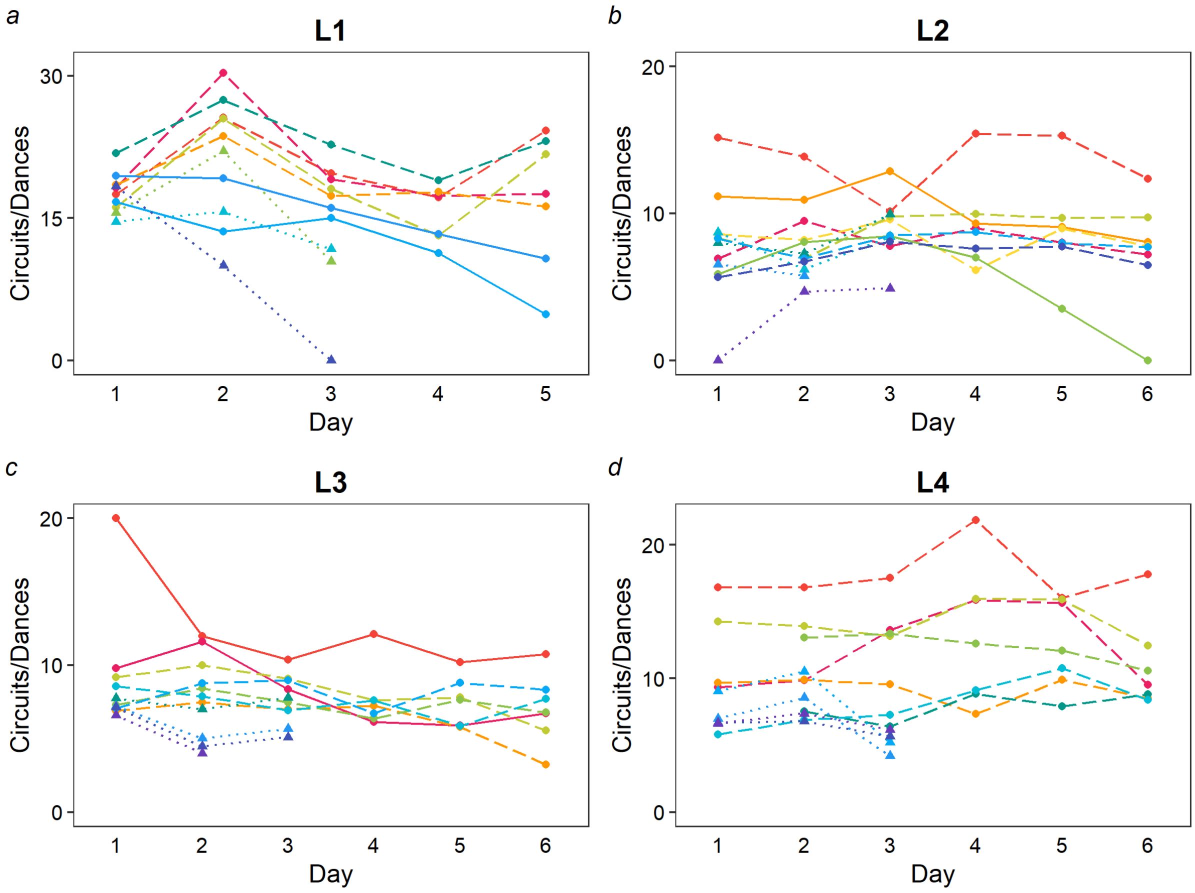

Figure 3.14: The circuits/dances of all individuals observed from each of the 4 forager groups in the Low Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Comparison of group level and individual level responses

In circuits/dances, only 3 forager groups (H6, L1 and L3) showed a change after the removal. Whereas some individual foragers in 5 forager groups (H1, H6, L1, L2 and L3) showed a significant change in their activity after the removal. Thus, in the case of 2 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity.

Circuits/Trips

High Removal and Low Removal

The removal of the more active dancers (HR) and the less active dancers (LR) had a similar effect on the circuits/trips at the forager group level (Fig. 3.2 *f*; HR vs LR: n = 8 vs 4, scaled mean ± standard deviation = 0.4859 ± 0.6472 vs -0.0539 ± 0.6799, difference estimate = -0.5399, CI = -1.4366 – 0.3669, *p* = 0.209). At the individual level also, both HR and LR experiments had a similar effect (Fig. 3.3 *f*; HR vs LR: n = 47 vs 29, scaled mean ± standard deviation = 0.3165 ± 0.8238 vs -0.0287 ± 0.8053, difference estimate = -0.4143, CI = -1.1737 – 0.3451, *p* = 0.2521).

Since both HR and LR had similar effects, data from all 12 experimental repeats were grouped together for further analysis.

Forager group level analysis

The removal of foragers led to a significant change in the average circuits/trips of 5 out of 12 forager groups observed (Fig. 3.4 *f*). 3 forager groups (H3, H4 and H7) showed an increase, whereas 2 forager groups (H6 and L1) showed a decrease in their average circuits/trips.

The model comparisons showed that the important predictors for the change in average circuits/trips of the forager group were the proportion of foragers removed, colony experience (number of experiments done with the colony), the forager group size and the number of foragers removed (Table 3.4). Of these, only forager group size had a significant correlation with the change in average circuits/trips after the removal, but with a small negative effect size (effect size = -0.322, CI = -0.616 – -0.029, *p* = 0.032). The proportion of foragers removed had a large positive effect size, but the correlation with the change in activity was not significant (effect size = 4.615, CI = -0.905 – 10.135, p = 0.101). Both the colony experience (effect size = 0.307, CI = -0.123 – 0.737, p = 0.161) and number of foragers removed (effect size = 0.485, CI = 0.149 – 1.119, p = 0.134) had small effect sizes and did not correlate significantly with the change in average circuits/trips after the removal.

Individual forager level analysis

The removal of foragers led to a significant change in the circuits/trips of 12 out of 76 foragers from 8 out of 12 forager groups observed (Fig. 3.15, 3.16 and 3.18 *c*). 9 foragers from 6 different forager groups (2 foragers from H1, 1 from H3, 2 from H4, 1 from H7, 2 from L2 and 1 from L4) showed an increase in their circuits/trips. 3 foragers from 3 different forager groups (1 each from H1, L1 and L3) showed a decrease in their circuits/trips.

To identify the correlation between the change in circuits/trips and the activity level of individual foragers before the removal, only data from the 8 forager groups in which at least one forager showed a significant change in its circuits/trips were considered. The change in circuits/trips strongly correlated with the ranking in pre-removal dance circuits (Fig. 3.19 *f*; effect size = -0.465, CI = -0.926 - -0.003, p = 0.048). The pre-removal ranking in the other parameters showed no correlation with the change in circuits/trips at the individual forager level.

Comparison of group level and individual level responses

In circuits/trips, only 5 forager groups (H3, H4, H6, H7 and L1) showed a change after the removal. Whereas some individual foragers in 8 forager groups (H1, H3, H4, H7, L1, L2, L3 and L4) showed a significant change in their activity after the removal. Thus, in the case of 3 forager groups, although there was no significant change in activity at the group level, some individual foragers showed a significant change in activity. Interestingly, even though one forager group (H6) showed a change at the group level, there were no individual foragers which showed a significant change in activity in this group.

Figure 3.15

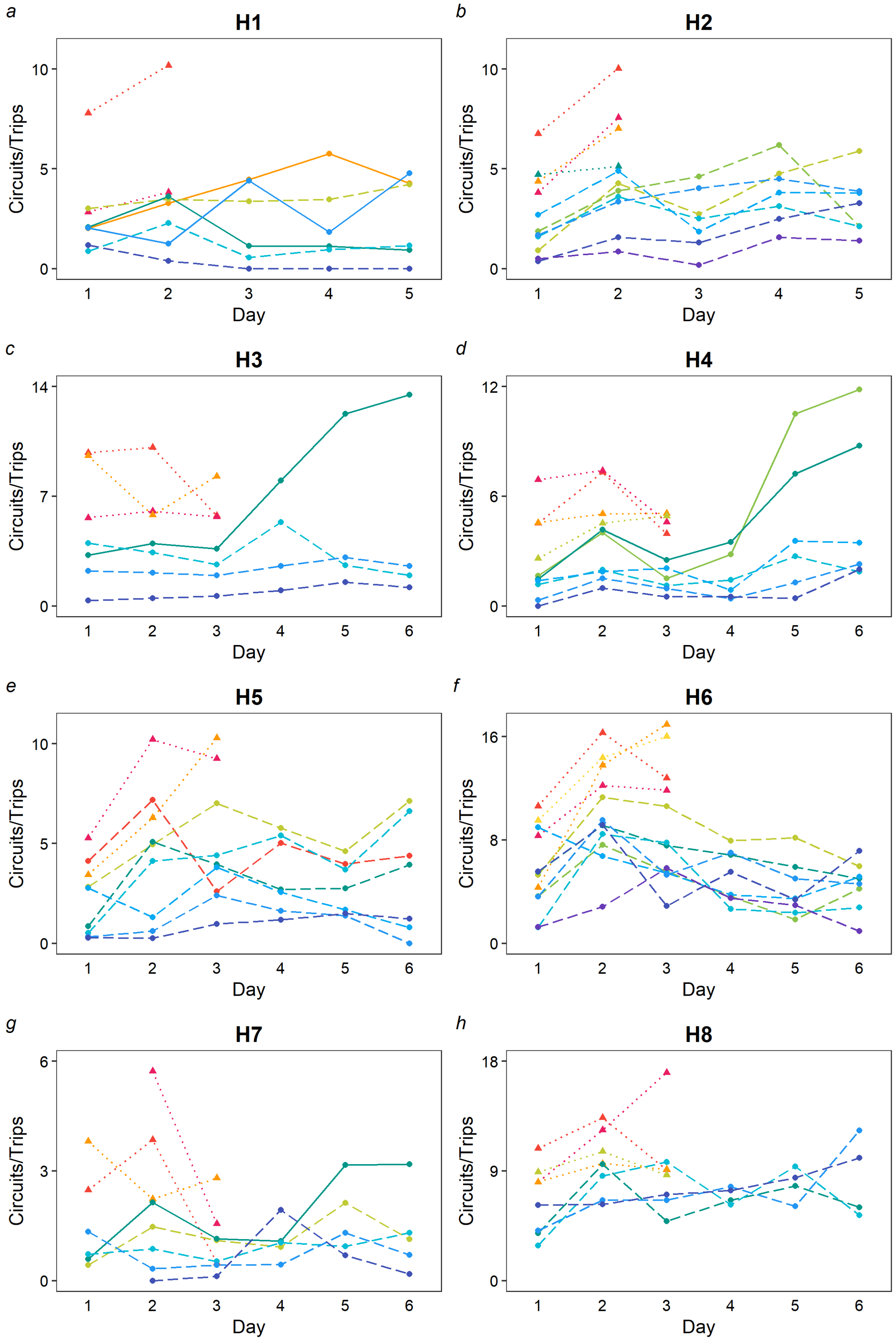
a
H1
b
H2
c
H3
d
H4
e
H5
f
H6
g
H7
h
H8
Circuits/Trips
Day
0
5
10
3
6
7
9
12
14
16
8
18
1
2
3
4
5
6

Figure 3.15: The circuits/trips of all individuals observed from each of the 8 forager groups in the High Removal (HR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.16

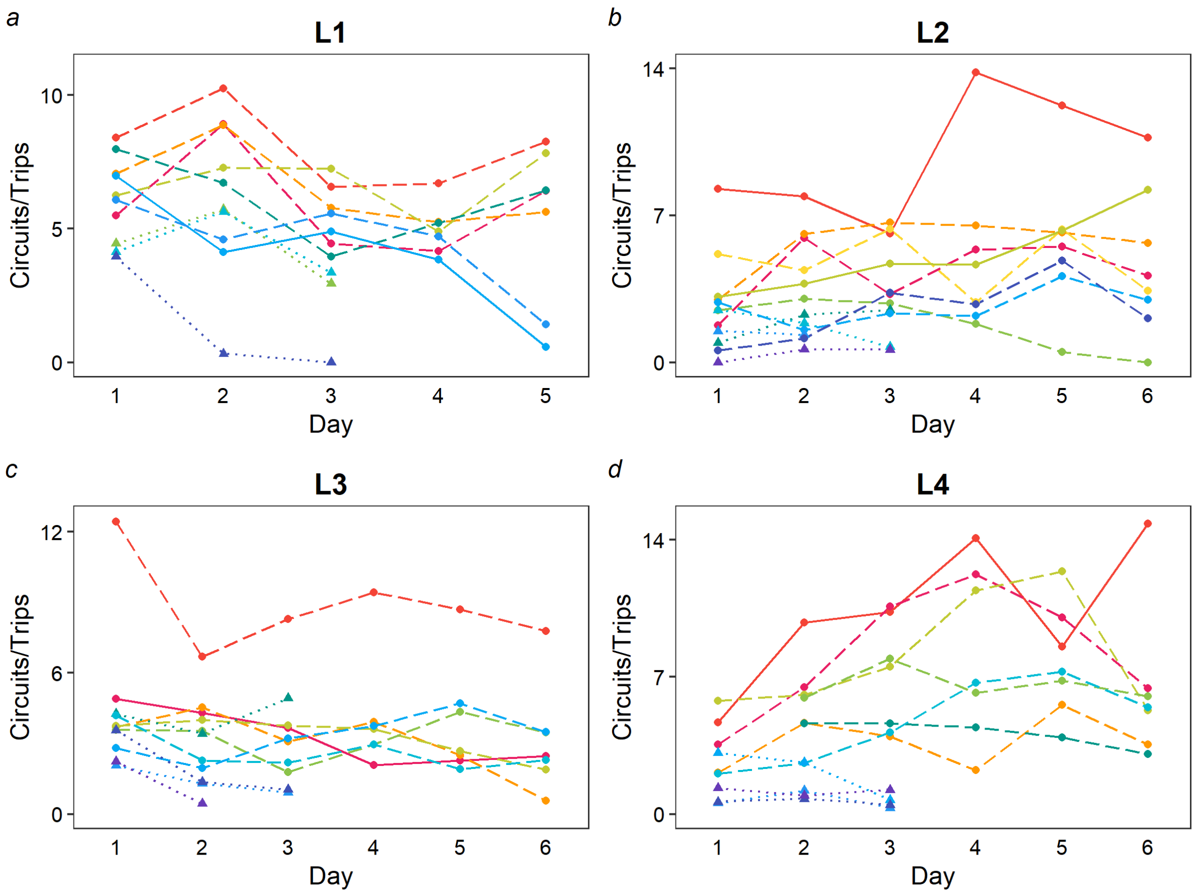

Figure 3.15: The circuits/trips of all individuals observed from each of the 4 forager groups in the Low Removal (LR) experiments. Each forager within a forager group is represented by a different colour. Triangles with dotted lines represent foragers which were removed as part of the experiment after the pre-removal phase. Circles with solid lines represent foragers which showed a significant change in activity after the removal. Circles with dashed lines represent foragers which did not show a significant change in activity after the removal.

Figure 3.17

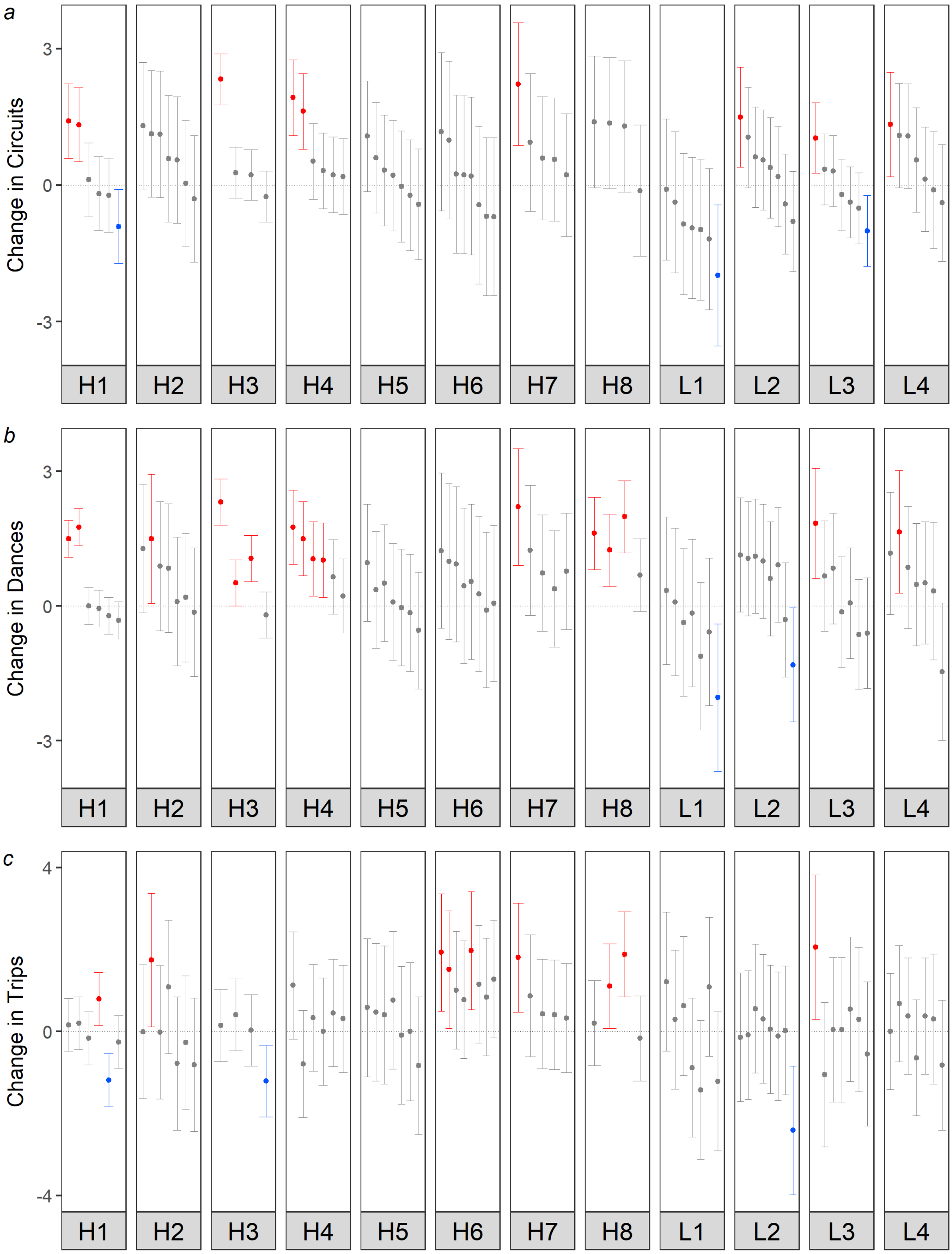

Figure 3.17: The difference in the average scaled activity between post-removal and pre-removal phase of 76 foragers from 12 forager groups in *(a)* total dance circuits, *(b)* number of dances and *(c)* number of foraging trips. Circles represent the difference estimate (effect size) obtained from linear mixed effects

models and the error bars represent the 95% confidence intervals around this estimate. Circles and error bars are colour coded based on the significance and the direction of change. Red represents foragers which showed a significant increase, blue represents foragers which showed a significant decrease and grey represents foragers which showed no significant change.

Comparison of effect of removal on probability versus intensity of dancing

The removal of foragers affected the probability of dancing of foragers more than the intensity of dancing. This was the case when probability was compared against both estimates of the intensity of dancing, circuits/dances and circuits/trips. In comparison with circuits/dances, individual foragers, on average, showed a greater increase in their probability (Fig. 3.20 *a*; mean ± standard deviation = 0.4129 ± 0.8508 vs -0.3192 ± 0.6757, difference estimate = 0.7322, CI = 0.5443 - 0.9201, p Value < 0.001). Similarly, in comparison with circuits/trips, individual foragers, on average, showed a greater increase in their probability (Fig. 3.20 *b*; mean ± standard deviation = 0.4129 ± 0.8508 vs 0.1848 ± 0.8288, difference estimate = 0.2281, CI = 0.1422 - 0.314, p Value < 0.001).

Discussion

The removal of the most and least active foragers had similar effects on foragers in all the parameters of dance activity. At least one forager, in 11 out of 12 forager groups observed, showed a change in dance activity in one of the parameters after the removal of foragers from the group. At the group level, this change in activity caused by the removal of foragers was most correlated with the proportion of foragers removed. At the individual level, those individuals which were more active (in the dance circuits) before the removal showed greater changes in activity (in probability of dancing and circuits/trips) after the removal.

Figure 3.18

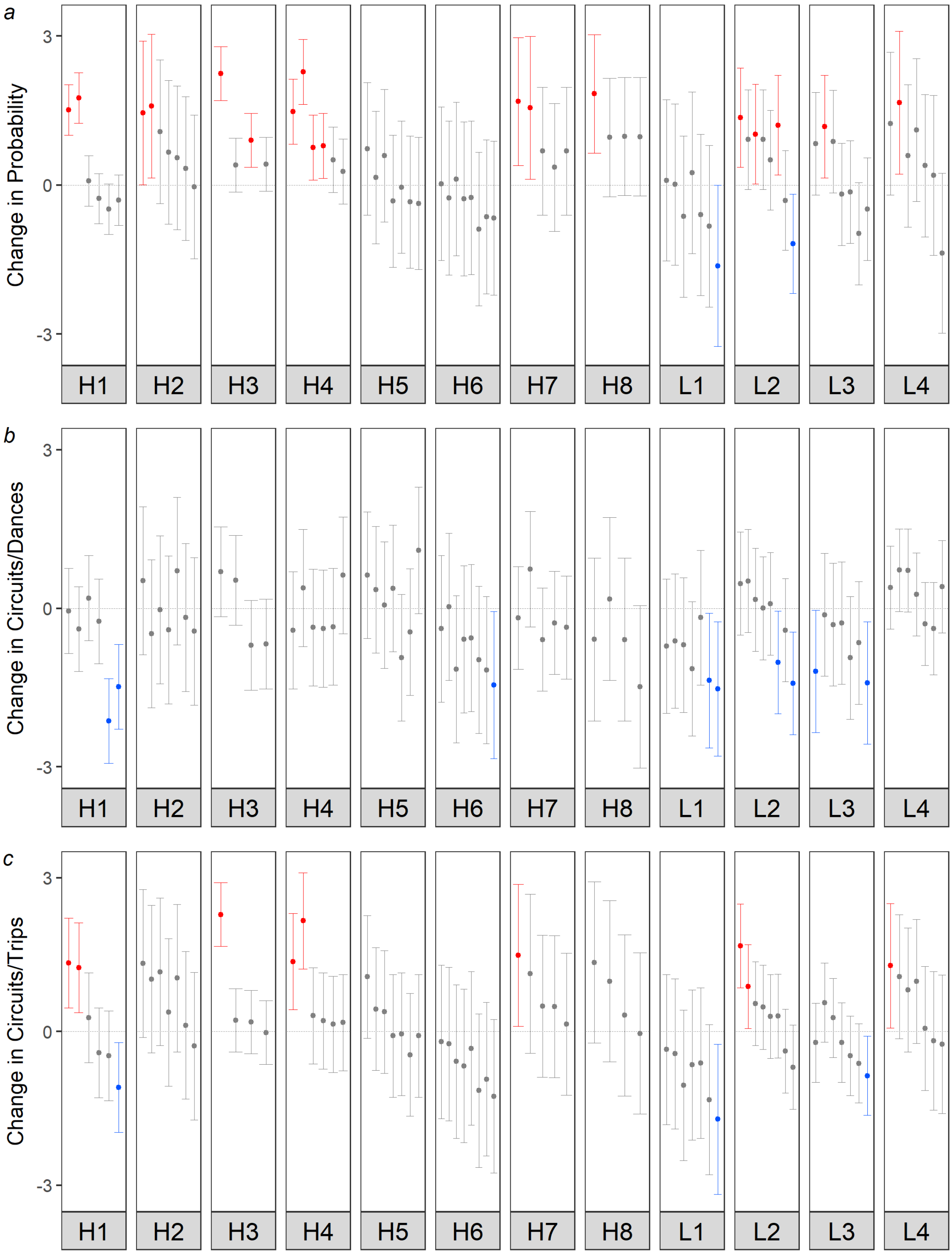

Figure 3.18: The difference in the average scaled activity between post-removal and pre-removal phase of 76 foragers from 12 forager groups in *(a)* probability of dancing, *(b)* circuits/dances and *(c)* circuits/trips. Circles represent the difference estimate (effect size) obtained from linear mixed effects

models and the error bars represent the 95% confidence intervals around this estimate. Circles and error bars are colour coded based on the significance and the direction of change. Red represents foragers which showed a significant increase, blue represents foragers which showed a significant decrease and grey represents foragers which showed no significant change.

Figure 3.19

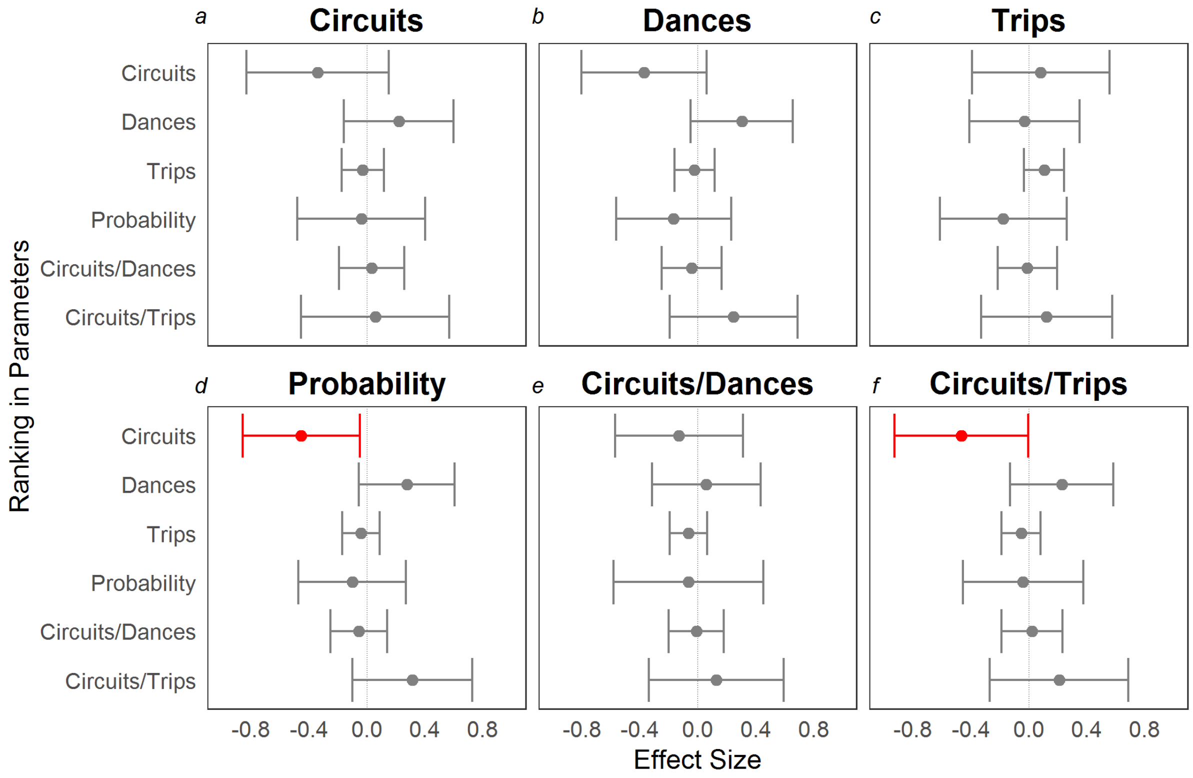

Figure 3.19: Results of linear mixed effects models correlating the change in activity of each of the 6 parameters with the pre-removal activity-based rank in any of the 6 parameters. Circles represent the effect size and error bars represent the 95% confidence interval. Red represents those parameters which showed a significant correlation while grey represents the parameters which did not show any significant correlation. Negative effect sizes indicate that higher pre-removal ranks (and hence lower numerical value of the ranks) correlated with a greater change in activity. The pre-removal activity-based rank in circuits correlated with the change in probability and circuits/trips. None of the other parameter-based ranks showed any correlation with the change in any of the parameters due to the removal.

Figure 3.20

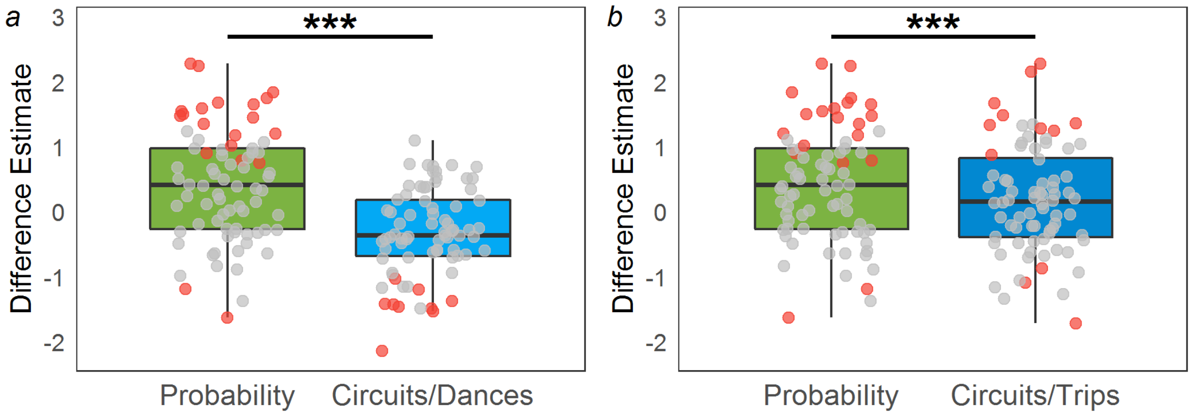

Figure 3.20: Boxplots comparing the change in activity between *(a)* probability and circuits/dances and *(b)* probability and circuits/trips of the remaining foragers due to the removal. Each circle represents the change in scaled activity shown by a single forager (n = 76). Red circles represent individuals which showed a significant change in activity while grey circles represent individuals which did not show a significant change in activity. Foragers showed a greater increase in their probability of dancing as compared to both estimates of the intensity of dancing (circuits/dances and circuits/trips).

High and Low removal experiments

Our results indicate that the more active individuals in honey bee forager groups are not 'key' individuals. Key individuals would have a disproportionate effect on the activity of the remaining foragers through intra-group interactions (Keiser et al., 2014). Thus, the presence or absence of these individuals would change the activity of the remaining individuals in a group (Couzin et al., 2005; Strandburg-Peshkin et al., 2018). In this scenario, the removal of the more active individuals should affect the remaining individuals, while the removal of other individuals should not. However, removal of both the most active and least active individuals similarly affected group and individual level activity in our experiments. The remaining foragers are more likely changing their activity in response to the general loss of foragers from the group and not the identity of the foragers removed (Grüter & Keller, 2016).

Effect on forager groups

In our experiments, the removal of foragers caused an increase in the average probability of dancing as well as average number of dances of 5 forager groups. This in turn led to an increase in the average total circuits of 2 forager groups. Two forager groups also concurrently increased the average number of foraging trips done. Interestingly, three forager groups decreased their average circuits/dances after the removal of foragers from these groups. However, three forager groups increased their circuits/trips while two decreased their average circuits/trips. These results indicate that groups prioritised increasing the number of recruitment events happening (probability of dances and hence number of dances) as opposed to the duration of these recruitment events (circuits/dances and circuits/trips) to compensate for the loss of foragers from the group.

In honey bee forager groups, removal of foragers leads to a decrease in the nectar influx into the colony. Previous work has shown that the nectar influx has a strong effect on the dance activity at the colony level (De Marco, 2006; W M Farina, 2000; Seeley, 1986, 1989, 1992; Seeley & Tovey, 1994). The increase in dance activity in the forager groups observed in our experiments was strongly correlated with the proportion of foragers removed and not with the absolute number of foragers removed from the groups. Thus, the relative change in nectar influx rather than the absolute change in nectar influx affected the dance activity of foragers in the group. The absolute nectar influx into the colony on any day can vary widely based on the season (Al-Ghamdi, Adgaba, Tadesse, Getachew, & Al-Maktary, 2017; Coffey & Breen, 1997; Khoury, Barron, & Myerscough, 2013; Sherman & Visscher, 2002). Therefore, to be more sensitive to sudden changes in its environment, it is more adaptive for the colony to be responsive to the relative change in nectar influx rather than the absolute change.

Effect on individual foragers

Individual foragers also responded to the removal of other foragers in a manner similar to forager groups. Most foragers which showed a change increased their probability of dancing (18 foragers from 9 groups) and hence the number of dances they performed (16 foragers from 8 groups). This in turn led to an increase in the total number of dance circuits they performed (9 foragers from 7 groups). Some foragers also increased the number of foraging trips that they did (9 foragers from 6 groups). Foragers also mostly showed an increase in their circuits/trips (9 foragers from 6 groups). However, foragers which showed a change in circuits/dances only showed a decrease in this parameter (9 foragers from 5 groups). Thus, foragers responded to the removal of some foragers from the group by increasing the number of recruitment events they performed, while some foragers also showed a decrease in the duration of these recruitment events.

Social cues and signals from nest mates provide valuable information to nectar foragers which can change their motivation to forage and recruit (W M Farina, 2000; Lindauer, 1949; Nieh, 2010; Seeley, 1989). The wait time that a forager faces before unloading nectar to a receiver is a reliable indicator of the nectar influx into the colony as well as the nutritional state of the colony (Lindauer, 1949; Seeley, 1989; Seeley & Tovey, 1994). At the same time, trophallactic interactions with receivers also can provide information to the forager about the colony need for that food source (De Marco, 2006; W M Farina, 2000; Walter M Farina, 1996; Lindauer, 1954). Experimental manipulations have shown that foragers modulate their dance activity in response to a change in these cues (De Marco, 2006; W M Farina, 2000; Seeley, 1989).

Our results indicate that the more active individuals were more responsive to changes in these social cues and signals. Behavioural differences in responses to the same social cues could potentially be driven by differences in individual response thresholds to the stimulus (Cappa, Bruschini, Cipollini, Pieraccini, & Cervo, 2014; Crall et al., 2018; Farine, Aplin, Garroway, Mann, & Sheldon, 2014; Firth, Voelkl, Farine, & Sheldon, 2015; Michelena, Jeanson, Deneubourg, & Sibbald, 2010; Pendrel & Plowright, 1981; Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012). Our results could be explained by differences in individual thresholds to respond to cues from receivers causing some individuals to increase their dance activity more (Değirmenci, Thamm, & Scheiner, 2018; Scheiner, Page, & Erber, 2001, 2004; Thamm & Scheiner, 2014). Alternatively, foragers could have one response threshold linking food reward to dance activity which is then further modulated by social cues, in a manner similar to social inhibition models (Beshers & Fewell, 2001; Beshers, Huang, Oono, & Robinson, 2001; Gordon, Goodwin, & Trainor, 1992; Naug & Gadagkar, 1999). Another factor that may play a role in driving these different responses is individual spatial fidelity, which would directly affect the interactions and hence information accessible to individuals (Crall et al., 2018; Pinter-Wollman, Penn, Theraulaz, & Fiore, 2018).

The correlation between the pre-removal activity level in dance circuits and the change in activity was seen only in the probability of dancing and circuits/trips. The lack of a correlation between the change in activity and the predictors tested in the other parameters indicates that the exact stimulus that foragers are responding to has not been measured in these observations. For example, no social interactions that foragers experienced in the hive have been quantified. Future experiments with a focus on more detailed observation of the interactions of foragers, particularly with receivers, over multiple days is essential to understand the relation between the social cues received by foragers and their behavioural decisions. Newer experimental methods, like automated tracking of larger numbers of individuals (Wario, Wild, Couvillon, Rojas, & Landgraf, 2015), would be especially useful, as the exact function of many cues and signals that foragers experience in the hive is still uncertain (De Marco, 2006; Rivera, Donaldson-Matasci, & Dornhaus, 2015).

Forager groups and individual foragers

There were interesting differences in the group level and individual level responses. Even in forager groups which did not increase their average activity after the removal, there were individual foragers which showed an increase in dance activity. Thus, an analysis of only group level changes in dance activity would have led us to conclude that the removal has no effect in these forager groups. So far, most studies on the honey bee dance communication have focussed on responses at the group level (Granovskiy, Latty, Duncan, Sumpter, & Beekman, 2012; Kirchner & Sommer, 1992; Seefeldt & De Marco, 2008; Seeley, 1989). The removal experiments show that these group level responses do not adequately reflect individual level responses (Gallistel, Fairhurst, & Balsam, 2004). This serves to further highlight the need for individual level studies on the waggle dance as well as on other behaviours in eusocial insects.

Comparisons with other social groups

The response of honey bee forager groups to the removal of foragers is similar to how other social groups respond to perturbations (Charbonneau, Sasaki, & Dornhaus, 2017; Crall et al., 2018; Firth et al., 2017; Rudin, Tomkins, & Simmons, 2018). In eusocial insects, a loss of individuals from a group of workers engaged in a task led to new individuals performing the same task (Breed, Williams, & Queral, 2002; O'Donnell, 1998) or to some of the workers in the group increasing their task performance (Gardner, Foster, & O'Donnell, 2007; Pendrel & Plowright, 1981; Pinter-Wollman et al., 2012). After the removal of individuals from groups of great tits (Parus major), their positions in the network were occupied by those individuals closest to them (Firth et al., 2017). Spatial positions occupied by individuals can also play a role in determining which individuals respond to a change in stimuli by affecting the interactions that an individual will be exposed to (Crall et al., 2018; Ireland & Garnier, 2018; Kamath et al., 2018; Mersch, Crespi, & Keller, 2013; Pinter-Wollman et al., 2018; Smith-Aguilar, Ramos-Fernández, & Getz, 2016). The removal of foragers from bumble bee colonies caused more centrally located individuals, exposed to more interactions in the colony, to initiate foraging (Crall et al., 2018). Thus, individual thresholds and spatial fidelity may both play a role in determining which individuals respond to perturbations. Individual differences in responding to changing stimuli should play an important role in the resilience shown by such diverse groups to perturbations in their structure.

Flexibly responding to changing food availability is essential for the survival of any individual or group (Kamil & Roitblat, 1985; Ryer & Olla, 1995; Stephens, Brown, & Ydenberg, 2007; J. F.A. Traniello, 1989; Venkataraman, Kraft, Dominy, & Endicott, 2017). The results of the removal experiments highlight three mechanisms by which honey bee colonies respond to changing food availability. First, forager groups are more sensitive to changes in the relative nectar influx, rather than the absolute nectar influx. Second, forager groups and individual foragers responded to a decrease in nectar influx by increasing the number of recruitment events (probability of dancing), as opposed to the duration of these events (intensity of dances). Finally, foragers which were more responsive to environmental cues (food reward) were also more sensitive to the social cues (interactions with receivers). Both the sucrose concentration (or perception of the food reward) and social cues (or perception of the same) might be represented by neuromodulator signalling which could affect the same reward system in honey bee foragers (Perry & Barron, 2012). Thus, these experiments can serve as a starting point to understand the mechanistic processes underlying the integration of social and environmental cues in the decision to perform a behaviour.

Future Directions

The experiments described in this chapter can be expanded further to understand how foragers respond to changing social interactions. The first step would be to mark individual receivers in the hive and combine removal experiments with observations of forager-receiver interactions. This would help in pinpointing the exact cue or signal that is involved in modulating the dance activity of the foragers. Further, observations of individually marked receivers would also reveal if inter-individual differences in interactions exist amongst receivers. These differences can be expected to play an important role in the regulation of foraging. Automated tracking techniques can be used to quantify various aspects of the interactions between foragers and receivers like antennation, trophallaxis etc.

Another direction in which these experiments can be further expanded would be to more carefully observe the correlation between changing nectar influx and the change in dance activity of the remaining foragers. One possibility could be to sequentially remove foragers one after the other over the course of multiple days. The gradual change in nectar influx can correlate with the number of foragers which change their activity or with the extent of the change. Another possibility would be to have two active forager groups at two different feeders and then remove different proportions of foragers from either of these groups. This set up can also be used to observe the effect of feeder reward on the change in activity. Once forager groups are active at the two feeders (with different sucrose concentrations at each), the same proportion of foragers can be removed from both feeders to observe whether the change in activity of the remaining foragers would be different based on the feeder reward.

The results from chapter 2 and 3 also raise an interesting question pertaining to the composition of forager groups and its effect on individual dance activity. Forager groups in natural conditions would be transient, as food sources can bloom and deplete over the course of days. In such a situation, foragers can be expected to form part of multiple forager groups within their lifetime. Foragers might show a change in their dance activity from one group to the other due to the different group compositions at these food sources. This behavioural flexibility of individual foragers would have important implications for the formation and maintenance of forager groups in honey bee colonies.

References

Al-Ghamdi, A. A., Adgaba, N., Tadesse, Y., Getachew, A., & Al-Maktary, A. A. (2017). Comparative study on the dynamics and performances of Apis mellifera jemenitica and imported hybrid honeybee colonies in southwestern Saudi Arabia. *Saudi Journal of Biological Sciences*, *24*(5), 1086–1093. https://doi.org/10.1016/j.sjbs.2017.01.008

Barton, K. (2017). MuMIn: Multi-Model Inference. Retrieved from https://cran.r-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using {lme4}. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. *Annual Review of Entomology*, *46*, 413–440. https://doi.org/10.1146/annurev.ento.46.1.413

Beshers, S. N., Huang, Z. Y., Oono, Y., & Robinson, G. E. (2001). Social inhibition and the regulation of temporal polyethism in honey bees. *Journal of Theoretical Biology*, *213*(3), 461–479. https://doi.org/10.1006/jtbi.2001.2427

Breed, M. D., Williams, D. B., & Queral, A. (2002). Demand for task performance and workforce replacement: Undertakers in honeybee, Apis mellifera, colonies. *Journal of Insect Behavior*, *15*(3), 319–329. https://doi.org/10.1023/A:1016261008322

Brown, C., & Irving, E. (2014). Individual personality traits influence group exploration in a feral guppy population. *Behavioral Ecology*, *25*(1), 95–101. https://doi.org/10.1093/beheco/art090

Cappa, F., Bruschini, C., Cipollini, M., Pieraccini, G., & Cervo, R. (2014). Sensing the intruder: A quantitative threshold for recognition cues perception in honeybees. *Naturwissenschaften*, *101*(2), 149–152. https://doi.org/10.1007/s00114-013-1135-1

Charbonneau, D., Sasaki, T., & Dornhaus, A. (2017). Who needs 'lazy' workers? Inactive workers act as a 'reserve' labor force replacing active workers, but inactive workers are not replaced when they are removed. *PLoS ONE*, *12*(9), 1–20. https://doi.org/10.1371/journal.pone.0184074

Chate, H., Theraulaz, G., Calovi, D. S., Sire, C., Schuhmacher, P., & Lopez, U. (2015). Collective response to perturbations in a data-driven fish school model. *Journal of The Royal Society Interface*, *12*(104), 20141362–20141362. https://doi.org/10.1098/rsif.2014.1362

Clarke, F. M., & Faulkes, C. G. (1997). Dominance and queen succession in captive colonies of the eusocial naked mole-rat, Heterocephalus glaber. *Proceedings of the Royal Society B: Biological Sciences*, *264*(1384), 993–1000. https://doi.org/10.1098/rspb.1997.0137

Claus O. Wilke. (2018). cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2." *R Package*

Version 0.9.3. Retrieved from https://cran.r-project.org/package=cowplot

Coffey, M. F., & Breen, J. (1997). Seasonal variation in pollen and nectar sources of honey bees in Ireland. *Journal of Apicultural Research*, *36*(2), 63–76. https://doi.org/10.1080/00218839.1997.11100932

Conradt, L., & Roper, T. J. (2003). Group decision-making in animals. *Nature*, *421*, 155–158. https://doi.org/10.1038/nature01294

Couzin, I. D., Krause, J., Franks, N. R., & Levin, S. A. (2005). Effective leadership and decision-making in animal groups on the move. *Nature*, *433*(February), 513–516. https://doi.org/10.1038/nature03236

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Spatial fidelity of workers predicts collective response to disturbance in a social insect. *Nature Communications*, *9*(1), 1–13. https://doi.org/10.1038/s41467-018-03561-w

De Marco, R. J. (2006). How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' `eagerness'. *Journal of Experimental Biology*, *209*(3), 421–432. https://doi.org/10.1242/jeb.02025

De Marco, R. J., & Farina, W. M. (2001). Changes in food source profitability affect the trophallactic and dance behavior of forager honeybees (Apis mellifera L.). *Behavioral Ecology and Sociobiology*, *50*(5), 441–449. https://doi.org/10.1007/s002650100382

Değirmenci, L., Thamm, M., & Scheiner, R. (2018). Responses to sugar and sugar receptor gene expression in different social roles of the honeybee (Apis mellifera). *Journal of Insect Physiology*, *106*(May 2017), 65–70. https://doi.org/10.1016/j.jinsphys.2017.09.009

Farina, W M. (2000). The interplay between dancing and trophallactic behavior in the honey bee Apis mellifera. *Journal of Comparative Physiology - A*, *186*(3), 239–245. https://doi.org/10.1007/s003590050424

Farina, Walter M. (1996). Food-exchange by foragers in the hive – a means of communication among honey bees? *Behavioral Ecology*, *38*, 59–64. https://doi.org/10.1007/s002650050217

Farine, D. R., Aplin, L. M., Garroway, C. J., Mann, R. P., & Sheldon, B. C. (2014). Collective decision making and social interaction rules in mixed-species flocks of songbirds. *Animal Behaviour*, *95*, 173–182. https://doi.org/10.1016/j.anbehav.2014.07.008

Fernald, R. D. (2014). Communication about social status. *Current Opinion in Neurobiology*. Elsevier Ltd. https://doi.org/10.1016/j.conb.2014.04.004

Fewell, J. H. (2003). Social Insect Networks. *Science*, *301*(5639), 1515–1519. https://doi.org/10.1126/science.1088945

Firth, J. A., Voelkl, B., Crates, R. A., Aplin, L. M., Biro, D., Croft, D. P., & Sheldon, B. C. (2017). Wild birds respond to flockmate loss by increasing their social network associations to others. *Proceedings. Biological Sciences*, *284*(1854), 20170299. https://doi.org/10.1098/rspb.2017.0299

Firth, J. A., Voelkl, B., Farine, D. R., & Sheldon, B. C. (2015). Experimental evidence that social relationships determine individual foraging behavior. *Current Biology*, *25*(23), 3138–3143. https://doi.org/10.1016/j.cub.2015.09.075

Gallistel, C. R., Fairhurst, S., & Balsam, P. (2004). The learning curve: Implications of a quantitative analysis. *Proceedings of the National Academy of Sciences*, *101*(36), 13124–13131. https://doi.org/10.1073/pnas.0404965101

Gardner, K. E., Foster, R. L., & O'Donnell, S. (2007). Experimental analysis of worker division of labor in bumblebee nest thermoregulation (Bombus huntii, Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology*, *61*(5), 783–792. https://doi.org/10.1007/s00265-006-0309-7

Gardner, K. E., Seeley, T. D., & Calderone, N. W. (2008). Do honeybees have two discrete dances to advertise food sources? *Animal Behaviour*, *75*(4), 1291–1300. https://doi.org/10.1016/j.anbehav.2007.09.032

Garrison, L. K., Kleineidam, C. J., & Weidenmüller, A. (2018). Behavioral flexibility promotes collective consistency in a social insect. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-33917-7

Giardina, I., Santagati, R., Viale, M., Cimarelli, A., Cavagna, A., Parisi, G., & Stefanini, F. (2010). Scale-free correlations in starling flocks. *Proceedings of the National Academy of Sciences*, *107*(26), 11865–11870. https://doi.org/10.1073/pnas.1005766107

Gordon, D. M., Goodwin, B. C., & Trainor, L. E. H. (1992). A parallel distributed model of the behaviour of ant colonies. *Journal of Theoretical Biology*, *156*(3), 293–307. https://doi.org/10.1016/S0022-5193(05)80677-0

Granovskiy, B., Latty, T., Duncan, M., Sumpter, D. J. T., & Beekman, M. (2012). How dancing honey bees keep track of changes: the role of inspector bees. *Behavioral Ecology*, *23*(3), 588–596. https://doi.org/10.1093/beheco/ars002

Grüter, C., & Keller, L. (2016). Inter-caste communication in social insects. *Current Opinion in Neurobiology*, *38*, 6–11. https://doi.org/10.1016/j.conb.2016.01.002

Hölldobler, B., & Wilson, E. O. (2009). *The superorganism*. *W W Norton* (Vol. 456). W.W. Norton & Company, Inc. https://doi.org/10.1038/456320a

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Ireland, T., & Garnier, S. (2018). Architecture, space and information in constructions built by humans and social insects: A conceptual review. *Philosophical Transactions of the Royal Society B: Biological Sciences*. https://doi.org/10.1098/rstb.2017.0244

Kamath, A., Primavera, S. D., Wright, C. M., Doering, G. N., Sheehy, K. A., Pinter-wollman, N., & Pruitt, J. N. (2018). Collective behavior and colony persistence of social spiders depends on their physical environment. *Behavioral Ecology*, 1–8. https://doi.org/10.1093/beheco/ary158

Kamil, A. C., & Roitblat, H. L. (1985). The Ecology of Foraging Behavior: Implications for Animal Learning and Memory. *Avian Cognition Papers*, *36*, 141–169. https://doi.org/10.1146/annurev.psych.36.1.141

Kavaliers, M., & Choleris, E. (2018). The role of social cognition in parasite and pathogen avoidance. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1751). https://doi.org/10.1098/rstb.2017.0206

Keiser, C. N., Pruitt, J. N., Sih, A., Watters, J. V., & Modlmeier, A. P. (2014). The keystone individual concept: an ecological and evolutionary overview. *Animal Behaviour*, *89*, 53–62. https://doi.org/10.1016/j.anbehav.2013.12.020

Khoury, D. S., Barron, A. B., & Myerscough, M. R. (2013). Modelling Food and Population Dynamics in Honey Bee Colonies. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0059084

Kietzman, P. M., & Visscher, P. K. (2015). The anti-waggle dance: use of the stop signal as negative feedback. *Frontiers in Ecology and Evolution*, *3*(February), 1–5. https://doi.org/10.3389/fevo.2015.00014

King, A. J., & Cowlishaw, G. (2009). Leaders, followers and group decision-making. *Communicative & Integrative Biology*, *2*(2), 147–150. Retrieved from www.landesbioscience.com

Kirchner, W. H., & Sommer, K. (1992). The dance language of the honeybee mutant diminutive wings. *Behavioral Ecology and Sociobiology*, *30*(3–4), 181–184. https://doi.org/10.1007/BF00166701

Kocher, S. D., & Cocroft, R. B. (2019). Signals in Insect Social Organization. *Encyclopedia of Animal Behavior*, 558–567. https://doi.org/10.1016/B978-0-12-809633-8.90781-7

Lau, C. W., & Nieh, J. C. (2010). Honey bee stop-signal production: temporal distribution and effect of feeder crowding. *Apidologie*, *41*(1), 87–95. https://doi.org/10.1051/apido/2009052

Leonhardt, S. D., Menzel, F., Nehring, V., & Schmitt, T. (2016). Ecology and Evolution of Communication in Social Insects. *Cell*, *164*(6), 1277–1287. https://doi.org/10.1016/j.cell.2016.01.035

Lindauer, M. (1949). Über die Einwirkung von Duft- und Geschmacksstoffen sowie anderer Faktoren auf die Tänze der Bienen. *Zeitschrift Für Vergleichende Physiologie*, *31*(3), 348–412. https://doi.org/10.1007/BF00297951

Lindauer, M. (1954). Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Zeitschrift Für Vergleichende Physiologie*, *36*(1954), 391–432. https://doi.org/10.1007/BF00345028

Mersch, D. P., Crespi, A., & Keller, L. (2013). Tracking Individuals Shows Spatial Fidelity Is a Key Regulator of Ant Social Organization. *Science*, *340*(6136), 1090–1093. https://doi.org/10.1126/science.1234316

Michelena, P., Jeanson, R., Deneubourg, J. L., & Sibbald, A. M. (2010). Personality and collective decision-making in foraging herbivores. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 1093–1099. https://doi.org/10.1098/rspb.2009.1926

Moore, D., Siegfried, D., Wilson, R., & Rankin, M. A. (1989). The Influence of Time of Day on the Foraging Behavior of the Honeybee, Apis mellifera. *Journal of Biological Rhythms*, *4*(3), 305–325. https://doi.org/10.1177/074873048900400301

Morand-Ferron, J., Doligez, B., Dall, S. R. X., & Reader, S. M. (2010). Social Information Use. *Encyclopedia of Animal Behavior*, 394–402. https://doi.org/10.1016/B978-0-12-813251-7.00281-9

Naug, D., & Gadagkar, R. (1999). Flexible Division of Labor Mediated by Social Interactions in an Insect Colony—a Simulation Model. *Journal of Theoretical Biology*, *197*, 123–133. https://doi.org/10.1006/jtbi.1998.0862

Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology*, *20*(4), 310–315. https://doi.org/10.1016/j.cub.2009.12.060

O'Donnell, S. (1998). Effects of experimental forager removals on division of labour in the primitively eusocial wasp *Polistes instabilis* (*Hymenoptera*: *Vespidae*). *Behaviour*, *135*(2), 173–193. https://doi.org/10.1163/156853998793066348

Pendrel, B. A., & Plowright, R. C. (1981). Larval feeding by adult bumble bee workers (Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology*, *8*(2), 71–76. https://doi.org/10.1007/BF00300817

Perry, C. J., & Barron, A. B. (2012). Neural Mechanisms of Reward in Insects. *Annual Review of Entomology*, *58*(1), 543–562. https://doi.org/10.1146/annurev-ento-120811-153631

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R.-C. (2018). Linear and nonlinear mixed effects models. *URL Https://Cran.r-Project.Org/Web/Packages/Nlme/Nlme.Pdf*. Retrieved from https://cran.r-project.org/package=nlme

Pinter-Wollman, N., Hubler, J., Holley, J.-A., Franks, N. R., & Dornhaus, A. (2012). How is activity distributed among and within tasks in Temnothorax ants? *Behavioral Ecology and Sociobiology*, *66*(10), 1407–1420. https://doi.org/10.1007/s00265-012-1396-2

Pinter-Wollman, N., Penn, A., Theraulaz, G., & Fiore, S. M. (2018). Interdisciplinary approaches for uncovering the impacts of architecture on collective behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1753). https://doi.org/10.1098/rstb.2017.0232

Pruitt, J. N., & Goodnight, C. J. (2014). Site-specific group selection drives locally adapted group compositions. *Nature*, *514*(7522), 359–362. https://doi.org/10.1038/nature13811

R Core Team. (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rivera, M. D., Donaldson-Matasci, M. C., & Dornhaus, A. (2015). Quitting time: When do honey bee foragers decide to stop foraging on natural resources? *Frontiers in Ecology and Evolution*, *3*(May), 1–9. https://doi.org/10.3389/fevo.2015.00050

Rudin, F. S., Tomkins, J. L., & Simmons, L. W. (2018). The effects of the social environment and physical disturbance on personality traits. *Animal Behaviour*, *In press*, 109–121. https://doi.org/10.1016/j.anbehav.2018.02.013

Ryer, C. H., & Olla, B. L. (1995). Influences of food distribution on fish foraging behaviour. *Animal Behaviour*, *49*, 411–418. https://doi.org/https://doi.org/10.1006/anbe.1995.0054

Scheiner, R., Page, R. E., & Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (Apis mellifera L.). *Neurobiology of Learning and Memory*, *76*(2), 138–150. https://doi.org/10.1006/nlme.2000.3996

Scheiner, R., Page, R. E., & Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (Apis mellifera) Ricarda. *Apidologie*, *35*, 133–142. https://doi.org/10.1051/apido:2004001

Seefeldt, S., & De Marco, R. J. (2008). The response of the honeybee dance to uncertain rewards. *The Journal of Experimental Biology*, *211*(Pt 21), 3392–3400. https://doi.org/10.1242/jeb.017624

Seeley, T. D. (1986). Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, *19*(5), 343–354. https://doi.org/10.1007/BF00295707

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony’s nutritional status. *Behavioral Ecology and Sociobiology*, *24*(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1992). The tremble dance of the honey bee: message and meanings. *Behavioral Ecology and Sociobiology*, *31*(6), 375–383. https://doi.org/10.1007/BF00170604

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, *34*, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). *The Wisdom of the Hive*. *Harvard University Press*.

Seeley, T. D., & Tovey, C. A. (1994). Why search time to find a food-storer bee accurately indicates the relative rates of nectar collecting and nectar processing in honey bee colonies. *Animal Behaviour*, *47*, 311–316. https://doi.org/https://doi.org/10.1006/anbe.1994.1044

Sen Sarma, M., Esch, H. E., & Tautz, J. (2004). A comparison of the dance language in Apis mellifera carnica and Apis florea reveals striking similarities. *Journal of Comparative Physiology A*, *190*(1), 49–53. https://doi.org/10.1007/s00359-003-0470-7

Sherman, G., & Visscher, P. K. (2002). Honeybee colonies achieve fitness through dancing. *Nature*, *419*(October), 920–922. https://doi.org/10.1038/nature01127

Sinhuber, M., van der Vaart, K., & Ouellette, N. T. (2019). Response of insect swarms to dynamic illumination perturbations. *Journal of The Royal Society Interface*, *16*(150), 20180739. https://doi.org/10.1098/rsif.2018.0739

Smith-Aguilar, S. E., Ramos-Fernández, G., & Getz, W. M. (2016). Seasonal changes in socio-spatial structure in a group of free-living spider monkeys (Ateles geoffroyi). *PLoS ONE*, *11*(6), 1–28. https://doi.org/10.1371/journal.pone.0157228

Stephens, D. W., Brown, J. S., & Ydenberg, R. C. (2007). *Foraging: behavior and ecology*. University of Chicago Press.

Strandburg-Peshkin, A., Papageorgiou, D., Margaret, C., & Farine, D. R. (2018). Inferring influence and leadership in moving animal groups Main Text Summary, 1–20. https://doi.org/10.1098/not

Sumana, A., & Sona, C. (2013). Key relocation leaders in an Indian queenless ant. *Behavioural Processes*, *97*, 84–89. https://doi.org/10.1016/j.beproc.2013.03.006

Tan, K., Dong, S., Li, X., Liu, X., Wang, C., Li, J., & Nieh, J. C. (2016). Honey Bee Inhibitory Signaling Is Tuned to Threat Severity and Can Act as a Colony Alarm Signal. *PLOS Biology*, *14*(3), e1002423. https://doi.org/10.1371/journal.pbio.1002423

Thamm, M., & Scheiner, R. (2014). PKG in honey bees: Spatial expression, Amfor gene expression, sucrose responsiveness, and division of labor. *Journal of Comparative Neurology*, *522*(8), 1786–1799. https://doi.org/10.1002/cne.23500

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *Journal of Experimental Biology*, *206*(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Traniello, J. F.A. (1989). Foraging strategies of ants. *Annual Review of Entomology. Vol. 34*, 191–210.

Traniello, James F.A, & Rosengaus, R. B. (1997). Ecology, evolution and division of labour in social insects. *Animal Behaviour*, *53*(1), 209–213. https://doi.org/10.1006/anbe.1996.0289

Venkataraman, V. V., Kraft, T. S., Dominy, N. J., & Endicott, K. M. (2017). Hunter-gatherer residential mobility and the marginal value of rainforest patches. *Proceedings of the National Academy of Sciences*, *114*(12), 3097–3102. https://doi.org/10.1073/pnas.1617542114

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

Wagenmakers, E.-J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin and Review*, *11*, 192–196. https://doi.org/10.3758/BF03206482

Wario, F., Wild, B., Couvillon, M. J., Rojas, R., & Landgraf, T. (2015). Automatic methods for long-term tracking and the detection and decoding of communication dances in honeybees. *Frontiers in Ecology and Evolution*, *3*(September), 1–14. https://doi.org/10.3389/fevo.2015.00103

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. *Springer International Publishing* (2nd ed.). Springer. https://doi.org/10.1007/978-3-319-24277-4

Chapter

IV

Thresholds

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Chapter 4 – Thresholds

Title Page

Honey makes the bee go around: Inter-individual variation in honey bee dance intensity correlates with expression of the *foraging* gene

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Abstract

Individual behavioural differences in responding to the same stimuli is a key facet of division of labour in eusocial insect colonies. Although these differences have been shown across behaviours in social insects, not much is known about the mechanisms underlying these differences. In this study we looked at the possible mechanisms underlying inter-individual variation in the waggle dance activity of honey bee foragers. We first quantified the variation in dance activity amongst groups of foragers visiting an artificial feeder filled consecutively with different sucrose concentrations. We then looked at the sucrose responsiveness and the brain gene expression levels of *foraging* gene *Amfor*, octopamine receptor gene *AmoctαR1* and *insulin receptor AmInR*-2 of these foragers. As expected, foragers showed large inter-individual differences in their dance activity, irrespective of the reward offered at the feeder. The sucrose responsiveness correlated positively with the intensity of the dance activity at the higher reward condition, with the more responsive foragers having a higher intensity of dancing. Out of the three genes tested, *Amfor* expression significantly correlated with dance activity, with more active dancers having lower expression levels. Our results thus provide the first hints of the processes underlying variation in dance activity in honey bee foragers and can be used as a starting point for further experiments.

Keywords

Foraging, Response thresholds, Waggle dance, Sucrose responsiveness, Octopamine, Recruitment behaviour, Insulin receptor, Navigation, Eusocial insects, Information communication

Introduction

Individuals show variation in their response to the same stimulus in terms of either a change in their behaviour or a change in the intensity of the same behaviour [1]. This inter-individual variation is important as it forms the basis for adaptation to novel stimuli. Individual variation can persist across contexts and over time in a consistent manner, a phenomenon referred to as behavioural syndromes [2]–[4]. These syndromes are being extensively studied in vertebrates, while there is considerably less work on invertebrates [5].

Insects form the predominant terrestrial animal taxon on our planet in terms of both diversity and biomass [6], [7]. Traditionally, they had been viewed as simple "reflex machines" with very little inter-individual differences in behaviour [8]. But the phenomenon of individual behavioural differences amongst invertebrate species is slowly gaining acceptance as a valuable tool to study ecological and evolutionary consequences of behavioural decisions [9], [10]. This is particularly true in the case of social insects, wherein behavioural differences between workers of the same colony play a key role in division of labour within the colony [11], [12].

In honey bees, foraging involves a large proportion of the work force of the colony [13], [14]. Some foragers scout the environment for new food sources and then recruit their nest mates to these food sources through dance communication [15]. This recruitment communication was initially identified as being either a round dance or a waggle dance, for food sources which were nearer and farther, respectively [16]. But more recent work showed that round and waggle dances do not represent two different recruitment signals [17]. Round and waggle dances incorporate both spatial information as well as information about the perceived profitability of the food source in case of nectar sources [18]–[20]. The profitability of a nectar source is defined by its energetic value and hence sugar concentration [21], [22], its distance from the hive [23], its distance to other nectar sources [21] and the nectar flow rate [24]. The profitability of a food source relative to other food sources in the environment drives recruitment to that food source [25] through its effect on the dance activity of individual foragers [15], [20]. In addition, previous work showed that individual foragers differed in both their foraging and dance activity for the same food source [23], [26] and that the differences in dance activity were consistent across days [27]. Thus, the dance activity provides a robust experimental paradigm to explore the mechanistic underpinnings of inter-individual variation in behaviour.

Inter-individual variation in sensory response thresholds determine which worker responds to a stimulus and the intensity of their response in social insect colonies [11]. In large colonies made up of thousands of workers, only those individuals for whom the intensity of the stimulus exceeds their thresholds respond to that stimulus [28]. This ensures that the colony does not expend unnecessary resources in responding to any change in stimuli. Empirical evidence for theoretical models of division of labour based on response thresholds point to the prevalence of this mechanism across social insects [29]–[33].

In honey bees, response thresholds for gustatory stimuli have been studied extensively [34]–[39]. Responsiveness to sucrose, assayed through the proboscis extension reflex, correlates with division of labour at multiple scales in the colony. Nurses and foragers show differences in their sucrose responsiveness as do pollen and nectar foragers [34]–[36], [38], [40], [41]. Individual honey bees can also differ hugely in their evaluation of a sugar source [38], [42]. This individual sense of taste or evaluation of a sugar source can have drastic effects on their cognitive performance [37], [40], [43]. Bees with a higher responsiveness to sucrose, for example, learn to associate an odour or a tactile stimulus with a sugar reward faster [35], [44]. Both sucrose responsiveness and dance activity are tied to the reward value perceived by the individual [21], [45], hinting at a link between the two.

In this study, we looked at mechanisms underlying individual variation in the round dance activity of honey bee foragers. We first tested the hypothesis that individual variation in sucrose responsiveness is correlated with individual variation in both foraging and dance activity. Bees with a higher sucrose responsiveness should place a higher subjective reward value on a feeder and hence dance more than bees with a lower responsiveness. We then tried to identify molecular correlates of individual variation in dance activity. Bees performing different tasks in the hive differ in the expression of nutrition-relates genes such as the *foraging* gene *Amfor* [42] or the octopamine receptor gene *AmoctαR1* [46]. We tested the hypothesis that individual dance activity is correlated with the brain gene expression of either of these genes. Further, we studied the gene expression of the *insulin receptor AmInR-2*, as a possible mediator of nutrient-sensing molecular pathways involved in foraging decisions [47], [48].

The *Amfor* gene codes for a cGMP-dependent protein kinase (PKG) in the honey bee. PKG has been linked to the lengths of foraging paths in both *Drosophila melanogaster* [49] and *C. elegans* [50], indicating a role in foraging-related locomotor behaviour. At the same time, bees differing in their gustatory responsiveness also differed in their *Amfor* brain gene expression [42] and in their PKG protein expression [51]. In *Drosphila* wild-type variants and in mutants differing in their PKG activity, a direct link between PKG and gustatory responsiveness was demonstrated [52], [53].

Previous work showed a link between the neuromodulator octopamine and the dance activity of honey bee foragers [45]. Specifically, the topical application of octopamine increased the dance probability in honey bee foragers. The octopamine receptor AmOctαR1 is one out of five octopamine receptors in honey bees. Like *Amfor*, this octopamine receptor is also connected to gustatory responsiveness. Foragers, which differed in their gustatory responsiveness from nurse bees, also differed in their *AmoctαR1* gene expression [46]. Increasing octopamine levels in the brain enhanced gustatory responsiveness [54] and improved individual learning performance [55]. Finally, insulin receptors are highly likely involved in the decision of whether to forage for pollen or nectar. High-pollen hoarding strains showed higher levels of insulin receptor expression than low pollen hoarding strains [48]. In addition, bees from the high pollen strains also had a higher gustatory responsiveness than bees from the low pollen strain [36]. Taken together, these findings make *Amfor*, *AmoctαR1* and *AmInR-2* interesting candidates for controlling or modulating individual dance performance in honey bees.

Materials and Methods

Bees

Apis mellifera carnica colonies used in all experiments were obtained from the University of Würzburg's apiary. Colonies consisting of naturally mated queens and around 4,000 workers were set up in a two-frame observation hive connected to an outdoor flight cage (length: 9m, width: 5m, height: 3m). The colonies were provided with pollen and nectar at two different feeders within the flight cage. Nectar foragers were trained to an artificial feeder located around 5m away from the entrance of the hive. On each experimental day, 6-10 foragers were marked individually using paint marks. A wedge was used to direct the returning foragers to one side of the observation hive. This ensured that foragers only used this side as the dance floor [56]. Newly recruited, i.e. unmarked, foragers were caught, kept on ice and allowed back into the hive after the experiments on each day [15].

Behavioural analysis

A total of 110 foragers from 3 colonies (28, 27 and 55 individuals, respectively) were observed from July 2015 to September 2015. On each experimental day, 3 hours of behavioural observations were done, between 09:00 hours and 13:00 hours. The foragers were provided with 1M sucrose for the first hour, 2M for the second hour and 1M for the third hour (Fig. 4.1 a) at the feeder following established protocols for performing dance experiments [23]. Throughout the observation time, two observers were present, one at the feeder and one near the observation hive. The observer at the feeder would note down the trip made by the marker forager and capture recruits. The observer at the observation hive would video record the dance activity of the marked foragers. Video recordings were performed using a Sony Digital HD Video Camera Recorder HDR-CX405 (Sony Corporation, Japan) with 50 frames per second.

We measured *foraging intensity* (the number of foraging trips made in an hour), *number of dances*, *number of dance circuits*, *dance probability* (the ratio of the number of dances to the number of foraging trips) and *dance intensity* (the ratio of the number of circuits to the number of dances) for each individual forager for each hour of the observation time-period. After the behavioural observations, we quantified the individual sucrose responsiveness of each videotaped dancer. To do this, individuals were first immobilized on ice and mounted in a metal tube. After an hour, a series of water and six sucrose solutions were applied sequentially to both antennae of each bee, following established protocols [57]. Each individual forager was given a score of 1 or 0 for a solution depending on whether the solution elicited a proboscis extension response or not. The sum of the scores (maximum value of 7) is the gustatory response score (GRS) of the bee. After measuring sucrose responsiveness, bees were quickly transferred to liquid nitrogen and then stored individually at -80°C until their brains were dissected for mRNA expression analysis.

Gene expression

We first shortlisted individual foragers based on their dance activity and then looked at the messenger RNA expression of our genes of interest in their brains. The foragers were shortlisted based on their total circuits for the 2M feeder condition in such a manner that they had strong differences in their behavioural output. We used the total dance circuits as it is a very reliable indicator of the reward value perceived by foragers and used their activity in the 2M sucrose condition as foragers showed the greatest variation during this feeder condition [23].

Individual bee brains were then homogenized in 750 mL of Isol-RNA lysis reagent (5PRIME, Hilden, Germany). This homogenate was mixed with 150 µl chloroform to achieve a phase separation in which RNAs tend to move into the aqueous phase. This aqueous phase was transferred to 1 volume Ethanol (70% v/v). Afterwards, the RNA in this solution was purified using the peqGOLD Total RNA Kit (Peqlab, Erlangen, Germany) following standard protocol including the optional DNase I digestion step (DNase I Digest Kit, Peqlab, Erlangen, Germany). The purified RNA (100 ng/reaction) was then used as a template to generate cDNA using the QuantiTect® Reverse Transcription Kit (Qiagen, Hilden, Germany). Each individual cDNA (Table 4.1) was analysed in triplicates in a quantitative Real-Time PCR using a Rotor-Gene Q (Qiagen, Hilden, Germany). A PCR reaction (25 µL) contained each primer (0.25 µM), TaqMan© probes (0.1 µM) and Rotor-Gene Multiplex PCR Master Mix (Qiagen, Hilden, Germany). Sequences of primers and TaqMan probes are given in Table 4.1. The following protocol was followed for the PCR: 1 min at 60°C, 5 min at 95°C and 45 cycles consisting of 20 s at 95°C followed by 1 min at 60°C each. Afterwards, the gene of interest (GOI) and reference gene (REF, *Amef1α*, Reim et al. 2013) Ct values were determined. With these values we have calculated the GOI expression relative to REF expression with the ΔΔCt method [59]. These calculations were performed using the Rotor Gene Q software (Qiagen, Chatsworth, CA, USA). We performed the gene expression analysis on two replicates of foragers.

Table 4.1

Gene	**Oligo name**	**Oligo sequence (5'-3')**
EF1a	AmEF1alpha_qF [1]	gAACATTTCTgTgAAAgAgTTgAggC
	AmEF1alpha_qR [1]	TTTAAAggTgACACTCTTAATgACgC
	Ef1a_o_S [2]	CgATTgTCACACCgCTCATATC
	Ef1a_o_R [2]	TAAAggTgACACTCTTAATgACg
	AmEF1alpha_TM	6FAM-ACCgAggAgAATCCgAAgAgCATCAA-BBQ
Amfor	AmFOR_A_qF2	CTTgACACCgACgAAACCC
	AmFOR_A_qR1	CtgCTTTgATCAgTTCACgAgATC
	AmFOR_A_TM	YAK -TTgTCAgCgTggCAAgCTCTTgA-BBQ
AmoctαR1	AmOa1_F	gCAggAggAACAgCTgCgAg
	AmOa1_R	gCCgCCTTCgTCTCCATTCg
	AmOa1_TM	YAK-TCCCCATCTTCATCACCCTTggCTTCTCC-BBQ
AmInR-2	InR-2 A	CgATCAgCAAAATTTgTACgAATC
	InR-2 S	AAAgAAgAgggAgTgTgCCA
	InR-2 TM	YAK-TCggCATCCCTTCAgTCTTgAgAACT-BBQ

[1] together with *Amfor, and AmoctαR1.*

[2] together with *Amilp2 and AmInR-2.*

Table 4.1: Oligonucleotides used for quantitative real time PCR along with their sequence for the reference gene *EF1a*, foraging gene *Amfor*, octopamine receptor gene *AmoctαR1* and insulin receptor gene *AmInR-2*

Statistical Analysis

We used mixed effects models to analyse the effect of the sucrose concentration on the five different behavioural parameters we measured: foraging intensity, number of dances, number of circuits, dance probability and dance intensity. We fit separate models for each behavioural parameter, with the parameter as the response variable, the 3 hours (corresponding to the first 1M, 2M and second 1M) as the predictor and the bee identity nested within colony as a random effect. We fit generalized linear mixed effects models (GLMMs) for foraging intensity, number of dances, number of circuits and dance probability and fit a linear mixed effects model (LMM) for dance intensity, based on the best fitting error distribution for each parameter. The error distributions for the GLMMs were Poisson for foraging intensity, Negative Binomial for number of dances and number of circuits and Binomial for dance probability. We then used generalized linear hypothesis test with conservative single step adjusted p values to compare each behavioural parameter between the 3 hours. We also checked for correlations between dance intensity and foraging intensity during either of the 3 hours, using Spearman's correlation coefficient.

We used mixed effects models to analyse whether the GRS of an individual could predict its foraging intensity, number of dances, number of circuits, dance probability or dance intensity. The behavioural parameter was the response variable, the GRS was the predictor and the colony was a random effect in the models. Separate models were built for each of the 3 hours for each of the behavioural parameters. We fit GLMMs for foraging intensity, number of dances, number of circuits and dance probability and fit an LMM for dance intensity, based on the best fitting error distribution for each parameter. The error distributions for the GLMMs were Poisson for foraging intensity, Negative Binomial for number of dances and number of circuits and Binomial for dance probability. Our model for the number of circuits at the second 1M feeder condition showed a high degree of overdispersion. To account for this, we fit the model with an extra random intercept with one level per observation [60].

We used LMMs to compare to analyse whether the relative mRNA expression of any of the 3 different genes of interest could predict the total dance circuits of the individual foragers in the 2M feeder condition. We first used an LMM with the dance circuits as the response, the GRS as the predictor and the replicates as a random effect to verify that there was no correlation between the dance circuits and the sucrose responsiveness of the selected individuals. We then built separate models for each of the genes of interest with the dance circuits as the response variable, the relative mRNA expression as the predictor and the replicates as a random effect.

All of the statistical analyses were performed in R 3.5.1 using the RStudio IDE [61], [62]. We first fit different distributions to each behavioural parameter using the fitdistrplus package [63]. We then used lme4 to fit GLMMs [64] and nlme to fit LMMs [65] based on the appropriate error structure. We used the lattice [66] and DHARMa [67] packages to check model assumptions and the ggplot2 [68], ggpubr [69], cowplot [70] and grid (from base R) packages to produce plots. Spearman's correlation tests and t-tests were done using the stats package in base R.

Results

Foraging intensity varies with sugar concentration of the feeder and amongst individuals

Foraging intensity strongly depended on the sucrose concentration of the feeder. The number of foraging trips was significantly greater for both the first 1M and the second 1M sucrose solution than for the 2M sucrose solution (Table 4.2, Fig. 4.1 b, $p < 0.001$ for both). The foraging intensity was the same for both 1M condition. Foraging intensity also varied amongst individuals, with some individuals only performing a few foraging trips and others performing a greater number of foraging trips (range - first 1M: 7 to 28 trips per hour, 2M: 0 to 19, second 1M: 0 to 24).

Table 4.2

Behavioural Parameter	**Comparison of Concentrations**	**Difference Estimate**	**Confidence Interval**	***p* Value**
Foraging Intensity	Second 1M vs First 1M	-0.072	-0.149 – 0.006	0.078
	2M vs First 1M	*-0.357*	*-0.441 - -0.273*	*<0.001*
	2M vs Second 1M	*-0.285*	*-0.370 - -0.200*	*<0.001*
Number of Dances	Second 1M vs First 1M	0.007	-0.093 – 0.107	0.986
	2M vs First 1M	-0.055	-0.156 – 0.047	0.413
	2M vs Second 1M	-0.062	-0.163 – 0.039	0.325
Number of Waggle Runs	Second 1M vs First 1M	0.084	-0.192 – 0.359	0.758
	2M vs First 1M	*0.437*	*0.161 – 0 .712*	*< 0.001*
	2M vs Second 1M	*0.353*	*0.078 – 0.629*	*0.008*
Dance Probability	*Second 1M vs First 1M*	*0.214*	*0.038 – 0.389*	*0.012*
	2M vs First 1M	*1.172*	*0.959 – 1.384*	*<0.001*
	2M vs Second 1M	*0.958*	*0.741 – 1.174*	*<0.001*
Dance Intensity	Second 1M vs First 1M	0.039	-1.893 – 1.970	0.999
	2M vs First 1M	*10.884*	*8.952 – 12.815*	*<0.001*
	2M vs Second 1M	*10.845*	*8.914 – 12.776*	*<0.001*

Table 4.2: Estimated differences, confidence intervals and single step adjusted *p* values from mixed

effects models looking at the effect of different concentrations on the 5 different behavioural parameters. The difference estimates are provided in the link scale of the respective GLMM and hence are not in the scale of the behavioural parameter (except in the case of dance intensity, for which an LMM was fitted). The comparisons which showed a significant difference are highlighted in italics.

Figure 4.1

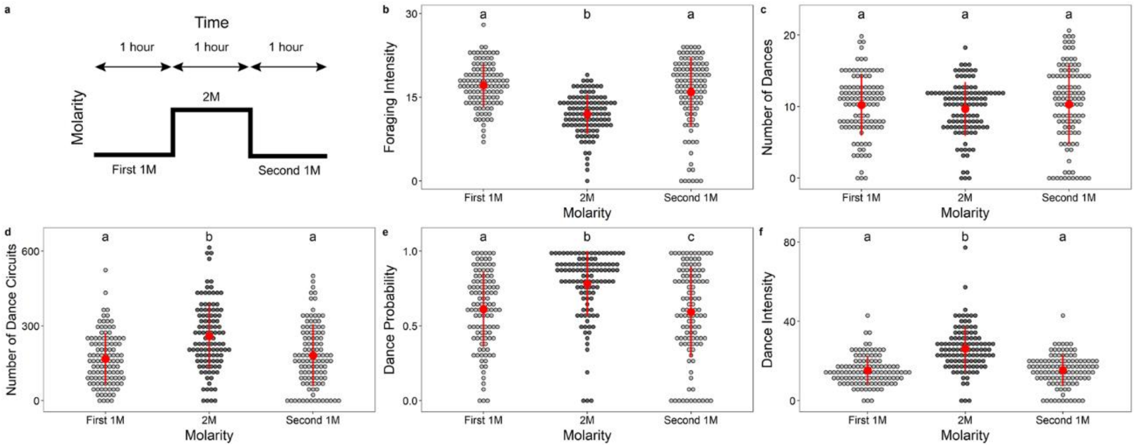


Figure 4.1 **a:** Schematic of experimental protocol. Honeybees in an observation hive were trained to a feeder in an outdoor flight cage. On each experimental day at the same time, bees were offered a 1M sucrose feeder for one hour. This was followed by a 2M sucrose feeder in the next hour. In the third hour, bees were again offered a 1M sucrose feeder. Bees were marked individually at the feeder. Their dance behaviour was subsequently videotaped in the hive. At the end of the observation, we quantified sucrose responsiveness of each dancer which had been videotaped. **b-f:** Dotplots representing the foraging intensity, the number of dances, the total number of circuits, the dance probability and the dance intensity, respectively of all 110 individuals for each of the 3 hours of experimental time. The mean and the standard deviation for each group is indicated by the red circle and vertical line respectively. Significant differences ($p < 0.05$) between groups are indicated by different letters above the boxplots. Foraging intensity was lower and the number of circuits, dance probability and dance intensity were higher in the 2M as compared to both 1M. In addition, the dance probability was higher during the second 1M as compared to the first. The number of dances did not differ during either of the 3 hours.

Dance activity varies with sugar concentration of the feeder and amongst individuals

Interestingly, the difference in foraging intensity did not translate to a difference in the number of dances. The number of dances performed for each concentration did not significantly differ from each other (Table 4.2, Fig. 4.1 c, $p > 0.05$). At the same time, individual bees strongly deviated from each other in their dance number. Some bees did not dance at all, whereas others performed up to 21 dances in the same time interval (range - first 1M: 0 to 21 dances per hour, 2M: 0 to 18, second 1M: 0 to 21).

We also found that the number of circuits showed an inverse relationship as compared to the foraging intensity. The total number of circuits was higher for 2M in comparison with both 1M situation (Table 4.2, Fig. 4.1 d, $p < 0.01$). The number of circuits was not significantly different between either of the 1M condition. Individuals strongly differed in the number of circuits they performed within an hour, with some individuals not performing any dances at all and some performing more than 400 circuits (range - first 1M: 0 to 520 circuits per hour, 2M: 0 to 617, second 1M: 0 to 510).

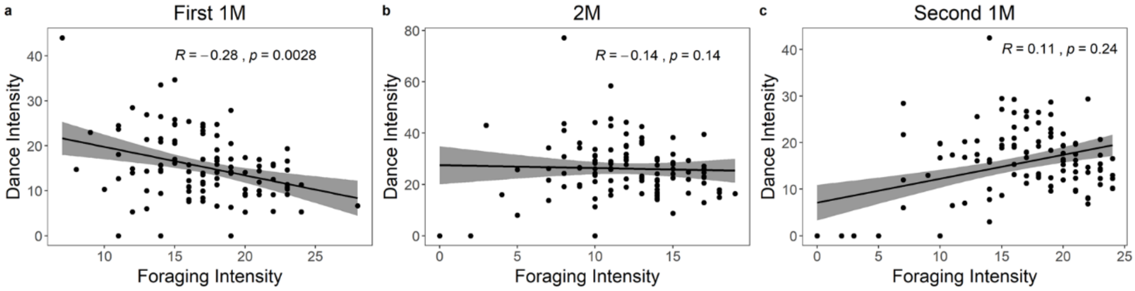
Similarly, we found that both the dance probability and the dance intensity were significantly higher for 2M as compared to both 1M condition (Table 4.2, Fig. 4.1 e and f, $p < 0.001$). In addition, the dance intensity was similar ($p > 0.05$) during the two hours with 1M sucrose solution at the feeder, but the dance probability differed, with a higher probability for the second 1M as compared to the first 1M sucrose solution ($p = 0.012$).

Individual foragers also differed strongly in their dance probability, with some individuals not performing any dance at all and some performing dances during all their foraging trips during the first 1M, 2M and the second 1M sucrose feeder. This inter-individual variation was also seen in the dance intensity, with some individuals performing fewer than 10 runs per dance and some individuals performing more than 25 runs in both 1M condition. This variation was stronger in the 2M condition, with some individuals performing more than 40 runs per dance and others performing fewer than 20 runs per dance.

Trade-off between dance activity and foraging activity

We hypothesized that individual foragers would show a trade-off between their foraging activity and dance intensity. In line with our hypothesis, we found that dance intensity correlated negatively with the foraging intensity during the first 1M sucrose solution (Fig. 4.2 a; Spearman's correlation coefficient = -0.28, $P = 0.002$). However, we did not find a correlation when the feeder offered 2M sucrose solution (Fig. 4.2 b; Spearman's correlation coefficient = -0.14, P = 0.14), or during the second 1M (Fig. 4.2 c; Spearman's correlation coefficient = 0.11, P = 0.24).

Figure 4.2



a-c: Correlations between the dance intensity and the foraging intensity of all 110 individual foragers for each of the 3 hours, respectively. Spearman's correlation coefficient (R) and the p value representing the significance of the correlation are provided within each plot. A linear regression along with the 95% confidence interval around it is provided as a black line and grey shaded region in each plot. Dance intensity was negatively correlated with foraging intensity for the first 1M sucrose feeder.

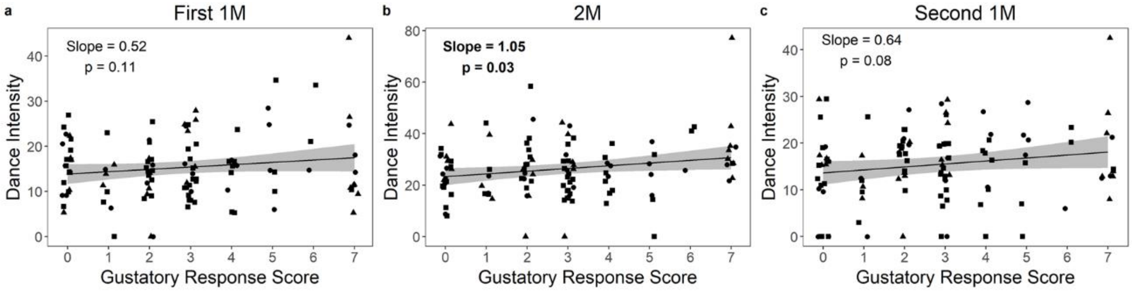
Individual sucrose responsiveness can explain variation in individual dance activity

We hypothesized that individual differences in the dance activity of foragers would be positively correlated with individual sucrose responsiveness. We first tested whether gustatory response scores (GRS), which are a measure for sucrose responsiveness [57], correlated with foraging intensity. We found that there was no correlation between foraging intensity and the GRS in either of the three feeder conditions (first 1M: effect size = -0.013, CI = -0.035 – 0.008, *p* = 0.225; 2M: effect size = -0.0096, CI = -0.035 – 0.016, *p* = 0.463; second 1M: effect size = 0.008, CI = -0.014 – 0.029, *p* = 0.485). Similarly, we found no correlation between the GRS and number of dances (first 1M: effect size = -0.014, CI = -0.054 – 0.025, *p* = 0.463; 2M: effect size = 0.0012, CI = -0.035 – 0.037, *p* = 0.947; second 1M: effect size = 0.003, CI = -0.057 – 0.064, *p* = 0.916) and GRS and number of circuits (first 1M: effect size = 0.0102, CI = -0.056 – 0.078, *p* = 0.763; 2M: effect size = 0.036, CI = -0.024 – 0.096, *p* = 0.245; second 1M: effect size = 0.104, CI = -0.047 – 0.255, *p* = 0.175).

We also found no correlation between the GRS and the dance probability at either the first 1M (odds ratio: 2.722, CI = 2.607 – 2.848, *p* = 0.952) or the second 1M condition (odds ratio: 2.686, CI = 2.568 – 2.816, *p* = 0.614). We found an observable but non-significant trend in dance probability at the 2M sucrose feeder (odds ratio: 2.886, CI = 2.697 – 3.108, *p* = 0.087), indicating that an increase in GRS by 1 increases the odds of dancing by a factor of around 3.

Finally, we found that the GRS of foragers correlated positively with the intensity of dancing of foragers at the 2M feeder (Fig. 4.3 b; slope: 1.054, CI = 0.086 – 2.022, *p* = 0.033). But the GRS did not correlate with the dance intensity at either the first 1M feeder (Fig. 4.3 a; slope: 0.515, CI = -0.127 – 1.158, *p* = 0.115) or the second 1M feeder (Fig. 4.3 c; slope: 0.636, CI = -0.068 – 1.341, *p* = 0.076).

Figure 4.3



a-c: Correlations between GRS and Dance Intensities of foragers for each of the 3 hours, respectively. The data for all 110 foragers are provided with the shape representing the colony they belonged to (Colony 1: circles, Colony 2: triangles, Colony 3: squares). The straight line represents the linear correlation obtained between GRS and dance intensity from linear mixed effects models, with the grey area representing the 95 % confidence interval around this correlation. The slope and the significance of the correlation is provided for each of the three comparisons. GRS correlated with the dance intensity only during foraging at the 2M sucrose-concentration feeder.

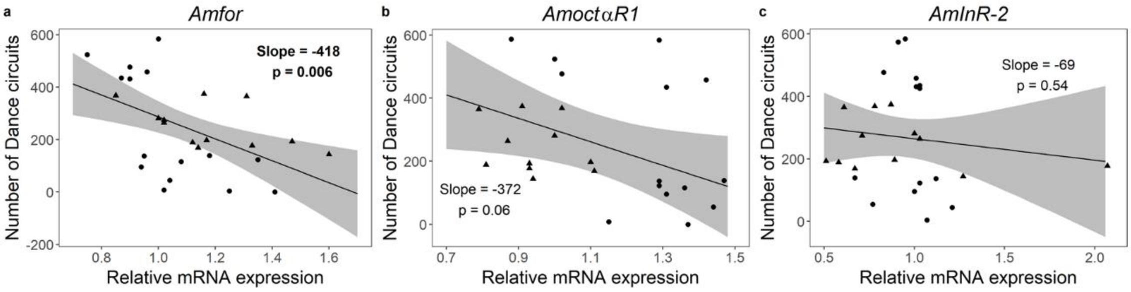
Gene expression correlates with differences in individual dance activity

We quantified mRNA expression of candidate genes in the brains of selected individual foragers to look for neuronal candidates underlying differences in dance activity. There was no correlation between the GRS and the total dance circuits in the 2M feeder condition of the selected individuals (slope: -7.183, CI = -44.405 – 30.039, *p* = 0.705).

We then checked for correlations between the dance circuits in the 2M feeder condition and the mRNA expression of the following genes: the *foraging* gene *Amfor*, the octopamine receptor gene *AmoctαR1* and the insulin receptor gene *AmInR-2*.

We found a strong negative correlation between the dance activity and the mRNA expression of *Amfor*. Individual foragers with lower expression levels had performed higher dance circuits in the 2M feeder condition (Fig. 4.4 a; slope: -418.027, CI = -694.209 – -141.844, *p* = 0.006). We did not find any correlation between the dance circuits and expression levels of *AmoctαR1* (Fig. 4.4 b; slope: -372.268, CI = -743.824 – 0.713, *p* = 0.059) or *AmInR-2* (Fig. 4.4 c; slope: -68.679, CI = -290.323 – 152.966, *p* = 0.544).

Figure 4.4



a-c: Correlations between the dance circuits in the 2M feeder condition and messenger RNA expression of *Amfor*, *AmOctαR1* and *AmInR-2*, respectively. The straight line represents the linear correlation obtained between the mRNA expression levels and dance circuits from linear mixed effects models, with the grey area representing the 95 % confidence interval around this correlation. The slope and the significance of the correlation is provided for each of the three comparisons. Circles represent the selected individuals in the first replicate and triangles represent the selected individuals in the second replicate. Only the mRNA expression levels of *Amfor* showed a significant negative with the dance circuits, with more active dancers having lower levels of *Amfor*.

Discussion

We found that honey bee foragers show large inter-individual variation in both foraging and dance activity. Our results are similar to findings from earlier studies on the foraging and dance activities of honey bee workers [23], [26], [71], and are in line with studies from other social groups [72]–[76]. In addition, recent work has shown a high degree of consistency in inter-individual activity variation among social insects [77], [78]. For example, such consistency in activity has been observed in the dance activity of honey bee foragers [27]. Consistent inter-individual differences across behavioural contexts have been linked to animal ‘personalities’ in vertebrates [3]. The role that such consistent inter-individual differences play in a eusocial insect colony’s functioning remains largely unexplored. Individual differences are hypothesized to play a key role in maintaining division of labour and hence ensure the efficient functioning of large colonies [79].

Our behavioural observations also revealed a trade-off between foraging and recruitment activity. Our results can be understood in the context of the colony’s need to ensure that each foraging group is efficiently exploiting a food source. A balance has to be struck between foraging and recruitment activity of the group, till an optimal number of foragers are active at the food site. This balance between these two parallel activities might be achieved by coupling individual variation in foraging and dance intensity negatively, as seen in our experiments. Individuals might invest more in foraging or dancing and at the same time contribute to the efficient exploitation of the food source. Our results indicate that such a trade-off does exist but might be easier to observe only under a situation when individuals have to invest maximally into either foraging or recruitment. We found a negative correlation between foraging and dance intensity at the first 1M feeder condition, but at 2M or the second 1M feeder condition. In the initial stages of foraging at a nearby food source, individuals do trade off foraging and dancing. However, since the food source is nearby, and individuals do not have to invest a lot of time in flying to the food source, this trade-off is not apparent in our experimental set-up after the first hour. We believe that a similar situation to our first 1M condition would arise if foraging was more energetically intensive, for example if foragers had to fly longer distances or forage at food sources with longer handling time. Nevertheless, our results are similar to an earlier study on ants which reported a trade-off between attacking a prey or calling for help from nest mates [80]. Thus, trade-offs between foraging and recruitment at the individual level might be a hallmark of eusocial foraging groups which rely on social communication to exploit food sources.

Response thresholds have been hypothesized to play a key role in driving individual variation in behaviour in eusocial colonies. Individual differences in response thresholds could underly individual variation in both the intensity and the probability of responding to a stimulus [81]. This in turn would help the colony invest an appropriate number of workers while responding to any change in stimulus and hence ensures the efficient functioning of the colony. Thus, the presence of such response thresholds across eusocial insect species points to prevalence of such thresholds in determining who does what work in such colonies. For example, such thresholds have been observed in thermoregulation in bumblebees [33], undertaking in honey bees [29] and foraging in ants [82].

Our results hint at a possible link between sucrose responsiveness and the intensity of dance activity. Individual sucrose responsiveness correlated positively with dance intensity when bees were foraging at a 2M sucrose feeder. This indicates that when foraging at a feeder offering a high sugar concentration (i.e. 2M sucrose), individuals with a high responsiveness perceived a relatively higher "reward" value than bees with a lower responsiveness. Interestingly, we did not find a correlation between sucrose responsiveness and dance intensity at the 1M feeder condition. The lesser inter-individual variation in dance intensity at 1M as compared to 2M (see Fig. 4. 1 f) might be an explanation for why we don't see a correlation between dance intensity and sucrose responsiveness at the lower concentration. We can also not rule out the role other factors, particularly social cues activity [19], [27], [83], [84], play in determining individual variation in dance activity. The relative strength of all these factors in modulating inter-individual variation might be reward-dependent and hence would differ based on the sucrose concentration.

Finally, we also investigated mRNA expression of three selected genes involved in locomotor behaviour, nutrition-related behaviours and sucrose responsiveness to explore possible molecular mechanisms underlying individual variation in dance activity. Among these genes, the mRNA expression of the *foraging* gene *Amfor* showed a significant negative correlation with dance activity. Individuals with higher mRNA expression had performed fewer dance circuits. At the same time, the sucrose responsiveness of the selected individuals did not correlate with the dance circuits.

Hence, the variation in gene expression is likely to be related to variation in locomotor behaviour rather than to variation in the evaluation of the food source. The *foraging* gene encodes one of two cGMP-dependent protein kinases in the honey bee. In *Drosophila melanogaster,* expression of the *foraging* gene was related to locomotor behaviour of larvae during food search. Larvae with higher PKG protein levels displayed longer food-search paths than those with lower PKG levels (referred to as "rovers" and "sitters" respectively) [49]. More importantly, adult sitters showed a higher turning rate than rovers in the sugar-elicited search behaviour [85]. Thus, it appears that turning frequency in flies and honey bees is correlated with low expression levels of the *foraging* gene. Interestingly, Dethier (1957) demonstrated that sugar-elicited search behaviour in flies and honey bee dance communication show many similarities. He proposed that behavioural modules of the search behaviour might have been incorporated into the honey bee dance behaviour [86]. Our experiments clearly showed a correlation between *foraging* gene expression and dance activity, suggesting that this gene not only plays a role in spatial exploration but also in the communication of navigational information. In addition, they might also provide support for Dethier's ideas on how one form of social communication might have evolved from solitary behaviour by using an already existing behavioural genetic toolkit [87], [88]. Future studies of this phenomenon using targeted knockdown through RNA interference [89] and knockout by CRISPR/Cas 9 [90] hold the promise of providing a deeper understanding of the neuronal and molecular mechanisms underlying PKG and recruitment behaviour in honey bees.

Data accessibility

The raw data obtained from these observations will be uploaded and made available at a Mendeley Data repository.

References

[1] M. J. Ryan, *An Introduction to Animal Behaviour: An Integrative Approach*, vol. 84, no. 5. 2012.

[2] A. Sih, A. Bell, and J. C. Johnson, "Behavioral syndromes: An ecological and evolutionary overview," *Trends Ecol. Evol.*, vol. 19, no. 7, pp. 372–378, 2004.

[3] A. Sih, K. J. Mathot, M. Moirón, P. O. Montiglio, M. Wolf, and N. J. Dingemanse, "Animal personality and state-behaviour feedbacks: A review and guide for empiricists," *Trends Ecol. Evol.*, vol. 30, no. 1, pp. 50–60, 2015.

[4] S. R. X. Dall, A. I. Houston, and J. M. McNamara, "The behavioural ecology of personality: Consistent

individual differences from an adaptive perspective,” *Ecol. Lett.*, vol. 7, no. 8, pp. 734–739, 2004.

[5] A. M. Bell, S. J. Hankison, and K. L. Laskowski, “The repeatability of behaviour: a meta-analysis,” *Anim. Behav.*, vol. 77, no. 4, pp. 771–783, Apr. 2009.

[6] K. J. Gaston, “The Magnitude of Global Insect Species Richness,” *Conserv. Biol.*, vol. 5, no. 3, pp. 283–296, 1991.

[7] R. M. May, N. Series, and N. Sep, “How Many Species are There on Earth ?,” vol. 241, no. 4872, pp. 1441–1449, 2007.

[8] B. Brembs, “Invertebrate behavior-actions or responses?,” *Front. Neurosci.*, vol. 7, no. 7 NOV, pp. 1–2, 2013.

[9] M. Wolf and F. J. Weissing, “Animal personalities: Consequences for ecology and evolution,” *Trends Ecol. Evol.*, vol. 27, no. 8, pp. 452–461, 2012.

[10] A. S. Griffin, L. M. Guillette, and S. D. Healy, “Cognition and personality: An analysis of an emerging field,” *Trends Ecol. Evol.*, vol. 30, no. 4, pp. 207–214, 2015.

[11] S. N. Beshers and J. H. Fewell, “Models of division of labor in social insects.,” *Annu. Rev. Entomol.*, vol. 46, pp. 413–40, Jan. 2001.

[12] B. R. Johnson, “Division of labor in honeybees: Form, function, and proximate mechanisms,” *Behav. Ecol. Sociobiol.*, vol. 64, no. 3, pp. 305–316, 2010.

[13] T. D. Seeley, *The Wisdom of the Hive*. 1995.

[14] C. Thom, T. D. Seeley, and J. Tautz, “A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity,” *Apidologie*, vol. 31, no. 6, pp. 737–738, 2000.

[15] K. von Frisch, *The dance language and orientation of bees*. Harvard University Press, 1967.

[16] K. von Frisch, “Decoding the language of the bee.,” in *Science*, 1974, vol. 185, no. 4152, pp. 663–668.

[17] K. E. Gardner, T. D. Seeley, and N. W. Calderone, “Do honeybees have two discrete dances to advertise food sources?,” *Anim. Behav.*, vol. 75, no. 4, pp. 1291–1300, Apr. 2008.

[18] T. D. Seeley, “Social foraging by honeybees: how colonies allocate foragers among patches of flowers,” *Behav. Ecol. Sociobiol.*, vol. 19, no. 5, pp. 343–354, Nov. 1986.

[19] T. D. Seeley, “Social foraging in honey bees: how nectar foragers assess their colony’s nutritional status,” *Behav. Ecol. Sociobiol.*, vol. 24, no. 3, pp. 181–199, Mar. 1989.

[20] T. D. Seeley, A. S. Mikheyev, and G. J. Pagano, “Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability,” *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.*, vol. 186, no. 9, pp. 813–819, Oct. 2000.

[21] K. D. Waddington, “Honey bee foraging profitability and round dance correlates,” *J. Comp. Physiol. A*, vol. 148, no. 3, pp. 297–301, 1982.

[22] J. Tautz and D. C. Sandeman, “Recruitment of honeybees to non-scented food sources.,” *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.*, vol. 189, no. 4, pp. 293–300, Apr. 2003.

[23] T. D. Seeley, “Honey bee foragers as sensory units of their colonies,” *Behav. Ecol. Sociobiol.*, vol. 34, pp. 51–62, 1994.

[24] A. J. Wainselboim and W. M. Farina, “Trophallaxis in honeybees, Apis mellifera (L.), as related to their past experience at the food source,” *Anim. Behav.*, vol. 66, no. 4, pp. 791–795, 2003.

[25] T. D. Seeley and W. F. Towne, “Tactics of dance choice in honey bees: do foragers compare dances?,” *Behav. Ecol. Sociobiol.*, vol. 30, no. 1, pp. 59–69, 1992.

[26] P. Tenczar, C. C. Lutz, V. D. Rao, N. Goldenfeld, and G. E. Robinson, “Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels,” *Anim. Behav.*, vol. 95, pp. 41–48, Sep. 2014.

[27] E. A. George and A. Brockmann, “Regulation of individual differences in recruitment behaviour within honey bee foraging groups,” *bioRxiv*, p. 241679, Jan. 2018.

[28] G. Theraulaz, E. Bonabeau, and J.-L. Deneubourg, “Response threshold reinforcements and division of labour in insect societies,” *Proc. R. Soc. B Biol. Sci.*, vol. 265, no. 1393, pp. 327–332, Feb. 1998.

[29] M. D. Breed, D. B. Williams, and A. Queral, “Demand for task performance and workforce replacement: Undertakers in honeybee, Apis mellifera, colonies,” *J. Insect Behav.*, vol. 15, no. 3, pp. 319–329, 2002.

[30] S. O’Donnell, “Effects of experimental forager removals on division of labour in the primitively eusocial wasp *Polistes instabilis* (*Hymenoptera*: *Vespidae*),” *Behaviour*, vol. 135, no. 2, pp. 173–193, 1998.

[31] K. E. Gardner, R. L. Foster, and S. O’Donnell, “Experimental analysis of worker division of labor in bumblebee nest thermoregulation (Bombus huntii, Hymenoptera: Apidae),” *Behav. Ecol. Sociobiol.*, vol. 61, no. 5, pp. 783–792, 2007.

[32] N. Pinter-Wollman, J. Hubler, J.-A. Holley, N. R. Franks, and A. Dornhaus, “How is activity distributed among and within tasks in Temnothorax ants?,” *Behav. Ecol. Sociobiol.*, vol. 66, no. 10, pp. 1407–1420, Aug. 2012.

[33] A. Weidenmüller, “The control of nest climate in bumblebee (Bombus terrestris) colonies: interindividual variability and self reinforcement in fanning response,” *Behav. Ecol.*, vol. 15, no. 1, pp. 120–128, Jan. 2004.

[34] L. Değirmenci, M. Thamm, and R. Scheiner, “Responses to sugar and sugar receptor gene expression in different social roles of the honeybee (Apis mellifera),” *J. Insect Physiol.*, vol. 106, no. May 2017, pp. 65–70, 2018.

[35] R. Scheiner, J. Erber, and R. E. Page, “Tactile learning and the individual evaluation of the reward in honey bees (Apis mellifera L.),” *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.*, vol. 185, no. 1, pp. 1–10, 1999.

[36] R. Scheiner, R. E. Page, and J. Erber, “The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (Apis mellifera L.).,” *Neurobiol. Learn. Mem.*, vol. 76, no. 2, pp. 138–150, 2001.

[37] R. Scheiner, R. E. Page, and J. Erber, “Responsiveness to sucrose affects tactile and olfactory learning in preforaging honey bees of two genetic strains,” *Behav. Brain Res.*, vol. 120, no. 1, pp. 67–73, 2001.

[38] R. Scheiner, M. Barnert, and J. Erber, “Variation in water and sucrose responsiveness during the foraging season affects proboscis extension learning in honey bees,” *Apidologie*, vol. 34, pp. 67–72, 2003.

[39] R. Scheiner, R. E. Page, and J. Erber, “Sucrose responsiveness and behavioral plasticity in honey bees (Apis mellifera) Ricarda,” *Apidologie*, vol. 35, pp. 133–142, 2004.

[40] R. Scheiner, T. Reim, E. Søvik, B. V. Entler, A. B. Barron, and M. Thamm, “Learning, gustatory responsiveness and tyramine differences across nurse and forager honeybees,” *J. Exp. Biol.*, vol. 220, no. 8, pp. 1443–1450, 2017.

[41] Y. Ben-Shahar and G. E. Robinson, “Satiation differentially affects performance in a learning assay by nurse and forager honey bees,” *J. Comp. Physiol. - A Sensory, Neural, Behav. Physiol.*, vol. 187, no. 11, pp. 891–899, 2001.

[42] M. Thamm and R. Scheiner, “PKG in honey bees: Spatial expression, Amfor gene expression, sucrose responsiveness, and division of labor,” *J. Comp. Neurol.*, vol. 522, no. 8, pp. 1786–1799, 2014.

[43] A. Behrends and R. Scheiner, “Evidence for associative learning in newly emerged honey bees (Apis mellifera),” *Anim. Cogn.*, vol. 12, no. 2, pp. 249–255, 2009.

[44] R. Scheiner, A. Kuritz-Kaiser, R. Menzel, and J. Erber, “Sensory responsiveness and the effects of equal subjective rewards on tactile learning and memory of honeybees,” *Learn. Mem.*, vol. 12, no. 6, pp. 626–635, 2005.

[45] A. B. Barron, R. Maleszka, R. K. Vander Meer, and G. E. Robinson, “Octopamine modulates honey bee dance behavior.,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 104, no. 5, pp. 1703–7, Jan. 2007.

[46] T. Reim and R. Scheiner, “Division of labour in honey bees: Age- and task-related changes in the expression of octopamine receptor genes,” *Insect Mol. Biol.*, vol. 23, no. 6, pp. 833–841, 2014.

[47] G. J. Hunt *et al.*, “Behavioral genomics of honeybee foraging and nest defense,” *Naturwissenschaften*, vol. 94, no. 4, pp. 247–267, 2007.

[48] Y. Wang *et al.*, “Down-regulation of honey bee IRS gene biases behavior toward food rich in protein,” *PLoS Genet.*, vol. 6, no. 4, pp. 24–26, 2010.

[49] K. A. Osborne *et al.*, “Natural behaviour polymorphism due to a cGMP-dependent protein kinase of Drosophila,” *Science (80-.).*, vol. 277, no. 1997, pp. 834–836, 1997.

[50] M. Fujiwara, P. Sengupta, and S. L. McIntire, “Regulation of body size and behavioral state of C. elegans by sensory perception and the egl-4 cGMP-dependent protein kinase,” *Neuron*, vol. 36, no. 6, pp. 1091–1102, 2002.

[51] M. Thamm, K. Sturm, J. Schlossmann, and R. Scheiner, “Levels and activity of cGMP-dependent protein kinase in nurse and forager honeybees,” *Insect Mol. Biol.*, vol. 0, pp. 1–9, 2018.

[52] R. Scheiner, M. B. Sokolowski, and J. Erber, “Activity of cGMP-Dependent Protein Kinase (PKG) Affects Sucrose Responsiveness and Habituation in Drosophila melanogaster Activity of cGMP-Dependent Protein Kinase (PKG) Affects Sucrose Responsiveness and Habituation in Drosophila melanogaster,” *Learn. Mem.*, vol. 11, no. 3, pp. 303–311, 2004.

[53] A. T. Belay *et al.*, “The foraging Gene of Drosophila melanogaster: Spatial-Expression Analysis and Sucrose Responsiveness,” *J Comp Neurol*, vol. 504, pp. 570–582, 2007.

[54] R. Scheiner, S. Plückhahn, B. Öney, W. Blenau, and J. Erber, “Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees,” *Behav. Brain Res.*, vol. 136, no. 2, pp. 545–553, 2002.

[55] A. Behrends and R. Scheiner, “Octopamine improves learning in newly emerged bees but not in old foragers,” *J. Exp. Biol.*, vol. 215, no. 7, pp. 1076–1083, 2012.

[56] P. K. Visscher and T. D. Seeley, “Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest,” *Ecology*, vol. 63, no. 6, pp. 1790–1801, 1982.

[57] R. Scheiner *et al.*, “Standard methods for behavioural studies of *Apis mellifera*,” *J. Apic. Res.*, vol. 52, no. 4, pp. 1–58, 2013.

[58] T. Reim, M. Thamm, D. Rolke, W. Blenau, and R. Scheiner, “Suitability of three common reference genes for quantitative real-time PCR in honey bees,” *Apidologie*, vol. 44, no. 3, pp. 342–350, 2013.

[59] K. J. Livak and T. D. Schmittgen, “Analysis of Relative Gene Expression Data Using Real- Time Quantitative PCR and the {2(-Delta} Delta {C(T))} Method,” *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[60] D. A. Elston, R. Moss, T. Boulinier, C. Arrowsmith, and X. Lambin, “Analysis of aggregation, a worked example: Numbers of ticks on red grouse chicks,” *Parasitology*, vol. 122, no. 5, pp. 563–569, 2001.

[61] R Core Team, “R: A Language and Environment for Statistical Computing.” R Foundation for Statistical Computing, Vienna, Austria, 2018.

[62] RStudio Team, “RStudio: Integrated Development Environment for R.” Boston, MA, 2016.

[63] M. L. Delignette-Muller and C. Dutang, “**fitdistrplus** : An *R* Package for Fitting Distributions,” *J. Stat. Softw.*, vol. 64, no. 4, 2015.

[64] D. Bates, M. Mächler, B. Bolker, and S. Walker, “Fitting Linear Mixed-Effects Models Using {lme4},” *J.*

Stat. Softw., vol. 67, no. 1, pp. 1–48, 2015.

[65] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, and R.-C. Team, “Linear and nonlinear mixed effects models,” *URL https://cran.r-project.org/web/packages/nlme/nlme.pdf*. 2018.

[66] D. Sarkar, *Lattice : multivariate data visualization with R*. Springer Science+Business Media, 2008.

[67] Florian Hartig, “DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models.” 2018.

[68] H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*, 2nd ed. Springer, 2016.

[69] Alboukadel Kassambara, “ggpubr: ‘ggplot2’ Based Publication Ready Plots.” 2018.

[70] Claus O. Wilke, “cowplot: Streamlined Plot Theme and Plot Annotations for ‘ggplot2,’” *R Packag. version 0.9.3*, 2018.

[71] A. Walton and A. L. Toth, “Variation in individual worker honey bee behavior shows hallmarks of personality,” *Behav. Ecol. Sociobiol.*, vol. 70, no. 7, pp. 999–1010, 2016.

[72] L. M. Guillette and C. B. Sturdy, “Individual differences and repeatability in vocal production: Stress-induced calling exposes a songbird’s personality,” *Naturwissenschaften*, vol. 98, no. 11, pp. 977–981, 2011.

[73] S. Potier, A. Carpentier, D. Grémillet, B. Leroy, and A. Lescroël, “Individual repeatability of foraging behaviour in a marine predator, the great cormorant, Phalacrocorax carbo,” *Anim. Behav.*, vol. 103, pp. 83–90, 2015.

[74] S. Tran and R. Gerlai, “Individual differences in activity levels in zebrafish (Danio rerio),” *Behav. Brain Res.*, vol. 257, pp. 224–229, 2013.

[75] R. Izhar and D. Eilam, “Together they stand: A life-threatening event reduces individual behavioral variability in groups of voles,” *Behav. Brain Res.*, vol. 208, no. 1, pp. 282–285, 2010.

[76] B. D. Beverly, H. McLendon, S. Nacu, S. Holmes, and D. M. Gordon, “How site fidelity leads to individual differences in the foraging activity of harvester ants,” *Behav. Ecol.*, vol. 20, no. 3, pp. 633–638, 2009.

[77] J. D. Crall *et al.*, “Spatial fidelity of workers predicts collective response to disturbance in a social insect,” *Nat. Commun.*, vol. 9, no. 1, pp. 1–13, 2018.

[78] N. J. Dingemanse, A. J. N. Kazem, D. Réale, and J. Wright, “Behavioural reaction norms: animal personality meets individual plasticity,” *Trends Ecol. Evol.*, vol. 25, no. 2, pp. 81–89, 2010.

[79] G. E. Robinson, “Regulation Of Division Of Labor In Insect Societies,” *Annu. Rev. Entomol.*, vol. 37, no. 1, pp. 637–665, Jan. 1992.

[80] V. Witte, D. Schliessmann, and R. Hashim, “Attack or call for help? Rapid individual decisions in a group-hunting ant,” *Behav. Ecol.*, vol. 21, no. 5, pp. 1040–1047, 2010.

[81] R. Jeanson and A. Weidenmüller, “Interindividual variability in social insects - proximate causes and ultimate consequences.,” *Biol. Rev.*, Dec. 2013.

[82] E. J. H. Robinson, O. Feinerman, and N. R. Franks, “Flexible task allocation and the organization of work in ants.,” *Proc. R. Soc. B Biol. Sci.*, vol. 276, no. 1677, pp. 4373–80, Dec. 2009.

[83] R. J. De Marco, “How bees tune their dancing according to their colony’s nectar influx: re-examining the role of the food-receivers’ `eagerness’,” *J. Exp. Biol.*, vol. 209, no. 3, pp. 421–432, 2006.

[84] M. D. Rivera, M. C. Donaldson-Matasci, and A. Dornhaus, “Quitting time: When do honey bee foragers decide to stop foraging on natural resources?,” *Front. Ecol. Evol.*, vol. 3, no. May, pp. 1–9, 2015.

[85] K. J. Nagle and W. J. Bell, “Genetic control of the search tactic of Drosophila melanogaster: An ethometric analysis of rover/sitter traits in adult flies,” *Behav. Genet.*, vol. 17, no. 4, pp. 385–408, 1987.

[86] V. G. Dethier, “Communication by Insects : Physiology of Dancing,” *Science (80-.).*, vol. 125, no. 3243, pp. 331–336, 1957.

[87] C. C. Rittschof and G. E. Robinson, *Behavioral Genetic Toolkits: Toward the Evolutionary Origins of Complex Phenotypes*, 1st ed., vol. 119. Elsevier Inc., 2016.

[88] A. Brockmann and G. E. Robinson, “Central projections of sensory systems involved in honey bee dance language communication.,” *Brain. Behav. Evol.*, vol. 70, no. 2, pp. 125–36, Jan. 2007.

[89] G. V. Amdam, K. Norberg, R. E. Page, J. Erber, and R. Scheiner, “Downregulation of vitellogenin gene activity increases the gustatory responsiveness of honey bee workers (Apis mellifera),” *Behav. Brain Res.*, vol. 169, no. 2, pp. 201–205, 2006.

[90] H. Kohno, S. Suenami, H. Takeuchi, T. Sasaki, and T. Kubo, “Production of Knockout Mutants by CRISPR/Cas9 in the European Honeybee, *Apis mellifera* L.,” *Zoolog. Sci.*, vol. 33, no. 5, pp. 505–512, 2016.

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Chapter

V

Simulations

Manuscript in preparation

Chapter 5 – Simulations

Introduction

Social communication plays an important role in the structure and dynamics of animal groups (Conradt & Roper, 2003; Pruitt & Goodnight, 2014). Inter-individual variation in producing and responding to social signals can help buffer groups against perturbations (Chate et al., 2015; Giardina et al., 2010; Sinhuber, van der Vaart, & Ouellette, 2019). Consistent inter-individual variation in behavioural responses, including communication, is present across the animal kingdom (Bell, Hankison, & Laskowski, 2009). However, experimentally pinpointing the adaptive value of individual variation in a variety of social systems is difficult (Jeanson & Weidenmüller, 2013; Wolf & Weissing, 2012). Theoretical models and simulations can provide valuable insights into how such systems work in the absence of empirical work (Bouwhuis et al., 2018).

Agent-based models (ABMs) are a useful toolkit to understand the role that consistent individual variation ("behavioural syndromes" or "personalities") plays in group dynamics. These models simulate independent agents interacting with each other and the environment in a spatially defined manner (Railsback & Grimm, 2011). Since agents can be simulated with any degree of complexity, these models have seen widespread usage in fields ranging from social learning and the dynamics of opinion formation to animal welfare and risk management (Janssen, Sharpanskykh, & Curran, 2019; Jędrzejewski, Marcjasz, Nail, & Sznajd-Weron, 2018; McLane, Semeniuk, McDermid, & Marceau, 2011; Van Der Post, Franz, & Laland, 2016). In animal behaviour, theoretical models have been built to study the underlying mechanisms behind empirical observations on foraging, group hierarchies, the spread of diseases and animal rhythms amongst others (Hunter, Namee, & Kelleher, 2018; Pitt, Box, & Knowlton, 2003; Ravignani & de Reus, 2019). In silico experiments using ABMs have thus helped understand complex phenomenon in different systems.

Eusocial insect colonies are characterised by large number of workers performing different tasks at the same time (Hölldobler & Wilson, 2009). Individual workers do not have complete information about the colony state and must rely on social interactions to make decisions regarding the task to perform (Grüter & Keller, 2016; Waters & Fewell, 2012). In such systems, ABMs have been useful in understanding how efficient division of labour can arise from the interactions between agents with limited information and simple behavioural rules (Matthias A. Becher et al., 2014; Anna Dornhaus, Klügl, Puppe, & Tautz, 1998; Johnson & Nieh, 2010; Schmickl & Crailsheim, 2008). ABMs have been used to gain a better understanding of processes like nest site selection and collective foraging (M. A. Becher et al., 2016; List, Elsholtz, & Seeley, 2009; Pratt, Sumpter, Mallon, & Franks, 2005; Van Nest & Moore, 2012) and translate this knowledge into optimisation algorithms employed in economics, engineering and management (Fathian & Amiri, 2007; Maia, De Castro, & Caminhas, 2012; Teodorović, 2009; Zengin, Sarjoughian, & Ekiz, 2013). Recent advances in the field in terms of standardisation of methods (Grimm et al., 2006) and the availability of multiple programming environments (Carneiro, Andrade, Câmara, Monteiro, & Pereira, 2013; Grignard et al., 2013; Tisue & Wilensky, 2004) have made ABMs even more accessible.

The waggle dance behaviour of honey bee foragers is one of the most complex form of insect communication and is an integral part of the collective foraging activity of honey bee colonies (Seeley, 1995; von Frisch, 1967). Foragers returning from a rewarding food source use it to communicate the spatial location of the source to nest mates. In addition, foragers integrates social cues from nest mates which contain information about the colony food stores, nectar influx and food source conditions (Farina, 2000; Nieh, 2010; Seeley, 1989; Thom, 2003). Empirical studies on the adaptive value of the waggle dance are difficult due to the need to non-invasively test colonies which are unable to utilise the waggle dance under different environmental conditions (A. Dornhaus & Chittka, 2004; Kirchner & Grasser, 1998). As a result, the adaptive benefits of spatial information transfer through the waggle dance has remained inconclusive so far (Donaldson-Matasci & Dornhaus, 2012; A. Dornhaus & Chittka, 2004; Grüter, Balbuena, & Farina, 2008).

Modelling the process of honey bee foraging under different conditions has provided insights about the advantages of the waggle dance as a recruitment mechanism. Dornhaus *et al.* (2006) and Beekman and Lew (2007) showed using simulations that the waggle dance is advantageous only when food patches are poor and heterogenous. In their simulations the recruitment process was not advantageous for the colony's foraging success under high resource density. More recently, simulations have shown that relying on private information and not relying on shared information (the waggle dance) leads to a greater diversity of food source exploitation, especially when the nectar availability per flower is low (Bailis, Nagpal, & Werfel, 2010). Schürch & Grüter (2014) showed that the foraging success of simulated colonies is sensitive to the duration for which the model is run. When models were run for longer time periods, spatial information transfer led to increased foraging success at the colony level under most environmental conditions.

ABMs on the honey bee waggle dance behaviour have so far not incorporated consistent individual variation in foraging and dance activity amongst the simulated agents. There is growing evidence that individual foragers differ in their foraging and dance activity (Seeley, 1994; Tenczar, Lutz, Rao, Goldenfeld, & Robinson, 2014) and that these differences are consistent over days (Buatois & Lihoreau, 2016; George & Brockmann, 2019; Walton & Toth, 2016). In this study, we introduce an ABM incorporating individual variation in the probability and intensity of dance activity. We compare the consistency of the total recruitment activity of agents in the simulation with empirical data, to understand the role of individual variation in probability and intensity in maintaining this consistency.

Material and Methods

We simulated an agent-based model (ABM) of honey bee nectar foragers that have individual variations in their dance probability and dance intensity for any given food reward. Our model is based on an earlier one (Schürch & Grüter, 2014), which we have modified to incorporate individual level parameters. We used NETLOGO 5.3.1 to implement the model, and follow the "Overview, Design Concepts and Details" protocol to describe it (Grimm et al., 2006).

Purpose

The purpose of this model was to understand the effect of two variables, the probability that an individual will dance for a given food reward and the intensity of the dances for a given food reward, in maintaining consistent inter-individual differences in the recruitment behaviour of honey bee foragers. Both probability of dancing and intensity of dances, which is the number of waggle runs per waggle dance, is correlated with the foragers perception of the food reward (Barron, Maleszka, Vander Meer, & Robinson, 2007; George & Brockmann, 2019; Seeley, 1989, 1994). In our model, we implemented two individual state parameters that corresponded to our variables of interest; a probability modulator and an intensity modulator. The probability modulator is the internal threshold that regulates the probability that a honey bee performs a waggle dance on returning to the hive after foraging. The intensity modulator regulates the number of waggle runs that a bee makes in each waggle dance for a given nectar source reward.

State variables and scales

In all our model runs we simulated 300 foragers from one colony. The foragers were divided into two different types, scouts (n = 30) and recruits (n = 270), based on typical proportions of scouts and recruits in an *A. mellifera* colony (Seeley, 1995). Although we were interested in the consistency of recruitment activity of the recruits themselves, we needed to incorporate scouts into the model to ensure that the food sources were discovered by the colony. The states that individual agents could take in our model was the same as in the previously published model. Scouts readily abandon food sources they have found in order to explore and find new ones (Liang et al., 2012; Seeley, 1983). They could, at any time step, be in any of the 6 states: idle in the colony, scouting for food sources, feeding at a food source, returning to the colony, recruiting of idle foragers to the newly discovered food source, returning to the nest without having discovered food. Scouts could only change from some states to another (Fig. 5.1 *a*). Recruits, on the other hand, have a very low probability of independently flying out to find a food patch, and usually follow a waggle dance before they leave the nest for foraging (Biesmeijer & De Vries, 2001; Seeley, 1983). Recruits could be in any of the following 8 states (Fig. 5.1 *b*) at any time step: idle in the nest, waiting to be recruited, flying to food source, feeding on food source, returning to colony after feeding, recruiting new idle foragers, scouting for forage, returning to the colony without having discovered food. All agents were characterized by an identity number, a dance probability modulator and a dance intensity modulator (Table 5.1).

Our agents were located on a two-dimensional square grid with 201×201 patches with a patch width of 100m. Thus, our agents could explore an area corresponding to a 20km x 20km square centred on the hive, similar to the previous model. Our agents could occupy any position in this area and were not restricted to the edges of the patch. The agents' hive was located at the central patch. We simulated a foraging environment for our agents with no temporal change in the distribution of nectar sources. This was done to ensure that we could easily compare our simulation results with previous experimental data that we had obtained (George & Brockmann, 2019). Our agents had access to four equally rewarding (0.8 M sucrose concentration) nectar sources at a distance of 80 patches from the nest. The four patches were distributed around the nest with a 90° angle between consecutive patches. These patches never got exhausted, similar to artificial feeders provided in flight cage experiments.

Simulations were run in discrete time-steps. We modelled time as it was done in the previous model, where one time-step corresponded to 10 seconds in real time. The agents were allowed to forage between 6:23 and 17:37. This was done by letting them forage when this condition was true: $\sin\left((2\cdot\pi\cdot\frac{t}{t_{day}})\cdot\frac{180}{\pi}\right)>0.1$. Weather was not simulated, and so the general foraging activity of the colony remained similar throughout the day.

Process overview and scheduling

Each simulation was run for five days, consisting of a two-day non-experimental and a three-day experimental phase. The non-experimental phase allowed foragers to learn about the location of food sources around their colony. Scouts and recruits followed similar processes while foraging (Fig. 5.1 *a* and *b*). The recruitment process in our model was based on the earlier model. After unloading the nectar, individual agents could dance to recruit one other agent to the food source. Each dance had a 25% chance to recruit a forager, in accordance with empirical data (A. Dornhaus et al., 2006; Gould, Henerey, & MacLeod, 1970; Seeley & Towne, 1992; Seeley & Visscher, 1988; Tautz, 1996).

Figure 5.1

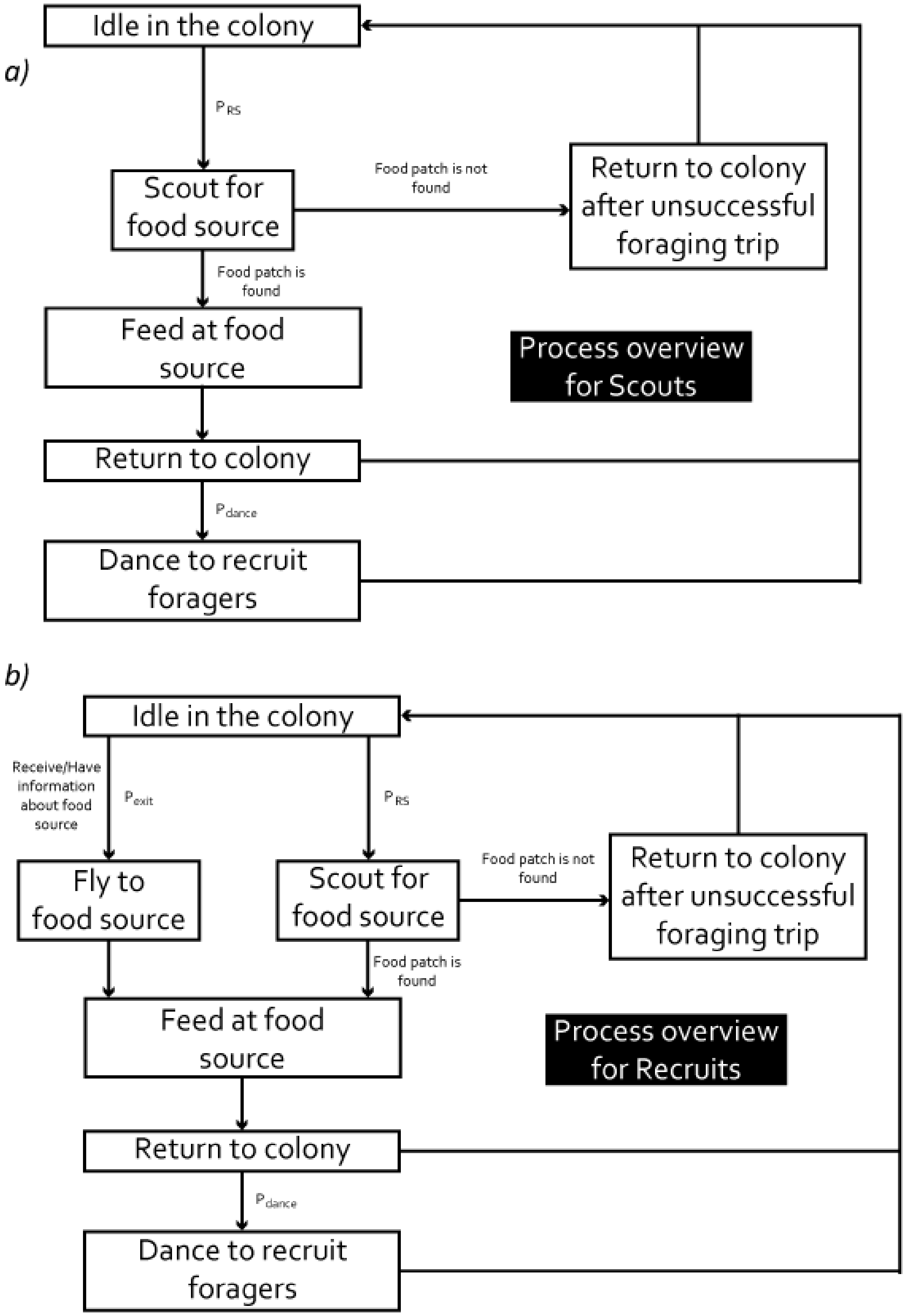

Figure 5.1: The process overview of *(a)* scouts and *(b)* recruits with the states that they can occupy at one time point in the model.

Table 5.1

Variable	**Value**
Number of scouts	30
Number of recruits	270
Mean ± SD individual probability modulator of bees*	0.255 ± 0.1
Mean ± SD individual dance intensity modulator of bees*	0.255 ± 0.1
Time steps per day	8640
Probability that a scout will leave the nest	0.815
Probability that an idle recruit will scout	0.00009
Mean ± SD handling time on patch	180±60
Nectar handling time in nest	6
Distance of feeders from nest$^+$	80 patches
Nectar quality at feeders$^+$	0.8 M
Agent Speed	0.7 patch width / time step
Levi flight parameter	2.4
Number of recruits following the dance of a bee	1
Probability of dance success	0.25

Table 5.1: The state variables used in the agent-based model. Variables marked with a (*) are individual specific variables unique to our model. Variables marked with a ($^+$) have been modified from the original model. The other parameters in our model are similar to the model in Schürch and Grüter, 2012.

The probability that a bee will dance on returning to the hive is determined by her probability modulator, the nectar quality in terms of sugar concentration (Tautz & Sandeman, 2003; Waddington, 1982), the distance of the nectar source from the hive (Seeley, 1994) and the current influx of returning foragers (Seeley, 1989). The quality of the food sources and their distances from the hive were kept the same throughout, and the current influx of returning foragers would be the same for bees dancing at the same time step. Thus, in our model, individual differences in dance probability were a direct result of individual differences in the probability modulator. If a bee decides to dance, the intensity of her dance is determined by nectar source distance, nectar quality (Seeley, 1994) and her dance modulator. Since nectar source distance and quality were kept constant, differences in dance intensity were due to individual differences in the intensity modulator between agents.

Scouts and recruits became idle after dancing. Agents in an idle state could leave the nest with probability $p_{exit.}$ Scouts constantly searched for new food sources, while recruits showed high patch fidelity (Al Toufailia, Grüter, & Ratnieks, 2013; Moore & Doherty, 2009). Each day recruits, that had previously foraged, decided to either continue foraging or abandon the patch. The probability to abandon the patch depended on the individual probability modulator, nectar quality and nectar source distance.

We tested four different experimental variations of our model. We ran 1200 simulations for each of our experimental variation. The experiments are described below:

Experiment 1: All agents had the same probability and intensity modulator. Honey bee foragers were all similar with no individual-specific state variable linked to differences amongst them.

Experiment 2: Every agent was characterised by an individual probability modulator. However, there was no variation in intensity modulator amongst the agents. Honey bee foragers showed inter-individual differences in their probability of dancing for any given food reward. The probability modulator for each agent was obtained from a normal distribution [mean ± standard deviation (sd) = 0.255 ± 0.1]. An agent with the mean value of this distribution as its probability modulator was similar to all agents in experiment 1 (Fig. 5.2 *a*).

Experiment 3: Every agent was characterised by an individual intensity modulator. However, there was no variation in probability modulator amongst the agents. Honey bee foragers showed inter-individual differences in their intensity of dances for any given food reward. The intensity modulator for each agent was obtained from a normal distribution (mean ± sd = 0.255 ± 0.1). An agent with the mean value of this distribution as its intensity modulator was similar to all agents in experiment 1 (Fig. 5.3 *a*).

Experiment 4: Each agent was characterized by an individual probability and intensity modulator, both of which were obtained from normal distributions (mean ± sd = 0.255 ± 0.1). An agent with the mean values of probability and intensity modulator was similar to an agent in experiment 1. In this experimental variation, honey bee foragers showed inter-individual differences in both the probability of dancing and intensity of dances for any given food reward.

Figure 5.2

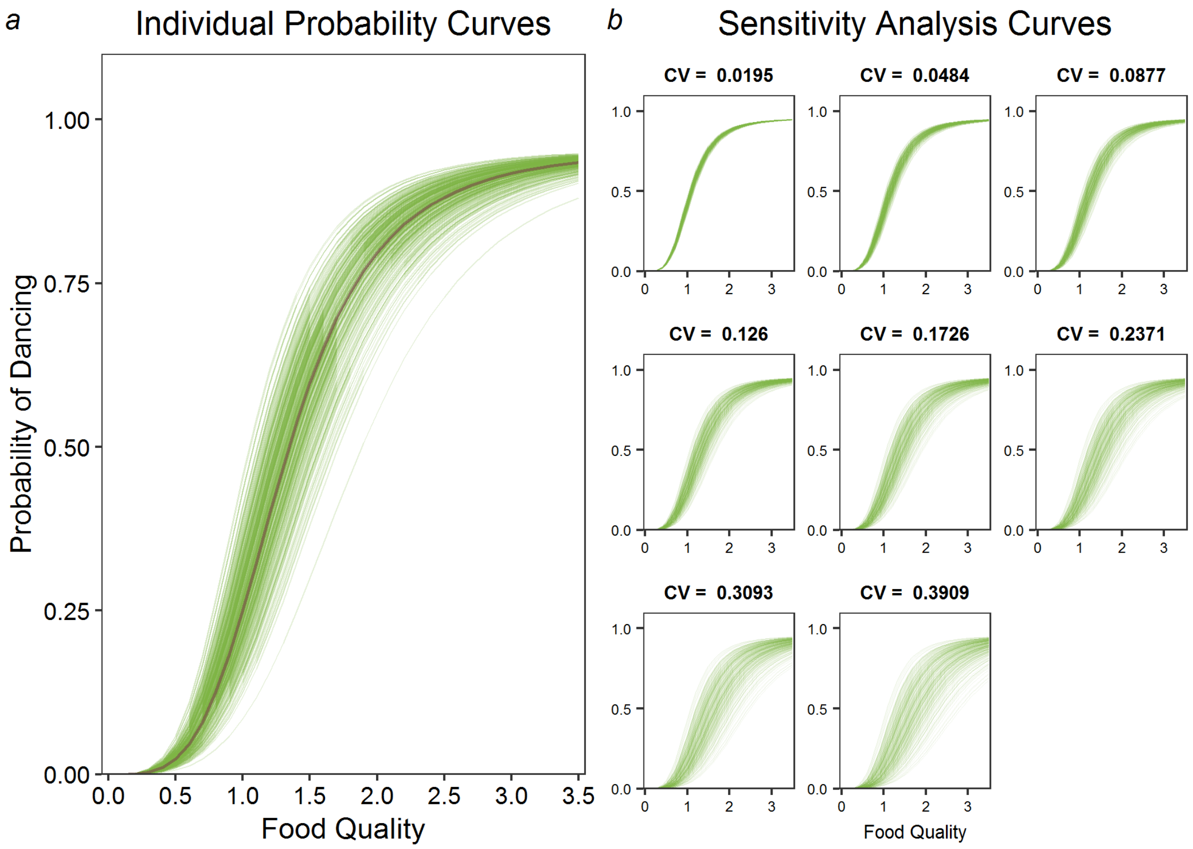

Figure 5.2: *(a)* The individual variation in the dance probability curves for 300 agents in one of the runs of experiment 2 and experiment 4. Each green curve corresponds to the relationship between probability of dancing and food quality for one of the agents, obtained from its probability modulator. The brown line corresponds to the relationship between probability of dancing and food quality for all agents in Model 1. *(b)* Individual variation in dance probability used in the sensitivity analysis. Each subplot corresponds to one particular coefficient of variation (CV) in the probability modulator. Green

lines within each subplot represents the relationship between the probability of dancing and food quality for all 300 agents at this coefficient of variation.

Design Concepts

In our model, honey bee foragers collected food from four food sources that were present throughout their foraging time period and brought it back to the hive for storage.

Environmental Stimuli: The honey bee foragers could evaluate the quality of the food sources. The probability and intensity of dancing depended on this quality.

Biotic stimuli: Scouts and recruits could pass on the information about nectar source location to other recruits. Recruits mostly flew out only after receiving this information.

Figure 5.3

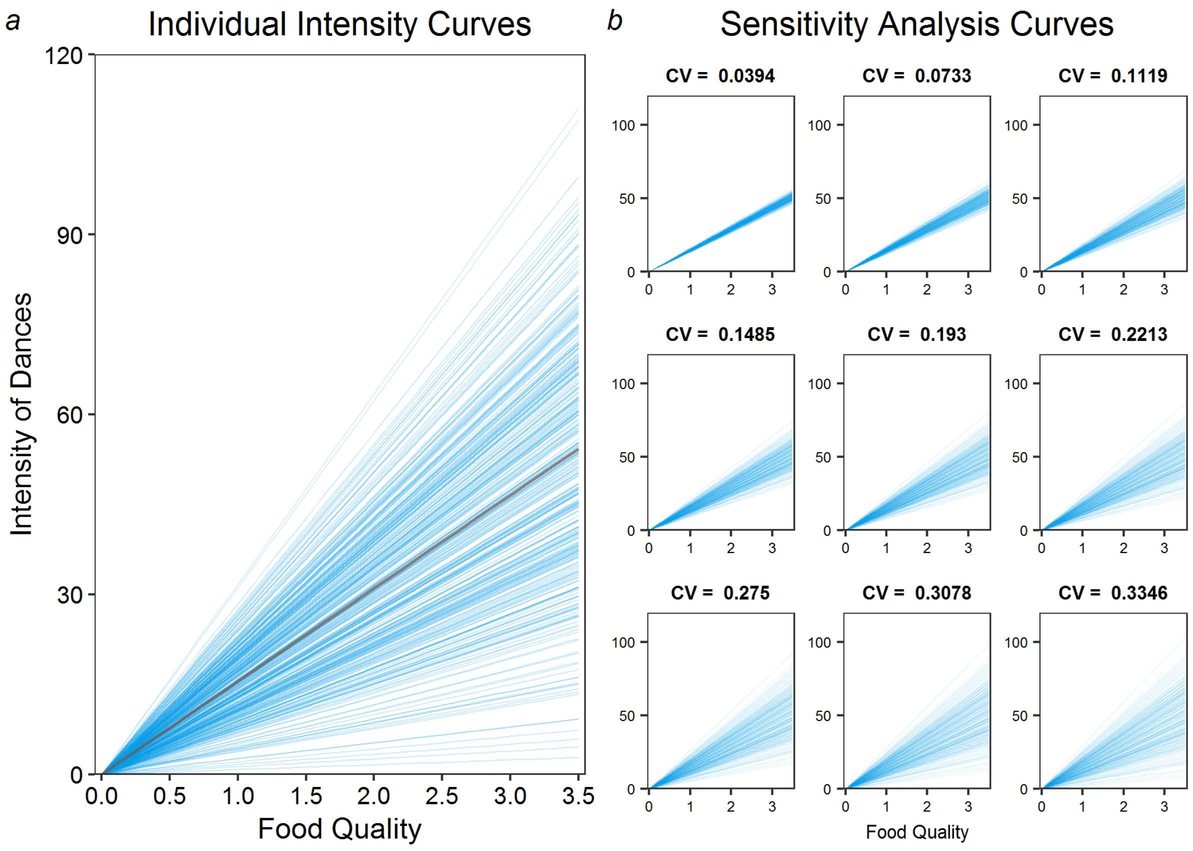

Figure 5.3: *(a)* The individual variation in the dance intensity curves for 300 agents in one of the runs of experiment 3 and experiment 4. Each blue curve corresponds to the relationship between intensity of dances and food quality for one of the agents, obtained from its intensity modulator. The brown line in the center corresponds to the relationship between intensity of dances and food quality for all agents in Model 1. *(b)* Individual variation in dance intensity used in the sensitivity analysis. Each subplot corresponds to one particular coefficient of variation (CV) in the intensity modulator. Blue lines within each subplot represents the relationship between the intensity of dances and food quality for all 300 agents at this coefficient of variation. The curves in *(a)* represent the variation in the intensity of dances at a coefficient of variation of 0.3705 of the intensity modulator.

Stochasticity: The probability and intensity modulators were assigned stochastically. They were picked randomly from a normal distribution and assigned to the individuals. Agent decisions with respect to leaving the hive, dancing for a food source, learning the location of a nectar source and abandoning a nectar source were all probabilistically determined.

Observation: At the end of each day, the number of foraging trips, the patch visited, number of dances and number of dance circuits in each dance made by every individual recruit bee were recorded. We then obtained the total number of dance circuits made by each forager for each day. We shortlisted foragers which consistently visited the same patch over the three days. As scouts abandoned food sources readily, they were automatically removed in our analysis. We then used this data to look at the repeatability of individuals in terms of the total dance circuits for a given food reward. We compared the intra-agent variation in dance activity for the same food source with the inter-agent variation in dance activity and obtained a measure of the consistency in activity (Nakagawa & Schielzeth, 2010).

Initialization

All agents were idle at the start of the simulation.

Sub models

The Lévy flight

The movement of agents during a search followed an optimal Lévy flight pattern (Reynolds, Swain, Smith, Martin, & Osborne, 2009). This sub model was used from Schürch and Grüter, 2012. Agents moved in bouts after initially choosing a random direction. Before the start of the bout, a total travel distance for the bout was calculated using the formula $d_{Lévy} = e^{\ln a \cdot \frac{1}{1-\mu_{Lévy}}}$. Here, *a* was obtained randomly from a uniform distribution bound by [0,1). The value for $\mu_{Lévy}$ was kept at 2.4 by default (Reynolds et al., 2009). Once the distance of the bout was calculated, agents would then move in the chosen direction in subsequent time steps, till they had reached the end of the distance. Then, the whole process was repeated till the agent found a food source.

The individual probability and intensity modulators for foragers

Each bee had an individual internal probability and intensity modulator, which decided the probability that she would dance and the number of waggle runs in each dance for a given food reward. A normal distribution was chosen to define the distribution of modulators present in a population of foragers (Beshers & Fewell, 2001; Page & Robinson, 1991), and each forager was randomly assigned one value from this normal distribution. Thus, all 300 individuals showed quantitative differences in their link between food quality and dance probability (Fig. 5.2 *a*) and dance intensity (Fig. 5.3 *a*).

The decision to dance for food sources

On returning to the hive, successful scouts and recruits would first unload the nectar that they had brought into the hive. They could then communicate information about the location of the food source to their nest-mates by performing a waggle dance. The forager would decide to dance only after she has finished unloading the nectar she has brought back while foraging. The equation that linked the probability of dancing to the food reward was

$$p_{qt} = \frac{0.956937}{1 + e^{(\frac{I_p - \ln(q_t)}{0.2624014})}}$$

Here I_p referred to the individual probability modulator and q_t was the energetic value of a food source, given by

$$q_t = 1.02 + \; q_{patch} - 0.04915919 - 2.851457.10^{-5}.d + 3.174098.d^2$$

where q_{patch} was the sucrose concentration of the food source and d was the distance of the food source from the hive.

Our equation was modified from the earlier model, which in turn used data from previous empirical studies (Boch, 1956; Seeley, Camazine, & Sneyd, 1991). We incorporated individual probability modulators, thereby giving each individual agent a different quantitative relationship between food quality and dance probability. The mean value for the probability modulator was obtained by making two assumptions: 1) all individual agents had the same qualitative relationship between patch quality and dance probability as described in Schürch and Grüter, 2012 and 2) the probability modulators of the population of agents was normally distributed. We chose the mean and standard deviation of the probability modulator based on the average value of the curves fit for each agent, such that our two assumptions were met.

The dance probability also depended on the influx of other foragers,

$$p_{Influx} = \begin{cases} 1 - 0.035.N_{Influx} & N_{Influx} \leq 20 \\ \quad 0.3 & N_{Influx} > 20 \end{cases}$$

Thus, final dance probability was given by

$$p_d = p_{qt}.p_{Influx}$$

The decision to abandon food sources

The probability of abandoning the nectar source was modelled analogous to the probability of dancing (Schürch & Grüter, 2014; Seeley, 1989) with the probability modulator playing a role in the decision to abandon a food source:

$$p_a = 1 - \frac{0.956937}{1 + e^{\left(\frac{I_p - \ln(q_t + 0.6687)}{0.2624014}\right)}}$$

Individual dance intensity curves for foragers.

Dance intensity was defined as the number of waggle runs that a bee makes per dance. The relationship between food quality and number of dance turns is linear (Seeley, 1994). In our model each bee had a quantitatively different slope that predicted how much she would dance after returning to the hive.

$$dance_{intensity} = 57 \times q_t \times I_i$$

Here I_i referred to the individual intensity modulator and q_t was the energetic value of a food source.

To obtain the value of the intensity modulator, we made 4 assumptions: 1) all individual agents had the same qualitative relationship between patch quality and dance intensity as observed in Seeley, 1994, 2) the intensity modulators of the population of agents was normally distributed, 3) all agents had the same intercept of zero for the linear link between patch quality and dance intensity, i.e., there was no dance activity for a patch of zero quality and 4) there is a maximal value of the dance intensity for the most rewarding food source [38]. Based on these assumptions, we fit individual curves for each agent and chose the mean and standard deviation of the intensity modulator.

Sensitivity Analysis

In order to test how sensitive our model was to changes in our two parameters of interest we ran a sensitivity analysis. We varied the distribution of the probability (Fig. 5.2 *b*) and intensity modulators (Fig. 5.3 *b*) and observed its effect on consistent inter-individual differences in the total dance activity. We varied the spread of the two parameters in such a way as to keep the coefficient of variation of both parameters similar across all comparisons.

The probability equation was modified as shown below in order to vary the spread.

$$p_a = 1 - \frac{0.956937}{1 + e^{\left(\frac{(m * I_p) - \ln(q_t)}{0.2624014}\right)}}$$

The value of m was varied from 0.25 to 2 in steps of 0.25 to change the spread of the distribution (Fig. 5.2 *b*).

To change the spread of the individual intensity curves (Fig. 5.3 *b*), we changed the standard deviation of the normal distribution of intensity modulator, while keeping the mean constant. The values of standard deviation were changed from 0.01 to 0.1 in steps of 0.01.

We thus tested 80 different versions (all combinations of 8 levels of variation in individual probability modulator and 10 levels of variation in individual intensity modulator) and ran 100 simulations of each version for a total of 8000 simulations.

Statistical Analysis

We used generalized linear mixed effects models to quantify the repeatability in dance circuits of agents visiting the same food source in each simulation. Individual agent identity was used as a random effect to obtain the ratio of within-agent variation to amongst-agent variation (Nakagawa & Schielzeth, 2010). We obtained a single repeatability estimate for each of the 1200 simulations runs of each experiment. The repeatability estimates were then compared using a linear model with the estimate as the response variable and the experiment as the predictor. We corrected for multiple comparisons between the experiments using Tukey's HSD.

For the sensitivity analysis, we first obtained the repeatability estimates for all 8000 simulation runs as before. Then, a LOESS model was fit to this data with the estimate as the response variable and an interaction between the coefficient of variation in the probability modulator and the coefficient of variation in the intensity modulator at 0.8M as the predictor variable. We then predicted the repeatability estimates for other combinations of variation in the probability and intensity modulator from this fitted model. Finally, contour plots were used to examine the effect of the variation in probability and intensity modulators on repeatability of dance activity.

We built these generalized linear mixed effects models using the rptR package in R (Stoffel, Nakagawa, & Schielzeth, 2017). For linear models, we used the base package in R and for multiple comparisons, we used the glht function in the multcomp package (Hothorn, Bretz, & Westfall, 2008). We fit the LOESS model also using the base package in R. For data visualisation, we used the ggplot2 (Wickham, 2016) and cowplot (Claus O. Wilke, 2018) packages in R.

Results

Comparison of the different experiments

The four experimental variations of the model significantly differed in the consistency of the total dance circuits made by the agents (Table 5.2, Fig. 5.4). Repeatability estimates were lowest in experiment 1 in which all agents were similar and had no pre-defined individual specific modulators of dance activity (mean ± sd = 0.0328 ± 0.0327, $p < 0.001$). Incorporating individual variation in the probability of dancing in experiment 2 increased the repeatability estimate slightly (mean ± sd = 0.1156 ± 0.0435, $p < 0.001$). However, in experiment 3 in which the agents had individual variation in the intensity of their dances, repeatability estimates were drastically higher (mean ± sd = 0.5965 ± 0.0459, $p < 0.001$). Surprisingly, in experiment 4, even though agents had individual variation in both the probability of dancing and intensity of dances, repeatability estimates were lower than in experiment 3 (mean ± sd = 0.4878 ± 0.0484, $p < 0.001$).

Table 5.2

Comparison	**Estimate**	**t Value**	**p Value**
Exp 2 – Exp 1	0.0828	47.10	<0.0001
Exp 3 – Exp 1	0.5638	320.63	<0.0001
Exp 4 – Exp 1	0.4550	258.77	<0.0001
Exp 3 – Exp 2	0.4809	273.51	<0.0001
Exp 4 – Exp 2	0.3721	211.65	<0.0001
Exp 4 – Exp 3	-0.1088	-61.89	<0.0001

Table 5.2: Results from multiple comparisons of the repeatability estimates from all four experimental

variations of our model. Comparisons were done using generalized linear hypothesis tests with Tukey's HSD correction for multiple comparisons. The difference estimates, t values and associated p values are given.

Comparison with empirical observations

The repeatability estimates obtained from experiment 3 and 4 were similar to the repeatability estimate obtained from empirical data (see results for total dance circuits in chapter 2). The point estimate of the repeatability from empirical data (mean = 0.5263, CI =0.4295 – 0.6002) was within the confidence intervals of the repeatability estimates from experiments 3 and 4 (Fig. 5.5).

Figure 5.4

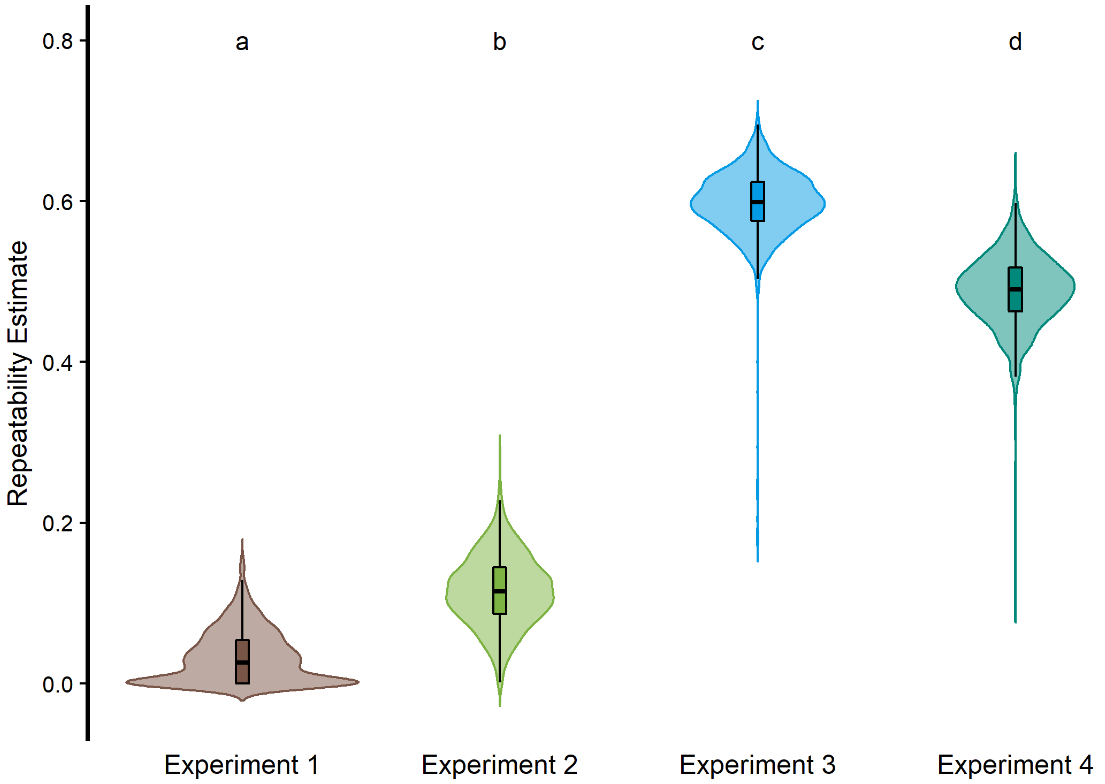

Figure 5.4: Box and violin plots comparing the repeatability estimates of 1200 runs from each of the 4 experiments. The boxplots give the median and the first and third quartile range, while the overlaid violin plot gives the distribution of the repeatability estimates from the 1200 runs. Different alphabets above the plots represent significant differences at the $p < 0.001$ level. All the experiments showed significant differences between each other in their repeatability estimates.

Sensitivity Analysis

The variation in individual intensities had a stronger effect on the repeatability estimates as compared to the variation in individual probabilities (Fig. 5.6). Higher variation in the intensity modulators amongst individual foragers led to repeatability estimates greater than 0.5. However, variation in probability modulators had very little effect on the repeatability estimates. Interestingly, repeatability estimates were highest when variation in intensity modulators were high and the corresponding variation in probability modulators were low.

Figure 5.5

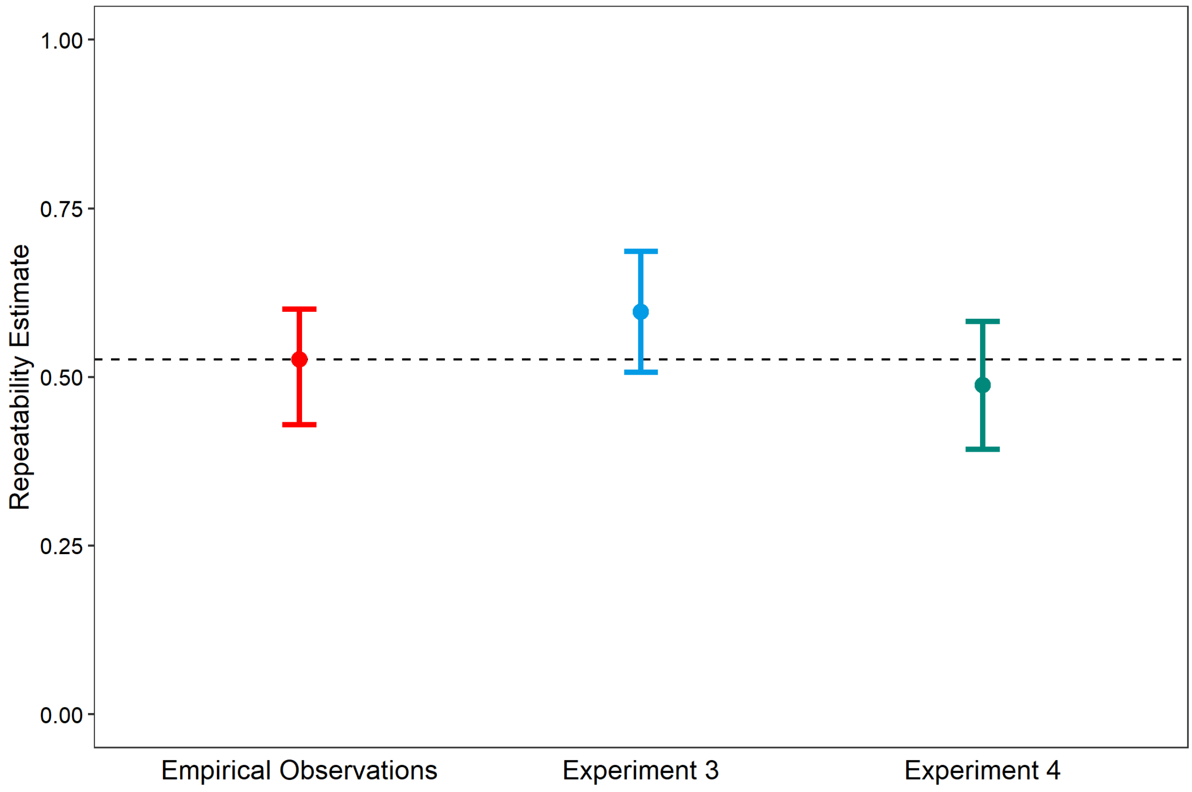

Figure 5.5: Comparison of the repeatability estimate and 95% confidence interval for the total dance circuits obtained from empirical observations (red, see chapter 2), experiment 3 (blue) and experiment 4 (dark green). The dashed horizontal line represents the repeatability estimate from empirical observations. This value was within the 95% confidence interval of the estimates obtained from experiments 3 and 4.

Discussion

The agent-based model showed that individual variation in the intensity modulator is sufficient to obtain highly repeatable inter-individual variation in the waggle dance activity. Even though the total waggle dance activity of an individual is a function of both the intensity and probability of dancing, the probability modulator had very little effect on consistent inter-individual variation in the waggle dance activity. Additionally, the individual variation in dance probability and intensity [based on other empirical data (Boch, 1956; Seeley, 1994; Seeley et al., 1991)] were able to reproduce the degree of consistent inter-individual differences in the total recruitment seen in our own observations of honey bee foragers (George & Brockmann, 2019).

The strong effect of individual variation in the intensity modulator on consistent inter-individual variation in the waggle dance activity supports results from other studies on the waggle dance activity. The intensity of dance activity is weakly correlated with the gustatory response threshold (see chapter 4). Gustatory response thresholds of individual honey bees can change over their lifetime, as they transition from one task to another (Reim & Scheiner, 2014; Scheiner, Page, & Erber, 2001). However, since these thresholds can directly affect an individual's response to a stimulus (Beshers & Fewell, 2001; Scheiner, Erber, & Page, 1999; Weidenmüller, 2004), they can be expected to remain relatively stable for the duration that individuals are engaged in the same task, like foraging. The link between the gustatory response thresholds and individual dance intensity would imply that individual variation in dance intensity would remain relatively stable.

In contrast, previous experiments showed no link between the probability of dancing and gustatory response thresholds (see chapter 4). Dance probability, in addition to being affected by other internal individual thresholds, could also be strongly influenced by other factors (Farina, 2000; Seeley, 1989). Previous studies showed a strong effect of changing social cues on individual dance probability (Farina, 2000; George & Brockmann, 2019). Interestingly, these studies showed no concurrent effect of social cues on individual dance intensity. These results along with our simulations suggest that consistent inter-individual variation in the recruitment activity of honey bee foragers is strongly driven by individual variation in their waggle dance intensity, which is further modulated by social cues through its effect on dance probability.

Figure 5.6

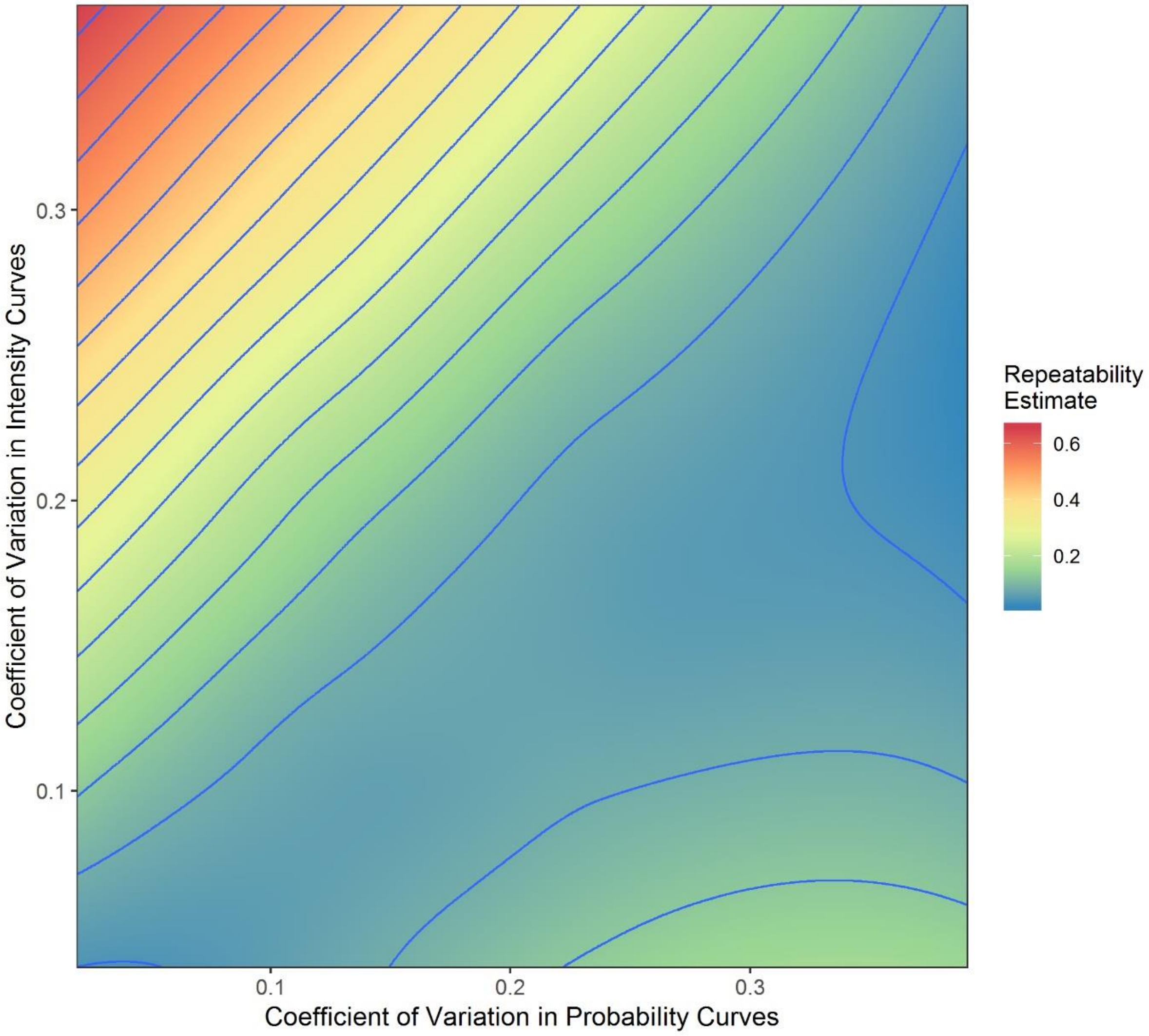

Figure 5.6: Contour plot showing results from the sensitivity analysis. The repeatability estimates obtained from varying the coefficient of variation in the probability and intensity modulators are plotted. The estimates are colour coded from blue (representing a low repeatability estimate, 0) to red (representing a high repeatability estimate, 0.6). Variation in the intensity modulator had a greater effect on the repeatability estimates than variation in the probability modulator.

Although there has been extensive work using ABMs to explore the collective foraging behaviour of honey bees, these models have not implemented consistent inter-individual variation in behavioural responses (Bailis et al., 2010; Beekman & Lew, 2007; A. Dornhaus et al., 2006; Schürch & Grüter, 2014). Our agent-based model on consistent inter-individual differences in the waggle dance activity of honey bee foragers is a first step in this direction. In the immediate future, we plan to implement two new dimensions in our model. The first would be to add more realistic environmental conditions with respect to food availability. Building on Schürch & Grüter's (2014) model, this would help us to understand the effect of inter-individual variation in recruitment activity on colony fitness. This could help us understand the adaptive function of variation in dance activity seen in empirical observations [9]. (George & Brockmann, 2019; Seeley, 1994). The second would be to add a new agent, in-hive receivers, to our model. Interactions with receivers can have a strong effect on the dance activity of foragers, particularly on the probability of dancing (Farina, 2000; George & Brockmann, 2019; Seeley, 1989). Thus, individual variation in receivers and the interactions between receivers and incoming foragers can be expected to play a role in maintaining consistent inter-individual variation in dance activity, and affect the collective foraging behaviour of a honey bee colony.

Our study adds to the growing body of literature of agent-based models focussed on individual variation and heterogeneity with respect to behavioural responses. Although models have explicitly simulated individual heterogeneity, very few studies have incorporated consistent inter-individual differences (Campos, Bartumeus, Mendez, Andrade, & Espadaler, 2016; Evers, de Vries, Spruijt, & Sterck, 2011). The studies which have focussed on consistent inter-individual variation have mostly stressed on the role played by it in key aspects of social groups like their structure and collective foraging (Couzin, Krause, James, Ruxton, & Franks, 2002; Michelena, Jeanson, Deneubourg, & Sibbald, 2010; Pinter-Wollman, 2015). Very few theoretical studies have sought to understand mechanisms underlying inter-individual variation in behavioural responses in social groups (Boumans, de Boer, Hofstede, & Bokkers, 2018; Boumans, Hofstede, Bolhuis, de Boer, & Bokkers, 2016).

In conclusion, our agent-based model highlights the parameters sufficient to produce consistent inter-individual differences in waggle dance activity. It adds to other empirical observations on how social cues and response thresholds can modulate the probability and intensity of an individual's activity differently (Farina, 2000; Garrison, Kleineidam, & Weidenmüller, 2018; George & Brockmann, 2019). Taking into account individual behavioural differences while modelling group dynamics can drastically alter predictions on how these groups can respond to perturbation (Vindenes & Langangen, 2015). Our model serves as a first step to bring together theoretical work on honey bee colony foraging dynamics with empirical observations of inter-individual differences in activity (M. A. Becher et al., 2016; George & Brockmann, 2019; Schürch & Grüter, 2014; Seeley, 1994; Tenczar et al., 2014; Walton & Toth, 2016).

Future Directions

The agent-based model that is described in this chapter can be expanded to answer more complex questions. One immediate next step would be to test the colony's foraging success with and without individual variation in the recruitment process under more natural conditions of food availability (Schürch & Grüter, 2014). These experiments would help to understand the adaptive benefit of the inter-individual variation under different conditions of food availability.

Another important question that can also be looked at would the role of inter-individual differences in how colonies respond to loss of foragers (see chapter 3). This would require the addition of a third type of agent, the receivers, and a more dynamic formulation of the interaction between receivers and foragers. Adding receivers to the model would also help in incorporating individual variation in responding to social cues, a factor that may also play a strong role in the efficient utilisation of the foragers available to the colony.

Finally, a third area worth exploring is the interplay between response thresholds, response probability and response intensity. The model described here only incorporates individual variation in the latter 2 parameters in determining the behavioural response of the waggle dance activity. A more realistic model should also incorporate individual variation in response thresholds with respect to the patch quality. The effect of varying the strength of the correlation between the 3 response parameters on consistent recruitment activity of individual agents would be particularly interesting to quantify.

References

Al Toufailia, H., Grüter, C., & Ratnieks, F. L. W. (2013). Persistence to Unrewarding Feeding Locations by Honeybee Foragers (Apis mellifera): the Effects of Experience, Resource Profitability and Season. *Ethology*, *119*(12), 1096–1106. https://doi.org/10.1111/eth.12170

Bailis, P., Nagpal, R., & Werfel, J. (2010). Positional communication and private information in honeybee foraging models. In *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* (Vol. 6234 LNCS, pp. 263–274). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-15461-4_23

Barron, A. B., Maleszka, R., Vander Meer, R. K., & Robinson, G. E. (2007). Octopamine modulates honey bee dance behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(5), 1703–1707. https://doi.org/10.1073/pnas.0610506104

Becher, M. A., Grimm, V., Knapp, J., Horn, J., Twiston-Davies, G., & Osborne, J. L. (2016). BEESCOUT: A model of bee scouting behaviour and a software tool for characterizing nectar/pollen landscapes for BEEHAVE. *Ecological Modelling*, *340*, 126–133. https://doi.org/10.1016/j.ecolmodel.2016.09.013

Becher, Matthias A., Grimm, V., Thorbek, P., Horn, J., Kennedy, P. J., & Osborne, J. L. (2014). BEEHAVE: A systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. *Journal of Applied Ecology*, *51*(2), 470–482. https://doi.org/10.1111/1365-2664.12222

Beekman, M., & Lew, J. B. (2007). Foraging in honeybees-when does it pay to dance? *Behavioral Ecology*, *19*(2), 255–261. https://doi.org/10.1093/beheco/arm117

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, *77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. *Annual Review of Entomology*, *46*, 413–440. https://doi.org/10.1146/annurev.ento.46.1.413

Biesmeijer, J. C., & De Vries, H. (2001). Exploration and exploitation of food sources by social insect colonies: A revision of the scout-recruit concept. *Behavioral Ecology and Sociobiology*. https://doi.org/10.1007/s002650000289

Boch, R. (1956). Die Tanze der Bienen bei nahen und fernen Trachtquellen. *Zeitschrift Für Vergleichende Physiologie*, *38*, 136–167.

Boumans, I. J. M. M., de Boer, I. J. M., Hofstede, G. J., & Bokkers, E. A. M. (2018). Unravelling variation in feeding, social interaction and growth patterns among pigs using an agent-based model. *Physiology and Behavior*, *191*(October 2017), 100–115. https://doi.org/10.1016/j.physbeh.2018.03.030

Boumans, I. J. M. M., Hofstede, G. J., Bolhuis, J. E., de Boer, I. J. M., & Bokkers, E. A. M. (2016). Agent-based modelling in applied ethology: An exploratory case study of behavioural dynamics in tail biting in pigs. *Applied Animal Behaviour Science*, *183*, 10–18. https://doi.org/10.1016/j.applanim.2016.07.011

Bouwhuis, S., Bassar, R. D., Caswell, H., Douhard, M., Steiner, U. K., Lee, P. C., … Gangloff, E. J. (2018). General conclusion to the special issue Moving forward on individual heterogeneity. *Oikos*, *127*(5), 750–756. https://doi.org/10.1111/oik.05223

Buatois, A., & Lihoreau, M. (2016). Evidence of trapline foraging in honeybees. *The Journal of Experimental Biology*, *219*(16), 2426–2429. https://doi.org/10.1242/jeb.143214

Campos, D., Bartumeus, F., Mendez, V., Andrade, J. S., & Espadaler, X. (2016). Variability in individual activity bursts improves ant foraging success. *Journal of the Royal Society Interface*, *13*(125). https://doi.org/10.1098/rsif.2016.0856

Carneiro, T. G. de S., Andrade, P. R. de, Câmara, G., Monteiro, A. Ô. M. V., & Pereira, R. R. (2013). An extensible toolbox for modeling nature-society interactions. *Environmental Modelling and Software*, *46*, 104–117. https://doi.org/10.1016/j.envsoft.2013.03.002

Chate, H., Theraulaz, G., Calovi, D. S., Sire, C., Schuhmacher, P., & Lopez, U. (2015). Collective response to perturbations in a data-driven fish school model. *Journal of The Royal Society Interface*, *12*(104), 20141362–20141362. https://doi.org/10.1098/rsif.2014.1362

Claus O. Wilke. (2018). cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2.” *R Package Version 0.9.3*. Retrieved from https://cran.r-project.org/package=cowplot

Conradt, L., & Roper, T. J. (2003). Group decision-making in animals. *Nature*, *421*, 155–158. https://doi.org/10.1038/nature01294

Couzin, I. D., Krause, J., James, R., Ruxton, G. D., & Franks, N. R. (2002). Collective Memory and Spatial Sorting in Animal Groups. *Journal of Theoretical Biology*, *218*(1), 1–11. https://doi.org/10.1006/yjtbi.3065

Donaldson-Matasci, M. C., & Dornhaus, A. (2012). How habitat affects the benefits of communication in collectively foraging honey bees. *Behavioral Ecology and Sociobiology*, *66*, 583–592. https://doi.org/10.1007/s00265-011-1306-z

Dornhaus, A., & Chittka, L. (2004). Why do honey bees dance? *Behavioral Ecology and Sociobiology*, *55*(4), 395–401. https://doi.org/10.1007/s00265-003-0726-9

Dornhaus, A., Klugl, F., Oechslein, C., Puppe, F., Chittka, L., Klu, F., & Klügl, F. (2006). Benefits of recruitment in honey bees: Effects of ecology and colony size in an individual-based model. *Behavioral Ecology*, *17*(3), 336–344. https://doi.org/10.1093/beheco/arj036

Dornhaus, Anna, Klügl, F., Puppe, F., & Tautz, J. (1998). Task Selection in Honeybees - Experiments Using Multi-Agent Simulation. *Gwal ’98*.

Evers, E., de Vries, H., Spruijt, B. M., & Sterck, E. H. M. (2011). Better safe than sorry - socio-spatial group structure emerges from individual variation in fleeing, avoidance or velocity in an agent-based model. *PLoS ONE*, *6*(11). https://doi.org/10.1371/journal.pone.0026189

Farina, W. M. (2000). The interplay between dancing and trophallactic behavior in the honey bee Apis mellifera. *Journal of Comparative Physiology - A*, *186*(3), 239–245. https://doi.org/10.1007/s003590050424

Fathian, M., & Amiri, B. (2007). Integration of Self Organizing Feature Maps and Honey Bee Mating Optimization Algorithm. *Technology*, 70–86.

Garrison, L. K., Kleineidam, C. J., & Weidenmüller, A. (2018). Behavioral flexibility promotes collective consistency in a social insect. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-33917-7

George, E. A., & Brockmann, A. (2019). Social modulation of individual differences in dance communication in honey bees. *Behavioral Ecology and Sociobiology*, *73*(4), 41. https://doi.org/10.1007/s00265-019-2649-0

Giardina, I., Santagati, R., Viale, M., Cimarelli, A., Cavagna, A., Parisi, G., & Stefanini, F. (2010). Scale-free correlations in starling flocks. *Proceedings of the National Academy of Sciences*, *107*(26), 11865–11870. https://doi.org/10.1073/pnas.1005766107

Gould, J. L., Henerey, M., & MacLeod, M. C. (1970). Communication of direction by the honey bee: Review of previous work leads to experiments limiting olfactory cues to test the dance language hypothesis. *Science*, *169*(3945), 544–554.

Grignard, A., Taillandier, P., Gaudou, B., Vo, D. A., Huynh, N. Q., & Drogoul, A. (2013). GAMA 1.6: Advancing the art of complex agent-based modeling and simulation. In *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* (Vol. 8291 LNAI, pp. 117–131). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-44927-7_9

Grimm, V., Berger, U., Bastiansen, F., Eliassen, S., Ginot, V., Giske, J., … DeAngelis, D. L. (2006). A standard protocol for describing individual-based and agent-based models. *Ecological Modelling*, *198*(1–2), 115–126. https://doi.org/10.1016/j.ecolmodel.2006.04.023

Grüter, C., Balbuena, M. S., & Farina, W. M. (2008). Informational conflicts created by the waggle dance. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1640), 1321–1327. https://doi.org/10.1098/rspb.2008.0186

Grüter, C., & Keller, L. (2016). Inter-caste communication in social insects. *Current Opinion in Neurobiology*, *38*, 6–11. https://doi.org/10.1016/j.conb.2016.01.002

Hölldobler, B., & Wilson, E. O. (2009). *The superorganism*. *W W Norton* (Vol. 456). W.W. Norton & Company, Inc. https://doi.org/10.1038/456320a

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hunter, E., Namee, B. Mac, & Kelleher, J. (2018). *An open-data-driven agent-based model to simulate infectious disease outbreaks*. *PLoS ONE* (Vol. 13). https://doi.org/10.1371/journal.pone.0208775

Janssen, S., Sharpanskykh, A., & Curran, R. (2019). AbSRiM: An Agent-Based Security Risk Management Approach for Airport Operations. *Risk Analysis*. https://doi.org/10.1111/risa.13278

Jeanson, R., & Weidenmüller, A. (2013). Interindividual variability in social insects - proximate causes and ultimate consequences. *Biological Reviews*. https://doi.org/10.1111/brv.12074

Jędrzejewski, A., Marcjasz, G., Nail, P. R., & Sznajd-Weron, K. (2018). Think then act or act then think? *PLoS ONE*, *13*(11), 1–19. https://doi.org/10.1371/journal.pone.0206166

Johnson, B. R., & Nieh, J. C. (2010). Modeling the Adaptive Role of Negative Signaling in Honey Bee Intraspecific Competition. *Journal of Insect Behavior*, *23*(6), 459–471. https://doi.org/10.1007/s10905-010-9229-5

Kirchner, W. H., & Grasser, A. (1998). The Significance of Odor Cues and Dance Language Information for the Food Search Behavior of Honeybees (Hymenoptera : Apidae). *Journal of Insect Behavior*, *11*(2), 169–178. https://doi.org/10.1023/A:1021098405564

Liang, Z. S., Nguyen, T., Mattila, H. R., Rodriguez-Zas, S. L., Seeley, T. D., & Robinson, G. E. (2012). Molecular determinants of scouting behavior in honey bees. *Science*, *335*(6073), 1225–1228. https://doi.org/10.1126/science.1213962

List, C., Elsholtz, C., & Seeley, T. D. (2009). Independence and interdependence in collective decision making: An agent-based model of nest-site choice by honeybee swarms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1518), 755–762. https://doi.org/10.1098/rstb.2008.0277

Maia, R. D., De Castro, L. N., & Caminhas, W. M. (2012). Bee colonies as model for multimodal continuous optimization: The OptBees algorithm. *2012 IEEE Congress on Evolutionary Computation, CEC 2012*, 10–15. https://doi.org/10.1109/CEC.2012.6252975

McLane, A. J., Semeniuk, C., McDermid, G. J., & Marceau, D. J. (2011). The role of agent-based models in wildlife ecology and management. *Ecological Modelling*, *222*(8), 1544–1556. https://doi.org/10.1016/j.ecolmodel.2011.01.020

Michelena, P., Jeanson, R., Deneubourg, J.-L., & Sibbald, A. M. (2010). Personality and collective decision-making in foraging herbivores. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1684), 1093–1099. https://doi.org/10.1098/rspb.2009.1926

Moore, D., & Doherty, P. (2009). Acquisition of a time-memory in forager honey bees. *Journal of Comparative Physiology A*, *195*(8), 741–751. https://doi.org/10.1007/s00359-009-0450-7

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, *85*(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology*, *20*(4), 310–315. https://doi.org/10.1016/j.cub.2009.12.060

Page, R. E., & Robinson, G. E. (1991). The Genetics of Division of Labour in Honey Bee Colonies. *Advances in Insect Physiology*, *23*(C), 117–169. https://doi.org/10.1016/S0065-2806(08)60093-4

Pinter-Wollman, N. (2015). Persistent variation in spatial behavior affects the structure and function of interaction networks. *Current Zoology*, *61*(1), 98–106. https://doi.org/10.1093/czoolo/61.1.98

Pitt, W. C., Box, P. W., & Knowlton, F. F. (2003). An individual-based model of canid populations: Modelling territoriality and social structure. *Ecological Modelling*, *166*(1–2), 109–121. https://doi.org/10.1016/S0304-3800(03)00130-3

Pratt, S. C., Sumpter, D. J. T., Mallon, E. B., & Franks, N. R. (2005). An agent-based model of collective nest choice by the ant Temnothorax albipennis. *Animal Behaviour*, *70*(5), 1023–1036. https://doi.org/10.1016/j.anbehav.2005.01.022

Pruitt, J. N., & Goodnight, C. J. (2014). Site-specific group selection drives locally adapted group compositions. *Nature*, *514*(7522), 359–362. https://doi.org/10.1038/nature13811

Railsback, S. F., & Grimm, V. (2011). *Agent-Based and Individual-Based Modeling : A Practical Introduction* (1st ed.). Princeton University Press.

Ravignani, A., & de Reus, K. (2019). Modelling Animal Interactive Rhythms in Communication. *Evolutionary Bioinformatics*, *15*, 117693431882355. https://doi.org/10.1177/1176934318823558

Reim, T., & Scheiner, R. (2014). Division of labour in honey bees: Age- and task-related changes in the expression of octopamine receptor genes. *Insect Molecular Biology*, *23*(6), 833–841. https://doi.org/10.1111/imb.12130

Reynolds, A. M., Swain, J. L., Smith, A. D., Martin, A. P., & Osborne, J. L. (2009). Honeybees use a Lévy flight search strategy and odour-mediated anemotaxis to relocate food sources. *Behavioral Ecology and Sociobiology*, *64*(1), 115–123. https://doi.org/10.1007/s00265-009-0826-2

Scheiner, R., Erber, J., & Page, R. E. (1999). Tactile learning and the individual evaluation of the reward in honey bees (Apis mellifera L.). *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, *185*(1), 1–10. https://doi.org/10.1007/s003590050360

Scheiner, R., Page, R. E., & Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (Apis mellifera L.). *Neurobiology of Learning and Memory*, *76*(2), 138–150. https://doi.org/10.1006/nlme.2000.3996

Schmickl, T., & Crailsheim, K. (2008). TaskSelSim: A model of the self-organization of the division of labour in honeybees. *Mathematical and Computer Modelling of Dynamical Systems*, *14*(2), 101–125. https://doi.org/10.1080/13873950701846662

Schürch, R., & Grüter, C. (2014). Dancing bees improve colony foraging success as long-term benefits outweigh short-term costs. *PloS One*, *9*(8), e104660. https://doi.org/10.1371/journal.pone.0104660

Seeley, T. D. (1983). Division of labor between scouts and recruits in honeybee foraging. *Behavioral Ecology and Sociobiology*, *12*(3), 253–259. https://doi.org/10.1007/BF00290778

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. *Behavioral Ecology and Sociobiology*, *24*(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, *34*, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). *The Wisdom of the Hive*. *Harvard University Press*.

Seeley, T. D., Camazine, S., & Sneyd, J. (1991). Collective decision-making in honey bees: how colonies choose among nectar sources. *Behavioral Ecology and Sociobiology*, *28*(4), 277–290. https://doi.org/10.1007/BF00175101

Seeley, T. D., & Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? *Behavioral Ecology and Sociobiology*, *30*(1), 59–69. https://doi.org/10.1007/BF00168595

Seeley, T. D., & Visscher, P. K. (1988). Assessing the Benefits of Cooperation in Honeybee Foraging: Search Costs, Forage Quality, and Competitive Ability. *Behavioral Ecology and Sociobiology*, *22*(22), 229–237. Retrieved from http://www.jstor.org/stable/4600144%5Cnhttp://www.jstor.org/stable/4600144?seq=1&cid=pdf-reference#references_tab_contents%5Cnhttp://about.jstor.org/terms

Sinhuber, M., van der Vaart, K., & Ouellette, N. T. (2019). Response of insect swarms to dynamic illumination perturbations. *Journal of The Royal Society Interface*, *16*(150), 20180739. https://doi.org/10.1098/rsif.2018.0739

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*. https://doi.org/10.1111/2041-210X.12797

Tautz, J. (1996). Honeybee waggle dance: recruitment success depends on the dance floor. *Journal of Experimental Biology*, *1381*, 1375–1381. Retrieved from http://jeb.biologists.org/content/199/6/1375.short

Tautz, J., & Sandeman, D. C. (2003). Recruitment of honeybees to non-scented food sources. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology*, *189*(4), 293–300. https://doi.org/10.1007/s00359-003-0402-6

Tenczar, P., Lutz, C. C., Rao, V. D., Goldenfeld, N., & Robinson, G. E. (2014). Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. *Animal Behaviour*, *95*, 41–48. https://doi.org/10.1016/j.anbehav.2014.06.006

Teodorović, D. (2009). Bee colony optimization (BCO). *Studies in Computational Intelligence*, *248*, 39–60. https://doi.org/10.1007/978-3-642-04225-6_3

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *Journal of Experimental Biology*, *206*(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Tisue, S., & Wilensky, U. (2004). NetLogo: Design and implementation of a multi-agent modeling environment. *Proceedings of the Agent 2004 Conference on Social Dynamics: Interaction, Reflexivity and Emergence, Chicago, IL.*, *1*(2), 564–577. https://doi.org/10.1109/ICHCI-IEEE.2013.6887799

Van Der Post, D. J., Franz, M., & Laland, K. N. (2016). Skill learning and the evolution of social learning mechanisms. *BMC Evolutionary Biology*, *16*(1), 1–19. https://doi.org/10.1186/s12862-016-0742-9

Van Nest, B. N., & Moore, D. (2012). Energetically optimal foraging strategy is emergent property of time-keeping behavior in honey bees. *Behavioral Ecology*, *23*(3), 649–658. https://doi.org/10.1093/beheco/ars010

Vindenes, Y., & Langangen, Ø. (2015). Individual heterogeneity in life histories and eco-evolutionary dynamics. *Ecology Letters*, *18*(5), 417–432. https://doi.org/10.1111/ele.12421

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

Waddington, K. D. (1982). Honey bee foraging profitability and round dance correlates. *Journal of Comparative Physiology A*, *148*(3), 297–301. https://doi.org/10.1007/BF00679014

Walton, A., & Toth, A. L. (2016). Variation in individual worker honey bee behavior shows hallmarks of personality. *Behavioral Ecology and Sociobiology*, *70*(7), 999–1010. https://doi.org/10.1007/s00265-016-2084-4

Waters, J. S., & Fewell, J. H. (2012). Information processing in social insect networks. *PLoS ONE*, *7*(7). https://doi.org/10.1371/journal.pone.0040337

Weidenmüller, A. (2004). The control of nest climate in bumblebee (Bombus terrestris) colonies:

interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, *15*(1), 120–128. https://doi.org/10.1093/beheco/arg101

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. *Springer International Publishing* (2nd ed.). Springer. https://doi.org/10.1007/978-3-319-24277-4

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution*, *27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Zengin, A., Sarjoughian, H., & Ekiz, H. (2013). Discrete event modeling of swarm intelligence based routing in network systems. *Information Sciences*, *222*, 81–98. https://doi.org/10.1016/j.ins.2011.06.012

Chapter

VI

Conclusion

Chapter 6 – Conclusion

Honey bee forager groups consist of individuals who show consistent differences in their recruitment behaviour. Forager groups responded to a decrease in the group size by increasing their recruitment activity. This was driven by an increase in the activity of foragers who were already more involved in the task of recruitment within the group. The increase in activity was greater in dance probability as compared to dance intensity and was likely in response to changing social cues associated with the decrease in nectar influx. In contrast, the dance intensity of foragers correlated with gustatory response thresholds (GRTs) thresholds, whereas there was no correlation between dance probability and the GRT. Agent-based model simulations showed that dance intensity correlated more with consistent inter-individual differences in the recruitment activity of foragers, under constant environmental and social conditions, rather than dance probability. This thesis is the first detailed study of the role of inter-individual differences in dance activity within forager groups, and specifically the interplay between social modulation (through dance probability) and internal behavioural states (dance intensity linked to thresholds) in the recruitment activity of honey bee foragers.

What is the role of individual variation in group responses?

Consistent inter-individual variation in the recruitment activity within forager groups can help the colony in various contexts during the natural foraging process. Once a new food source is discovered, the colony would need to simultaneously invest in both foraging and recruitment for a short duration till an appropriate number of foragers are active at this source (Kirchner & Lindauer, 1994; C. Thom, 2003; Corinna Thom, Gilley, & Tautz, 2003; von Frisch, 1967). Inter-individual differences in recruitment would allow some foragers to continue exploiting the food source while others invest in expanding the forager group (Fig. 6.1 *a*). Since food sources can be transient, this would help the colony ascertain the reward value of the source while maintaining a competitive advantage (Seefeldt & De Marco, 2008; Seeley & Visscher, 1988; Stabentheiner & Kovac, 2016; Tan et al., 2015; P. Kirk Visscher & Seeley, 1982).

The second advantage of inter-individual variation within the forager group would be when the group faces some perturbation (Fig. 6.1 *a*). The loss of foragers from a group decreases the nectar influx into the colony and changes the social interactions of the remaining foragers with receivers and other nest mates (De Marco & Farina, 2001; Seeley, 1986, 1989; P.K. Visscher & Dukas, 1997). Inter-individual variation in responding to these social cues would ensure that only some individuals initiate or increase recruitment for the food source. The colony can also benefit from having inter-individual differences amongst foragers when there is a change in the relative value of a food source (Fig. 6.1 *b*). Food sources available to the colony can bloom and deplete rapidly (P. Kirk Visscher & Seeley, 1982) and so the relative value of any food source can increase or decrease accordingly. In case of an increase in relative reward value, the forager group size has to be increased proportionately. Only a few individuals, who are more sensitive to social cues (De Marco & Farina, 2001; Seeley, 1986, 1989), need to initiate or increase recruitment to expand the forager group.

Inter-individual variation in recruitment would also be beneficial when the colony needs to abruptly reduce recruitment to a food source (Fig. 6.1 *b*). In case of predation at the food source, only the more active individuals need to experience stop signals to effectively shut down further recruitment to the food source (Nieh, 2010; Tan et al., 2016). Additionally, if the relative reward value decreases, then those foragers which perceive the food as being more rewarding will persist at the food source while others will abandon it (Al Toufailia, Grüter, & Ratnieks, 2013; Scheiner, Erber, & Page, 1999; Seeley, 1989, 1994; Townsend-Mehler, Dyer, & Maida, 2010). Thus, inter-individual differences within forager groups provide the colony with greater flexibility in responding to changing social and environmental conditions.

Consistent inter-individual differences in recruitment adds to our understanding of task partitioning in honey bee foragers (George & Brockmann, 2019; Seeley, 1994; Tenczar, Lutz, Rao, Goldenfeld, & Robinson, 2014). So far, 7 different behavioural phenotypes of foragers have been identified: novice foragers, scouts, recruits, employed foragers, unemployed experienced foragers, inspectors and reactivated foragers (Biesmeijer & Vries, 2001; de Vries & Biesmeijer, 1998). However, this classification fails to take into account the task of communication and recruitment.

Since the decision to recruit is subsequent to the decision to continue foraging, individuals more active in recruitment can be described as being more invested in the food source. Thus, employed foragers can be further divided into more invested and less invested employed foragers. Under this classification, the role of inspectors (more persistent) and reactivated foragers (more reticent) could also be considered as correlating with higher and lower levels of investment respectively (Granovskiy, Latty, Duncan, Sumpter, & Beekman, 2012; Townsend-Mehler et al., 2010; Wagner, Van Nest, Hobbs, & Moore, 2013). This broad classification can be a useful approximation to disentangle the complexity in the regulation of the foraging behaviour of honey bees.

My findings are in line with contemporary studies on how inter-individual differences can affect group responses (Crall et al., 2018; Firth et al., 2017; Garrison, Kleineidam, & Weidenmüller, 2018; Rudin, Tomkins, & Simmons, 2018). Perturbations to the group led to some individuals changing their consistent activity level. In honey bees (this thesis), crickets (Rudin et al., 2018) and great tits (Firth et al., 2017), inter-individual differences before the removal could reliably predict the individual's response to the changed social context. Additionally, removal experiments targeting foragers in honey bees (this thesis) and bumblebees (Crall et al., 2018) show how colonies can rapidly respond to the loss of foragers by changing social interactions in the colony. A more recent study on thermoregulation in bumblebees is in contrast with these previous studies (Garrison et al., 2018). Bumblebee workers respond to increasing brood temperature by fanning. Garrison *et al.*, (2018) found that there was no link between the behavioural response of individual workers tested in isolation and in social groups of 9 other workers. The authors suggest that in this specific behaviour, consistent group level responses can be obtained by behavioural flexibility of individual workers. One important caveat of these experiments is that they were not done in the nest but in an artificial set up. The spatial position in the nest and associated cues of bumblebee workers play a strong role in their behavioural decisions (Crall et al., 2018). Fanning behavioural responses in the nest might differ from responses in an artificial set up. Nevertheless, all the studies and this thesis point to how consistent inter-individual differences can help social groups to cope with changing conditions.

Figure 6.1

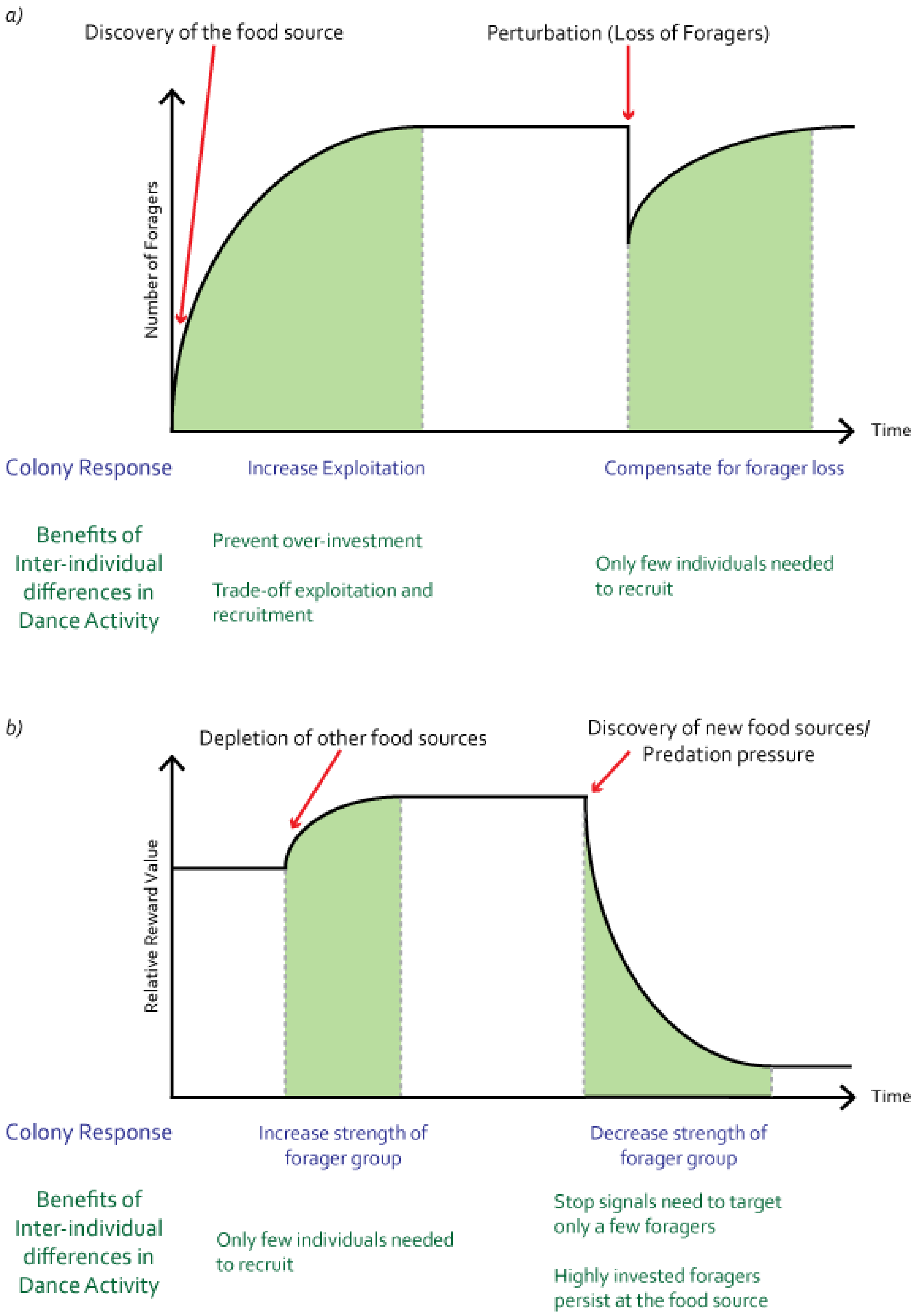

Figure 6.1: Representation of different contexts in which inter-individual variation within forager groups can help the colony. *(a)* The first two contexts involve flexibly changing the forager group size. Inter-

individual variation in the recruitment activity can help the colony during the initiation of foraging as colonies can continue to exploit the food source and expand the forager group size. The graded recruitment to a new food source will also give the colony time to ascertain the reward value of the food source and hence prevent over-investment. A loss of foragers is another context in which individual variation can be beneficial. In such a situation, the colony only needs to have a few individuals recruiting to maintain the forager group size. *(b)* The next two contexts involve the colony flexibly dealing with changing food availability. The depletion of existing food sources or the discovery of better food sources can increase and decrease the relative reward value of one particular food source respectively. In the former case, the colony will only need to have a few individuals recruiting to maintain the forager group size. In the latter case, stop signals only need to target the individuals more active in their dance behaviour to shut down further recruitment to the food source. This would be the case when there is predation at the food source also. In addition, individual variation in perception would mean that some of the most invested foragers would continue to persist at the food source which would allow the colony to keep a check on previously rewarding food sources.

The nascent body of literature on behavioural consistency in the context of social groups also provides some hints as to how these groups might trade-off the behavioural consistency of group members and the behavioural flexibility needed to adapt to changing conditions. Depending on the behavioural stimuli and the social group structure, there can either be a change in the responses of individuals already responding to the stimuli (Firth et al., 2017; Rudin et al., 2018, this thesis), or a turnover in the individuals responding to the stimuli (Crall et al., 2018; Garrison et al., 2018). In addition, if the perturbation is environmental, the individuals may respond directly to the environmental stimuli (Garrison et al., 2018; Rudin et al., 2018) or indirectly through social cues and signals (Crall et al., 2018; Firth et al., 2017, this thesis). Finally, individual responses to changing social cues and signals can be due to differences in perception of these social signals (Garrison et al., 2018; Rudin et al., 2018, this thesis), or due to differences in exposure to the social signals (Crall et al., 2018; Firth et al., 2017). However, more work needs to be done to establish the generality of these mechanisms across groups which differ in their structure and dynamics as well as across behavioural contexts. Further studies involving comparative experiments to either observe the responses of the same social groups to differing behavioural contexts (e.g., perturbation in the form of predation and a perturbation in the form of changing food availability) or to observe the responses of different groups in the same behavioural context (e.g., bumblebee and honey bee foragers to changing food availability) would be particularly useful.

Response thresholds, Probability and Intensity: are they correlated?

Response thresholds are widely considered to be the major physiological basis of division of labour in social insects (Beshers & Fewell, 2001; de Oliveira & Campos, 2019; Dornhaus, Klügl, Puppe, & Tautz, 1998; Gardner, Foster, & O'Donnell, 2007; Noa Pinter-Wollman, Hubler, Holley, Franks, & Dornhaus, 2012; Reim & Scheiner, 2014; Weidenmüller, 2004). However, response probability and response intensity might also play an important role in the division of labour within colonies (Jeanson & Weidenmüller, 2013; Weidenmüller, 2004). Most studies so far have neglected a link between these 3 response parameters, possibly due to the difficulty in dissociating response thresholds and probability in an experimental set up. While threshold represents the absolute intensity of a stimulus at which an individual responds, the probability and intensity can only be determined by testing the same individuals multiple times. Gustatory response thresholds (GRTs) or sucrose responsiveness correlates with division of labour in several species of eusocial insects (Josens, Lopez, Jofré, & Giurfa, 2018; Perez, Rolland, Giurfa, & D'Ettorre, 2013; Reim & Scheiner, 2014). The waggle dance behaviour provides a robust behavioural paradigm to explore whether the GRT is linked to the probability and intensity of individuals in the task of recruitment since forager responses to repeated exposures to the same stimuli (food reward) can be quantified easily under natural conditions.

The GRT and dance intensity showed a weak correlation, whereas the GRT and dance probability did not. These results likely reflect the process of recruitment in honey bee colonies. The total recruitment to a food source is dependent more on the number of dances than the duration of these dances since recruits follow multiple dances but only for a few waggle runs each before flying out (Seeley, 1995; Seeley & Towne, 1992). Hence, the probability of dancing should be expected to be more sensitive to changing social cues rather than the intensity.

An alternate explanation for the weak correlation is that other thresholds may be linked to the waggle dance behaviour (Fig. 6.2). Under free flying conditions, foragers make successive behavioural decisions related to the food reward. The first of these would be to probe the food source by extending their proboscis, a behavioural response similar to the proboscis extension reflex assay used for quantifying the GRT (Scheiner et al., 1999; Scheiner, Page, & Erber, 2004). The next behavioural decision would be to accept the food reward at the source. The final two decisions would be back in the hive, wherein the forager would decide to continue foraging at the food source and then decide to communicate about the food source. Different thresholds are likely associated with each of these behavioural decisions. Experiments with free flying foragers showed that the sucrose acceptance threshold of an individual forager (related to the behavioural decision to accept the food reward) is different from its GRT (Mujagic & Erber, 2009; Mujagic, Sarkander, Erber, & Erber, 2010). An additional 'dance initiation threshold', linked to the decision to communicate about the food source, might show a stronger link with dance activity.

Figure 6.2

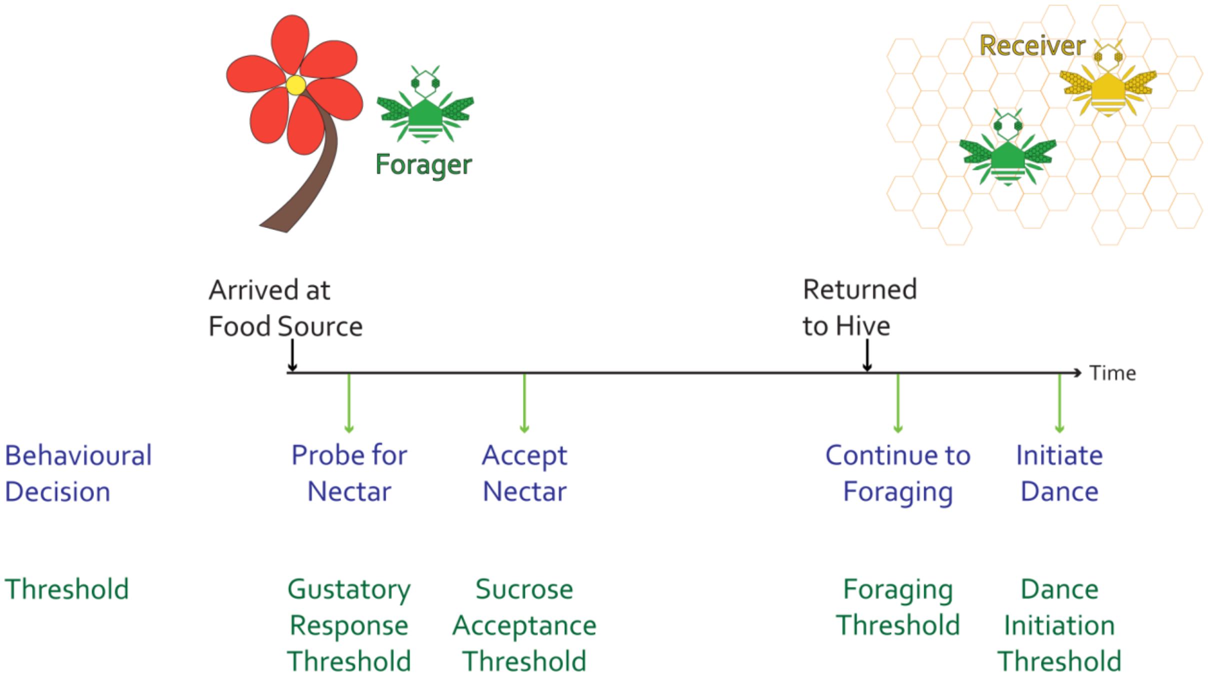

Figure 6.2: The different behavioural decisions that a forager makes during a trip to a food source and the thresholds that could be associated with each of these decisions. When it reaches the food source, the forager will first probe the food source, a behavioural response similar to the proboscis extension

reflex used to quantify gustatory response thresholds. The forager can then decide to accept this food source, a behavioural decision that correlates with the foragers sucrose acceptance threshold [43], [44]. After it gets the nectar load back to the hive the forager can then decide to continue foraging at the food source and further, recruit to the food source. A foraging threshold and a dance initiation threshold might show a strong correlation with the last two behavioural decisions.

Figure 6.3

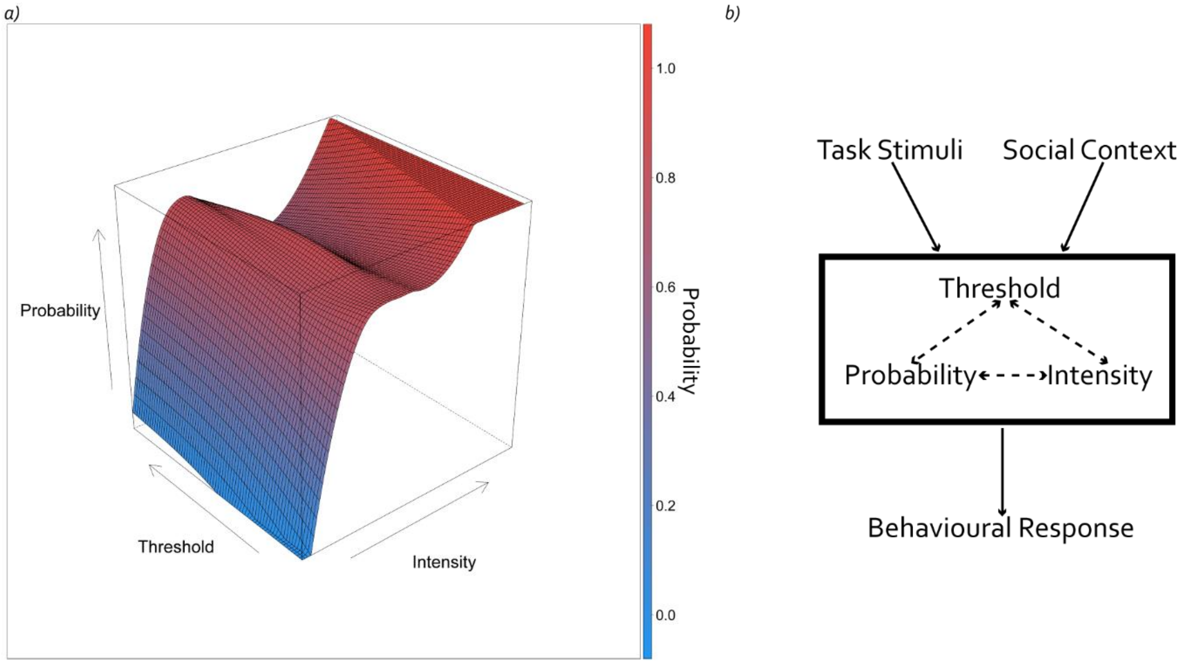

Figure 6.3: *(a)* The relationship between gustatory response thresholds, dance probability and dance intensity of 110 foragers from 3 different colonies (see chapter 4). A locally estimated scatterplot smoothing (LOESS) function was used to produce the surface depicting the relationship between the three response parameters. *(b)* Behavioural responses of individual workers to a task-associated stimulus in eusocial groups is dependent on the link between response thresholds, response probability and response intensity. These three response parameters can be further modulated by social cues and signals to varying degrees depending on the task. The interplay between the three response parameters and the social context would likely lead to division of labour in eusocial groups.

All three behavioural parameters, thresholds, probability and intensity, that determines an individual workers response to a stimulus are likely to be modulated by social cues (Beshers & Fewell, 2001; Beshers, Huang, Oono, & Robinson, 2001; Breed, Williams, & Queral, 2002; Naug & Gadagkar, 1999; O'Donnell, 1998; Noa Pinter-

Wollman et al., 2012; Seeley, 1989). Workers with different combinations of response threshold, probability and intensity would provide the colony with a wide range of behavioural responses to any change in stimuli (Fig. 6.3 *a*). The results of my thesis suggest that in recruitment in honey bees the response probability is strongly modulated by social cues, whereas the response threshold and response intensity are linked and likely represent a fixed behavioural 'state' of the individual for this task. The relationship between the three parameters, as well as the strength of their modulation by social cues may vary depending on the task.

Agent- based model simulations can provide a useful toolkit to explore the link between the three response parameters and the social context in a task specific manner at the colony level (de Oliveira & Campos, 2019). Apart from looking at how these parameters can lead to task partitioning, models can also be used to look at the conditions under which individual variation in these parameters can be beneficial (Boumans, de Boer, Hofstede, & Bokkers, 2018; Boumans, Hofstede, Bolhuis, de Boer, & Bokkers, 2016; Campos, Bartumeus, Mendez, Andrade, & Espadaler, 2016; Schürch & Grüter, 2014). The model described in chapter 5 can be expanded in the future to include individual variation in a 'dance initiation threshold', and thereby explore the link between the three response parameters in the task of recruitment. Further, ABMs can also be useful as a framework for designing experimental studies based on how various factors (like social interactions) can be expected to modulate the three response parameters (Allegue et al., 2017). Thus, a combination of theoretical models and empirical observations can be used to formulate a broader conceptual framework on division of labour in social insects and other social groups, taking into account the nature of the task and the link between social cues and response thresholds, probability and intensity (Fig. 6.3 *b*).

Outlook

In conclusion, this thesis adds a new aspect to the complex regulation of foraging in honey bee colonies and highlights the role of consistent inter-individual differences in this regulation. The relationship between consistent inter-individual differences in response thresholds, probability and intensity and division of labour in eusocial groups needs to be explored. In addition, at the level of social groups, the role played by consistent inter-individual differences in driving group responses needs to be understood further. This would need synergistic studies linking together behavioural syndromes and social context in animal groups using advanced technical and analytical methods (Arganda, Hinz, de Polavieja, Pérez-Escudero, & Vicente-Page, 2014; Farine & Whitehead, 2015; N. Pinter-Wollman et al., 2013; Sridhar, Roche, & Gingins, 2019; Wild et al., 2018). Such studies are all the more urgent given the drastically changing environmental conditions that group living animals are facing.

References

Al Toufailia, H., Grüter, C., & Ratnieks, F. L. W. (2013). Persistence to Unrewarding Feeding Locations by Honeybee Foragers (Apis mellifera): the Effects of Experience, Resource Profitability and Season. *Ethology*, *119*(12), 1096–1106. https://doi.org/10.1111/eth.12170

Allegue, H., Araya-Ajoy, Y. G., Dingemanse, N. J., Dochtermann, N. A., Garamszegi, L. Z., Nakagawa, S., … Westneat, D. F. (2017). Statistical Quantification of Individual Differences (SQuID): an educational and statistical tool for understanding multilevel phenotypic data in linear mixed models. *Methods in Ecology and Evolution*, *8*(2), 257–267. https://doi.org/10.1111/2041-210X.12659

Arganda, S., Hinz, R. C., de Polavieja, G. G., Pérez-Escudero, A., & Vicente-Page, J. (2014). idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nature Methods*, *11*(7), 743–748. https://doi.org/10.1038/nmeth.2994

Beshers, S. N., & Fewell, J. H. (2001). Models of division of labor in social insects. *Annual Review of Entomology*, *46*, 413–440. https://doi.org/10.1146/annurev.ento.46.1.413

Beshers, S. N., Huang, Z. Y., Oono, Y., & Robinson, G. E. (2001). Social inhibition and the regulation of temporal polyethism in honey bees. *Journal of Theoretical Biology*, *213*(3), 461–479. https://doi.org/10.1006/jtbi.2001.2427

Biesmeijer, J., & Vries, H. de. (2001). Exploration and exploitation of food sources by social insect colonies: a revision of the scout-recruit concept. *Behavioral Ecology and Sociobiology*, *49*(2), 89–99. Retrieved from http://link.springer.com/article/10.1007/s002650000289

Boumans, I. J. M. M., de Boer, I. J. M., Hofstede, G. J., & Bokkers, E. A. M. (2018). Unravelling variation in feeding, social interaction and growth patterns among pigs using an agent-based model. *Physiology and Behavior*, *191*(October 2017), 100–115. https://doi.org/10.1016/j.physbeh.2018.03.030

Boumans, I. J. M. M., Hofstede, G. J., Bolhuis, J. E., de Boer, I. J. M., & Bokkers, E. A. M. (2016). Agent-based modelling in applied ethology: An exploratory case study of behavioural dynamics in tail biting in pigs. *Applied Animal Behaviour Science*, *183*, 10–18. https://doi.org/10.1016/j.applanim.2016.07.011

Breed, M. D., Williams, D. B., & Queral, A. (2002). Demand for task performance and workforce replacement: Undertakers in honeybee, Apis mellifera, colonies. *Journal of Insect Behavior*, *15*(3), 319–329. https://doi.org/10.1023/A:1016261008322

Campos, D., Bartumeus, F., Mendez, V., Andrade, J. S., & Espadaler, X. (2016). Variability in individual activity bursts improves ant foraging success. *Journal of the Royal Society Interface*, *13*(125). https://doi.org/10.1098/rsif.2016.0856

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Spatial fidelity of workers predicts collective response to disturbance in a social insect. *Nature Communications*, *9*(1), 1–13. https://doi.org/10.1038/s41467-018-03561-w

De Marco, R. J., & Farina, W. M. (2001). Changes in food source profitability affect the trophallactic and dance behavior of forager honeybees (Apis mellifera L.). *Behavioral Ecology and Sociobiology*, *50*(5), 441–449. https://doi.org/10.1007/s002650100382

de Oliveira, V. M., & Campos, P. R. A. (2019). The emergence of division of labor in a structured response threshold model. *Physica A: Statistical Mechanics and Its Applications*, *517*, 153–162. https://doi.org/10.1016/j.physa.2018.11.023

de Vries, H., & Biesmeijer, J. C. (1998). Modelling collective foraging by means of individual behaviour rules in honey-bees. *Behavioral Ecology and Sociobiology*, *44*(2), 109–124. https://doi.org/10.1007/s002650050522

Dornhaus, A., Klügl, F., Puppe, F., & Tautz, J. (1998). Task Selection in Honeybees - Experiments Using Multi-Agent Simulation. *Gwal '98*.

Farine, D. R., & Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. *Journal of Animal Ecology*, *84*(5), 1144–1163. https://doi.org/10.1111/1365-2656.12418

Firth, J. A., Voelkl, B., Crates, R. A., Aplin, L. M., Biro, D., Croft, D. P., & Sheldon, B. C. (2017). Wild birds respond to flockmate loss by increasing their social network associations to others. *Proceedings. Biological Sciences*, *284*(1854), 20170299. https://doi.org/10.1098/rspb.2017.0299

Gardner, K. E., Foster, R. L., & O'Donnell, S. (2007). Experimental analysis of worker division of labor in bumblebee nest thermoregulation (Bombus huntii, Hymenoptera: Apidae). *Behavioral Ecology and Sociobiology*, *61*(5), 783–792. https://doi.org/10.1007/s00265-006-0309-7

Garrison, L. K., Kleineidam, C. J., & Weidenmüller, A. (2018). Behavioral flexibility promotes collective consistency in a social insect. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-33917-7

George, E. A., & Brockmann, A. (2019). Social modulation of individual differences in dance communication in honey bees. *Behavioral Ecology and Sociobiology*, *73*(4), 41. https://doi.org/10.1007/s00265-019-2649-0

Granovskiy, B., Latty, T., Duncan, M., Sumpter, D. J. T., & Beekman, M. (2012). How dancing honey bees keep track of changes: the role of inspector bees. *Behavioral Ecology*, *23*(3), 588–596. https://doi.org/10.1093/beheco/ars002

Jeanson, R., & Weidenmüller, A. (2013). Interindividual variability in social insects - proximate causes and ultimate consequences. *Biological Reviews*. https://doi.org/10.1111/brv.12074

Josens, R., Lopez, M. A., Jofré, N., & Giurfa, M. (2018). Individual size as determinant of sugar responsiveness in ants. *Behavioral Ecology and Sociobiology*, *72*(10), 1–9. https://doi.org/10.1007/s00265-018-2581-8

Kirchner, W. H., & Lindauer, M. (1994). The causes of the tremble dance of the honeybee , Apis mellifera. *Behavioral Ecology and Sociobiology*, *35*(5), 303–308. https://doi.org/https://doi.org/10.1007/BF00184419

Mujagic, S., & Erber, J. (2009). Sucrose acceptance, discrimination and proboscis responses of honey bees (Apis mellifera L.) in the field and the laboratory. *Journal of Comparative Physiology A*, *195*(4), 325–339. https://doi.org/10.1007/s00359-008-0409-0

Mujagic, S., Sarkander, J., Erber, B., & Erber, J. (2010). Sucrose acceptance and different forms of associative learning of the honey bee (apis mellifera L.) in the field and laboratory. *Frontiers in Behavioral Neuroscience*, *4*(July), 1–11. https://doi.org/10.3389/fnbeh.2010.00046

Naug, D., & Gadagkar, R. (1999). Flexible Division of Labor Mediated by Social Interactions in an Insect

Colony—a Simulation Model. *Journal of Theoretical Biology*, *197*, 123–133. https://doi.org/10.1006/jtbi.1998.0862

Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology*, *20*(4), 310–315. https://doi.org/10.1016/j.cub.2009.12.060

O'Donnell, S. (1998). Effects of experimental forager removals on division of labour in the primitively eusocial wasp *Polistes instabilis* (*Hymenoptera*: *Vespidae*). *Behaviour*, *135*(2), 173–193. https://doi.org/10.1163/156853998793066348

Perez, M., Rolland, U., Giurfa, M., & D'Ettorre, P. (2013). Sucrose responsiveness, learning success, and task specialization in ants. *Learning and Memory*, *20*(8), 417–420. https://doi.org/10.1101/lm.031427.113

Pinter-Wollman, N., Hobson, E. a., Smith, J. E., Edelman, a. J., Shizuka, D., de Silva, S., … McDonald, D. B. (2013). The dynamics of animal social networks: analytical, conceptual, and theoretical advances. *Behavioral Ecology*, *25*(2), 242–255. https://doi.org/10.1093/beheco/art047

Pinter-Wollman, Noa, Hubler, J., Holley, J.-A., Franks, N. R., & Dornhaus, A. (2012). How is activity distributed among and within tasks in Temnothorax ants? *Behavioral Ecology and Sociobiology*, *66*(10), 1407–1420. https://doi.org/10.1007/s00265-012-1396-2

Reim, T., & Scheiner, R. (2014). Division of labour in honey bees: Age- and task-related changes in the expression of octopamine receptor genes. *Insect Molecular Biology*, *23*(6), 833–841. https://doi.org/10.1111/imb.12130

Rudin, F. S., Tomkins, J. L., & Simmons, L. W. (2018). The effects of the social environment and physical disturbance on personality traits. *Animal Behaviour*, *In press*, 109–121. https://doi.org/10.1016/j.anbehav.2018.02.013

Scheiner, R., Erber, J., & Page, R. E. (1999). Tactile learning and the individual evaluation of the reward in honey bees (Apis mellifera L.). *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, *185*(1), 1–10. https://doi.org/10.1007/s003590050360

Scheiner, R., Page, R. E., & Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (Apis mellifera) Ricarda. *Apidologie*, *35*, 133–142. https://doi.org/10.1051/apido:2004001

Schürch, R., & Grüter, C. (2014). Dancing bees improve colony foraging success as long-term benefits outweigh short-term costs. *PloS One*, *9*(8), e104660. https://doi.org/10.1371/journal.pone.0104660

Seefeldt, S., & De Marco, R. J. (2008). The response of the honeybee dance to uncertain rewards. *The Journal of Experimental Biology*, *211*(Pt 21), 3392–3400. https://doi.org/10.1242/jeb.017624

Seeley, T. D. (1986). Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, *19*(5), 343–354. https://doi.org/10.1007/BF00295707

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. *Behavioral Ecology and Sociobiology*, *24*(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, *34*, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). *The Wisdom of the Hive. Harvard University Press*.

Seeley, T. D., & Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? *Behavioral Ecology and Sociobiology*, *30*(1), 59–69. https://doi.org/10.1007/BF00168595

Seeley, T. D., & Visscher, P. K. (1988). Assessing the Benefits of Cooperation in Honeybee Foraging: Search Costs, Forage Quality, and Competitive Ability. *Behavioral Ecology and Sociobiology*, *22*(22), 229–237. Retrieved from http://www.jstor.org/stable/4600144%5Cnhttp://www.jstor.org/stable/4600144?seq=1&cid=pdf-reference#references_tab_contents%5Cnhttp://about.jstor.org/terms

Sridhar, V. H., Roche, D. G., & Gingins, S. (2019). Tracktor: image-based automated tracking of animal movement and behaviour. *Methods in Ecology and Evolution*, (January), 1–16. https://doi.org/10.1101/412262

Stabentheiner, A., & Kovac, H. (2016). Honeybee economics: Optimisation of foraging in a variable world. *Scientific Reports*, *6*(June), 1–7. https://doi.org/10.1038/srep28339

Tan, K., Dong, S., Li, X., Liu, X., Wang, C., Li, J., & Nieh, J. C. (2016). Honey Bee Inhibitory Signaling Is Tuned to Threat Severity and Can Act as a Colony Alarm Signal. *PLOS Biology*, *14*(3), e1002423. https://doi.org/10.1371/journal.pbio.1002423

Tan, K., Latty, T., Dong, S., Liu, X., Wang, C., & Oldroyd, B. P. (2015). Individual honey bee (Apis cerana) foragers adjust their fuel load to match variability in forage reward. *Scientific Reports*, *5*(1), 1–7. https://doi.org/10.1038/srep16418

Tenczar, P., Lutz, C. C., Rao, V. D., Goldenfeld, N., & Robinson, G. E. (2014). Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. *Animal Behaviour*, *95*, 41–48. https://doi.org/10.1016/j.anbehav.2014.06.006

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *Journal of Experimental Biology*, *206*(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Thom, Corinna, Gilley, D. C., & Tautz, J. (2003). Worker piping in honey bees (Apis mellifera): The behavior of piping nectar foragers. *Behavioral Ecology and Sociobiology*, *53*(4), 199–205. https://doi.org/10.1007/s00265-002-0567-y

Townsend-Mehler, J. M., Dyer, F. C., & Maida, K. (2010). Deciding when to explore and when to persist: a comparison of honeybees and bumblebees in their response to downshifts in reward. *Behavioral Ecology and Sociobiology*, *65*(2), 305–312. https://doi.org/10.1007/s00265-010-1047-4

Visscher, P. Kirk, & Seeley, T. D. (1982). Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest. *Ecology*, *63*(6), 1790–1801. https://doi.org/10.2307/1940121

Visscher, P.K., & Dukas, R. (1997). Survivorship of foraging honey bees. *Insectes Sociaux*, *44*(1), 1–5. https://doi.org/10.1007/s000400050017

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

Wagner, A. E., Van Nest, B. N., Hobbs, C. N., & Moore, D. (2013). Persistence, reticence and the management of multiple time memories by forager honey bees. *The Journal of Experimental Biology*, *216*(Pt 7), 1131–1141. https://doi.org/10.1242/jeb.064881

Weidenmüller, A. (2004). The control of nest climate in bumblebee (Bombus terrestris) colonies: interindividual variability and self reinforcement in fanning response. *Behavioral Ecology*, *15*(1), 120–128. https://doi.org/10.1093/beheco/arg101

Wild, B., Rosemann, B., Landgraf, T., Wario, F., Dormagen, D., & Boenisch, F. (2018). Tracking All Members of a Honey Bee Colony Over Their Lifetime Using Learned Models of Correspondence. *Frontiers in Robotics and AI*, *5*(April), 1–10. https://doi.org/10.3389/frobt.2018.00035

Chapter

A1

Recruits

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Chapter A1 – Recruits

Introduction

Honey bee foragers make up a large proportion of the workforce of the colony (Seeley, 1995; Thom, Seeley, & Tautz, 2000). Foragers can be classified into pollen, nectar or sometimes even water foragers based on the resource they bring back to the colony (Seeley, 1995; von Frisch, 1967). Further, based on how they found the food source, foragers can also be classified into scouts and recruits (Seeley, 1983). Scouts are exploratory in nature and find novel food sources to which they recruit other foragers. Scouts show low food site fidelity and abandon food sources quickly to find new ones (Beekman & Gilchrist, 2007; Liang et al., 2012). Recruits are exploiters who forage at food sources to which they have been recruited to. They continuously visit a food site till it becomes less rewarding (Wells & Wells, 1986). The presence of scouts and recruits helps the colony flexibly explore and exploit food sources in the environment around it.

Efficient utilisation of the foraging force available to the colony depends on effectively responding to biotic and abiotic cues associated with foraging. Nectar foragers, on returning to the hive, interact with receivers who unload the nectar that the foragers have brought in (Seeley, 1982, 1995). Foragers get to know about the state of the colony food stores as well as the nectar influx into the colony from these interactions (De Marco & Farina, 2001; Fernandez, Gil, Farina, & Farina, 2003; Seeley, 1989). This information, along with the perceived value of the food source (Al Toufailia, Grüter, & Ratnieks, 2013; George & Brockmann, 2019), modulates the motivation of the foragers to recruit to the food source. The total recruitment to each food source is proportionate to the perceived reward value of the food source relative to all other food sources that the colony is exploiting (Seeley, 1986; Seeley & Towne, 1992). Thus, each food source is exploited by a group of foragers proportional to its current reward value for the colony.

Increasing recruitment to a food source will increase the nectar influx into the colony, which would have an inhibitory effect on the foragers' motivation to recruit. However, no study has quantified how individual foragers respond to increasing recruitment. In this chapter, I look at how individual foragers respond to the presence of recruits in the forager group and the time dynamics of the dance activity of foragers and recruits. This will give us a greater understanding of the regulation of foraging behaviour in a honey bee colony.

Materials and Methods

Apis mellifera colonies

The maintenance of the colonies used in these experiments followed the same protocol as in chapter 2.

Forager training

The protocol for training the foragers in these experiments were the same as described earlier in chapter 2.

Individual Identification

The protocol for tagging and identifying individuals in these experiments were the same as described earlier in chapter 2.

Experimental Protocol

The experiments were started 2 days after the tagging process and consisted of 2 consecutive days of observation of the individually marked foragers (Fig. A1.1). All experiments were started when there were at least 11 foragers coming to the feeder. After this, any other marked individual forager was caught and put on ice for the duration of the observations on that day. All unmarked recruits that were coming to the feeder during this time were also caught and put on ice till the end of the observation period on each day.

On each day, the observations lasted for 3 hours. The feeder was filled with 1M sucrose solution throughout the 3 hours of observation. The sucrose concentration was maintained at 1M to observe the effects of recruitment on dance activity without the confounding effect of changing reward conditions. The foraging and dance activity of the tagged foragers were observed during these 3 hours. In addition, the total number of dances made by unmarked individuals in the hive were also observed.

The two days of the recruit experiments differed in the size of the forager group that was active at the food source. On the first day of the experiment (Pre-recruit phase), a protocol similar to the consistency experiments were followed, in which all unmarked recruits coming to the feeder were caught and only the individually marked foragers were allowed to feed. On the second day of the experiment (Post-recruit phase), the recruitment process was unhindered and none of the recruits coming to the feeder were caught.

Figure A1.1

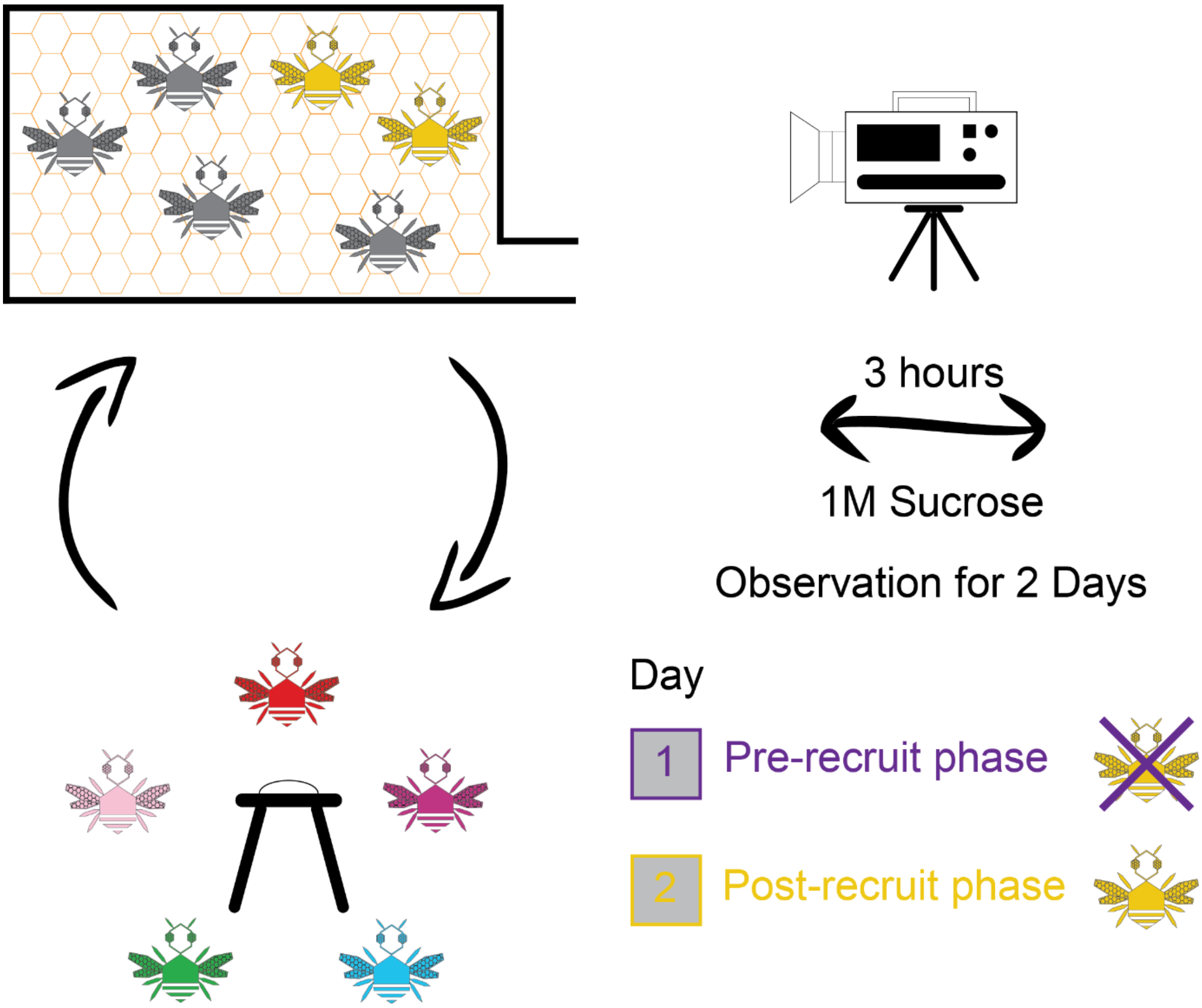

Figure A1.1: Experimental protocol for the recruit experiments. The basic experimental protocol is the same as the consistency experiments with 3-hour observations per day. But the feeder was provided with 1 M sucrose throughout the 3 hours. The recruit experiments were done over 2 days and consisted of 2 phases, a pre-recruit phase (day 1) and a post-recruit phase (day 2). Similar to the consistency experiments, in the pre-recruit phase, recruits coming to the feeder were caught and kept on ice. In the post-recruit phase, the recruits were allowed to join the forager group at the feeder.

One the first day, two observers were present at the feeder and one was present at the hive. At the feeder, one of the observers noted the time when each forager landed at the feeder with a resolution of one minute. The other caught all the recruits that were coming to the feeder to keep the individually marked foragers motivated to dance throughout the experiments (Seeley, 1995). The observer near the hive would video record the dances of the individually marked foragers. Recordings were made using a Sony Handycam (HDR CX260/HDR CX240) at 1080p and 25/50 frames per second. The recordings were started when any of the marked individuals started dancing and were stopped when none of the marked individuals were dancing. On the second day, one observer was present at the feeder and another at the hive. The observer at the feeder kept a note of the landing times of the individual foragers. The observer at the hive recorded the waggle dances of individually tagged foragers as described above. At the same time, the observer also noted down the number of dances made by the recruits in time-bins of 2 minutes. The dances of the recruits were not recorded, as individual recruits could not be identified. In total 32 individuals from 4 different forager groups from 2 colonies were tested (Table A1.1).

Table A1.1

Forager Group Name	**Month**	**Year**	**Forager Group Size**	**Colony**
A1	March	2016	7	4
A2	May	2017	6	9
A3	June	2017	11	9
A4	June	2017	8	9

Table A1.1: The identity of the forager group, the month and year when the group was observed, the forager group size and the colony to which the forager group belonged to in the consistency experiments.

Video Analysis

The recorded dances of the foragers were then analysed manually using the open source VLC Media Player. Since the feeder location was very close to the hive, most foragers did dances with very short waggle phases (Gardner, Seeley, & Calderone, 2008). The total number of circuits performed by each forager during each dance was obtained from the videos. Each dance circuit involved the forager walking in a circular path with a short waggling motion of her abdomen at the end of the path (Sen Sarma, Esch, & Tautz, 2004).

Statistical Analysis

The statistical analysis focussed on 6 different behavioural parameters estimated for each bee for each day. They were: 1) *total number of dance circuits*, 2) *total number of dances*, 3) *total number of foraging trips*, 4) *the probability of dancing* (the ratio of the total number of dances to the total number of trips), 5) the ratio of the total number of circuits to the total number of dances (referred to as *Circuits/Dances*) and 6) the ratio of the total number of circuits to the total number of trips (referred to as *Circuits/Trips*). The last two ratios are different methods of calculating the intensity of the dances made by the foragers. Circuits/dances gives an estimate of how much a forager dances on average and is a good proxy for how rewarding the food source is (Seeley, 1994). On the other hand, the circuits/trips parameter provides an estimate for the intensity of the dances normalised to differences in the number of foraging trips made by different foragers over the 6 days. All 6 parameters were calculated from the total activity of each forager over 3 hours.

In the recruit experiments, the data for 5 parameters (except foraging trips) were zero-inflated, as most foragers stopped their dance activity in the post-recruit phase. Hence GLMMs were fitted using Tweedie error distributions to account for the zero-inflation. GLMMs were built with each of the parameters as the response variable, presence of recruits (a categorical variable of two levels; pre and post) as the predictor and the forager group as a random effect. For foraging trips, GLMMs were built with a Poisson error distribution. In the case of the number of dances, the fitted GLMM with a

Tweedie distribution was computationally singular. Hence, a GLMM with Gaussian error distribution was fit after adding a small noise value to the number of dances.

In addition, in the forager group A1, some of the individually marked foragers made recruitment dances in the beginning of the post-recruitment phase. So, in this repeat the time dynamics of the dances made by marked foragers and recruits in the 3-hour observation period of the 2^nd^ day (post-recruit phase) were compared. First, the number of dances made by marked foragers and recruits were binned int 2-minute time intervals. Then, the cumulative proportion of the dances in each bin were obtained for both recruits and foragers. Finally, these two distributions were compared using a chi-squared test. In the other forager groups, none of the marked foragers made any dances in the post-recruit phase, and hence this comparison could not be made. Further, since only the number of dances of recruits were observed, this comparison could not be done for any of the other 5 parameters.

The Tweedie distribution was fitted using the tweedie package (Dunn & Smyth, 2005, 2008) and GLMMs were built using the glmmPQL function in the MASS package (Venables & Ripley, 2002) and the statmod package (Smyth, 2002) in R (R Core Team, 2018). All the graphs were made in R using the ggplot2 (Wickham, 2016) and cowplot (Claus O. Wilke, 2018) package.

Results

Allowing recruits to join the forager group had an immediate strong effect on the dance activity of foragers. Most of the marked foragers (27 out of 32 foragers) completely stopped dancing in the post-recruit phase (day 2).

Dance Circuits

The power variance index of the fitted Tweedie distribution for the total dance circuits was 1.312. The GLMM fit with this error distribution showed that individuals decreased the total dance circuits in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.2 *a*, difference estimate = 162.845, CI = 133.075 – 192.616, $p < 0.001$). The dance circuits performed by the foragers in the post-recruit phase was also not significantly different from zero (mean = 0.0295, $p = 0.9134$).

Figure A1.2

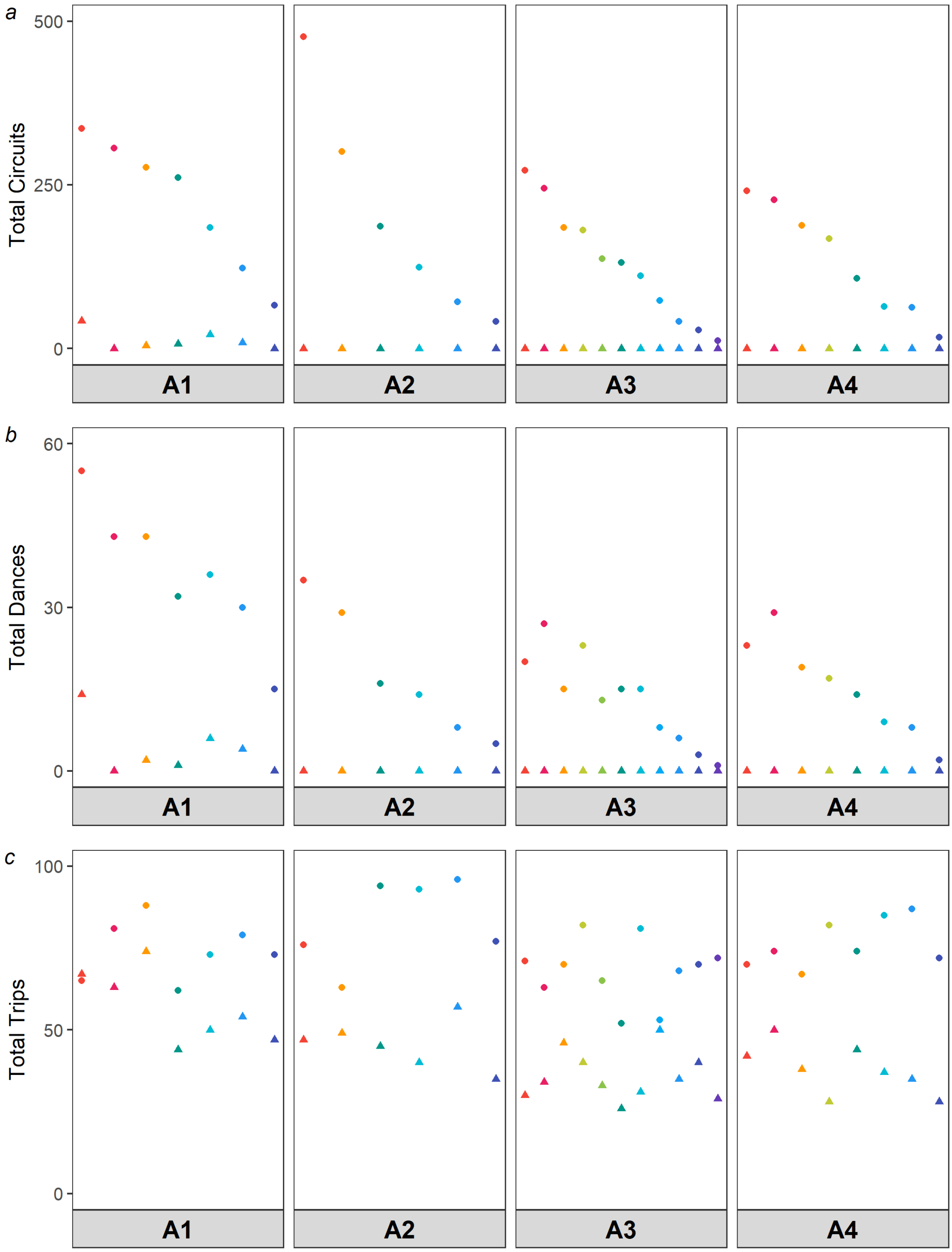

Figure A1.2: The activity of foragers in the pre (circles) and post-recruit phase (triangles) in *(a)* total dance circuits, *(b)* number of dances and *(c)* number of foraging trips of all 32 foragers from 4 forager groups. Colours represent different individuals, with the same columns representing the activity of the same individuals in the different parameters.

Number of Dances

The GLMM showed that individuals decreased the number of dances in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.2 *b*, difference estimate = 18.781, CI = 14.756 – 22.806, $p < 0.001$). The number of dances performed by the foragers in the post-recruit phase was also not significantly different from zero (mean = 1.340, $p = 0.6485$).

Foraging Trips

The GLMM fit with the Poisson error distribution showed that individuals decreased the foraging trips in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.2 *c*, difference estimate = 31.957, CI = 27.096 – 36.817, $p < 0.001$). But the number of foraging trips they performed was significantly higher than zero (mean = 43.524, $p < 0.001$).

Probability of Dancing

The power variance index of the fitted Tweedie distribution for the probability of dancing was 1.246. The GLMM fit with this error distribution showed that individuals decreased the probability of dancing in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.3 *a*, difference estimate = 0.259, CI = 0.205 – 0.312, $p < 0.001$). The probability of dancing of the foragers in the post-recruit phase was also not significantly different from zero (mean = 0.0001, $p = 0.884$).

Circuits/Dances

The power variance index of the fitted Tweedie distribution for circuits/dances was 1.03. The GLMM fit with this error distribution showed that individuals decreased their circuits/dances in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.3 *b*, difference estimate = 8.861, CI = 7.911 – 9.811, $p < 0.001$). The circuits/dances of the foragers in the post-recruit phase was also not significantly different from zero (mean = 0.003, $p = 0.852$).

Figure A1.3

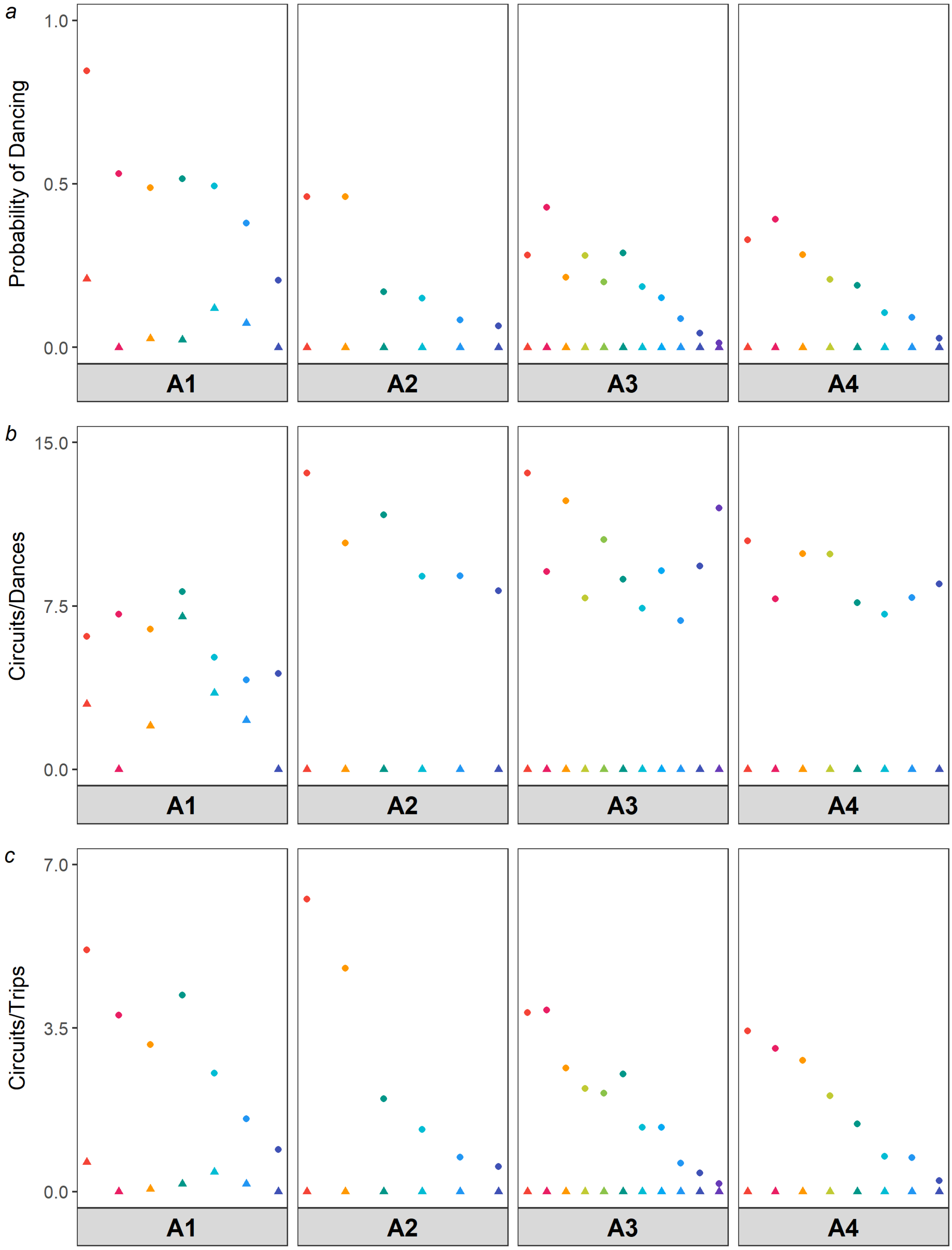

Figure A1.3: The activity of foragers in the pre (circles) and post-recruit phase (triangles) in *(a)* probability of dancing, *(b)* circuits/dances and *(c)* circuits/trips of all 32 foragers from 4 forager groups.

Colours represent different individuals, with the same columns representing the activity of the same individuals in the different parameters.

Figure A1.4

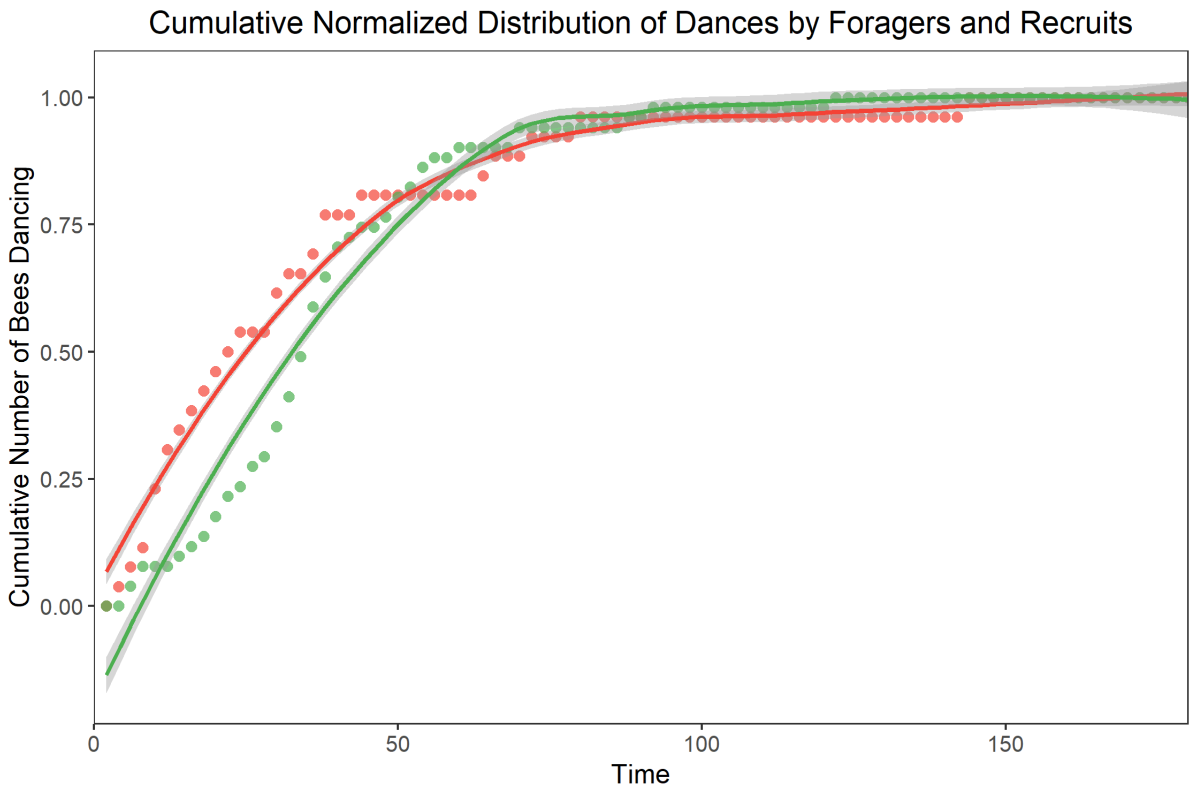

Figure A1.4: The normalised cumulative distribution of dances by foragers and recruits in forager group A1 in the post-recruit phase (day 2). The distributions were significantly different, with the foragers initially dancing more than recruits. Recruits increased their dance activity later but continued to dance for longer.

Circuits/Trips

The power variance index of the fitted Tweedie distribution for circuits/trips was 1.296. The GLMM fit with this error distribution showed that individuals decreased their circuits/trips in the post-recruit phase as compared to the pre-recruit phase (Fig. A1.3 *c*, difference estimate = 2.250, CI = 1.828 – 2.673, p < 0.001). The circuits/trips of the foragers in the post-recruit phase was also not significantly different from zero (mean = 0.0004, p = 0.92).

Time dynamics of dances by foragers and recruits

The time dynamics of dances made by foragers and recruits were different (χ^2 = 44.801, df = 15, p < 0.001). The cumulative proportion of dances distribution of foragers increased and saturated earlier as compared to the distribution of recruits (Fig. A1.4).

Discussion

Allowing recruits to join the forager group led to a drastic reduction in the dance activity in all forager groups tested. Most experimental foragers in these groups completely stopped their dance activity. In contrast to the removal experiments, the whole forager group was affected and not just specific individuals. In the experimental repeat where some foragers continued dancing, the time dynamics of the dances by foragers and recruits differed, with foragers dancing initially, followed by recruits.

The dramatic reduction in dance activity can be explained by the same mechanism as in the removal experiments. Allowing recruits to join the forager group increased the nectar influx into the colony (Seeley, 1989) which caused a change in the interactions of foragers and receivers. In these experiments, the increase in nectar influx and hence the change in stimulus was so large that even those individuals which were perceiving the food as being more rewarding stopped their dance communication.

The exact magnitude of this change could not be quantified since the total number of individuals that were recruited could not be identified. Nevertheless, the number of recruits coming to the feeder were more than 20 in all the repeats of these experiments (personal observation). Thus, in contrast with the removal experiments, mass recruitment had a stronger effect due to the magnitude of the change in nectar influx.

Most experiments on dance communication so far involved catching and removing recruits to keep the foragers motivated to dance throughout the experiment (von Frisch, 1967). In contrast, under natural conditions, recruitment to any food source continues till an appropriate number of foragers are active at that source (Mailleux, Deneubourg, & Detrain, 2003). Otherwise, recruitment would lead to overcrowding and diminishing returns on the investment from the colony in that food source (Kirchner & Lindauer, 1994; C. Thom, 2003; Corinna Thom, Gilley, & Tautz, 2003; von Frisch, 1967). Our results show that the colony can drastically reduce recruitment to an over-exploited food source in a rapid manner. In our experiments, there was a strong and immediate recruitment to the food source in the beginning of the post-recruit phase. This could be due to the constraints of the flight cage, which would lead to most foragers finding the food source rapidly. Under more natural conditions, this process can be expected to be longer, with a gradual increase in recruitment to a new food source.

Interestingly, in the experimental repeat in which foragers did not stop dancing immediately, the dynamics of the dances by foragers and recruits were different. Initially, most of the dances were by foragers, but they stopped dancing after a short duration. However, recruits drastically increased their dance activity later as compared to the foragers. They stopped dancing, but after a longer duration (possibly due to the larger number of recruits as compared to the foragers). These results suggest that there is an inherent negative feedback within foragers dancing for a food source which is linked to the increasing recruitment to the food source. Limited and decreasing dance activity by an individual is a prominent feature of the waggle dance behaviour for nest sites in honey bee swarms (Seeley, 2003; Seeley & Buhrman, 1999). Thus, the dance activity of honey bee foragers for a food source and honey bee scouts for a nest site are more similar than previously appreciated (Beekman, Gloag, Even, Wattanachaiyingchareon, & Oldroyd, 2008).

Future Directions

As a next step, similar experiments could be conducted with colonies foraging under natural conditions, and not in a flight cage. The results of these experiments would provide a more detailed understanding of how the regulation of foraging occurs under natural conditions. In addition, the effect of different feeder rewards on the dynamics of recruitment should be explored. A higher feeder reward would lead to an increase in the motivation of foragers to recruit for longer. At the same time, the increased recruitment to a higher feeder reward might decrease the motivation of foragers faster. The interaction between these two factors could be analysed to understand if foraging dynamics at any food source remains the same, irrespective of the reward offered.

Another dimension that can be explored is the effect of controlled increase in the number of recruits that join the forager group. These experiments would be similar to the removal experiments in chapter 3. A different individual identification process would be needed to tag individuals and observe them on the same day (as the current tagging process involves keeping foragers on ice). The results could indicate if an increase in the nectar influx also has an individual specific effect and further, which individuals are affected first.

References

Al Toufailia, H., Grüter, C., & Ratnieks, F. L. W. (2013). Persistence to Unrewarding Feeding Locations by Honeybee Foragers (Apis mellifera): the Effects of Experience, Resource Profitability and Season. *Ethology*, *119*(12), 1096–1106. https://doi.org/10.1111/eth.12170

Beekman, M., & Gilchrist, A. (2007). What makes a honeybee scout? *Behavioral Ecology and Sociobiology*, *61*, 985–995. https://doi.org/10.1007/s00265-006-0331-9

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? *Behavioral Ecology and Sociobiology*, *62*(8), 1259–1265. https://doi.org/10.1007/s00265-008-0554-z

Claus O. Wilke. (2018). cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2.” *R Package Version 0.9.3*. Retrieved from https://cran.r-project.org/package=cowplot

De Marco, R. J., & Farina, W. M. (2001). Changes in food source profitability affect the trophallactic and dance behavior of forager honeybees (Apis mellifera L.). *Behavioral Ecology and Sociobiology*, *50*(5), 441–449. https://doi.org/10.1007/s002650100382

Dunn, P. K., & Smyth, G. K. (2005). Series evaluation of Tweedie exponential dispersion models. *Statistics and Computing*, *15*(4), 267–280. https://doi.org/10.1007/s11222-005-4070-y

Dunn, P. K., & Smyth, G. K. (2008). Evaluation of Tweedie exponential dispersion model densities by Fourier inversion. *Statistics and Computing*, *18*(1), 73–86. https://doi.org/10.1007/s11222-007-9039-6

Fernandez, P. C., Gil, M., Farina, W. M., & Farina, P. C. F. M. G. W. M. (2003). Reward rate and forager activation in honeybees: recruiting mechanisms and temporal distribution of arrivals. *Behavioral Ecology and Sociobiology*, *54*(1), 80–87. https://doi.org/10.1007/s00265-003-0607-2

Gardner, K. E., Seeley, T. D., & Calderone, N. W. (2008). Do honeybees have two discrete dances to advertise food sources? *Animal Behaviour*, *75*(4), 1291–1300. https://doi.org/10.1016/j.anbehav.2007.09.032

George, E. A., & Brockmann, A. (2019). Social modulation of individual differences in dance communication in honey bees. *Behavioral Ecology and Sociobiology*, *73*(4), 41. https://doi.org/10.1007/s00265-019-2649-0

Kirchner, W. H., & Lindauer, M. (1994). The causes of the tremble dance of the honeybee , Apis mellifera. *Behavioral Ecology and Sociobiology*, *35*(5), 303–308. https://doi.org/https://doi.org/10.1007/BF00184419

Liang, Z. S., Nguyen, T., Mattila, H. R., Rodriguez-Zas, S. L., Seeley, T. D., & Robinson, G. E. (2012). Molecular determinants of scouting behavior in honey bees. *Science*, *335*(6073), 1225–1228. https://doi.org/10.1126/science.1213962

Mailleux, A.-C., Deneubourg, J.-L., & Detrain, C. (2003). Regulation of ants' foraging to resource productivity. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1524), 1609–1616. https://doi.org/10.1098/rspb.2003.2398

R Core Team. (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Seeley, T. D. (1982). Adaptive Significance of the Age Polyethism Schedule in Honeybee Colonies. *Behavioral Ecology and Sociobiology*, *11*, 287–293. https://doi.org/https://doi.org/10.1007/BF00299306

Seeley, T. D. (1983). Division of labor between scouts and recruits in honeybee foraging. *Behavioral Ecology and Sociobiology*, *12*(3), 253–259. https://doi.org/10.1007/BF00290778

Seeley, T. D. (1986). Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, *19*(5), 343–354. https://doi.org/10.1007/BF00295707

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. *Behavioral Ecology and Sociobiology*, *24*(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1994). Honey bee foragers as sensory units of their colonies. *Behavioral Ecology and Sociobiology*, *34*, 51–62. https://doi.org/10.1007/BF00175458

Seeley, T. D. (1995). *The Wisdom of the Hive*. *Harvard University Press*.

Seeley, T. D. (2003). Consensus building during nest-site selection in honey bee swarms: the expiration of dissent. *Behavioral Ecology and Sociobiology*, *53*(6), 417–424. https://doi.org/10.1007/s00265-003-0598-z

Seeley, T. D., & Buhrman, S. C. (1999). Group decision making in swarms of honey bees. *Behavioral Ecology and Sociobiology*, *45*(1), 19–31. https://doi.org/10.1007/s002650050536

Seeley, T. D., & Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? *Behavioral Ecology and Sociobiology*, *30*(1), 59–69. https://doi.org/10.1007/BF00168595

Sen Sarma, M., Esch, H. E., & Tautz, J. (2004). A comparison of the dance language in Apis mellifera carnica and Apis florea reveals striking similarities. *Journal of Comparative Physiology A*, *190*(1), 49–53. https://doi.org/10.1007/s00359-003-0470-7

Smyth, G. K. (2002). An efficient algorithm for reml in heteroscedastic regression. *Journal of Computational and Graphical Statistics*, *11*(4), 836–847. https://doi.org/10.1198/106186002871

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *Journal of Experimental Biology*, *206*(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Thom, C, Seeley, T. D., & Tautz, J. (2000). A scientific note on the dynamics of labor devoted to nectar foraging in a honey bee colony: number of foragers versus individual foraging activity. *Apidologie*, *31*(6), 737–738. https://doi.org/10.1051/apido:2000158

Thom, Corinna, Gilley, D. C., & Tautz, J. (2003). Worker piping in honey bees (Apis mellifera): The behavior of piping nectar foragers. *Behavioral Ecology and Sociobiology*, *53*(4), 199–205. https://doi.org/10.1007/s00265-002-0567-y

Venables, W. N., & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4th ed.). New York: Springer-Verlag. Retrieved from http://www.stats.ox.ac.uk/pub/MASS4 von Frisch, K. (1967). *The dance language and orientation of bees*. *Harvard University Press*. Harvard University Press.

Wells, H., & Wells, P. H. (1986). Optimal Diet, Minimal Uncertainty and Individual Constancy in the Foraging of Honey Bees, Apis mellifera. *The Journal of Animal Ecology*, *55*(3), 881–891. https://doi.org/10.2307/4422

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. *Springer International Publishing* (2nd ed.). Springer. https://doi.org/10.1007/978-3-319-24277-4

Chapter

A2

Followers

Manuscript in preperation

Chapter A2 – Followers

Introduction

Communication between group members plays a key role in determining the structure and dynamics of social groups (Duboscq, Romano, MacIntosh, & Sueur, 2016; Morand-Ferron, Doligez, Dall, & Reader, 2010; Pruitt & Goodnight, 2014). Individuals in the group can obtain information from other members through cues or signals (Chittka & Leadbeater, 2005; Conradt & Roper, 2003). Information transfer through interactions might be of particular importance in large groups (Kao, Miller, Torney, Hartnett, & Couzin, 2014; King & Cowlishaw, 2007) like social insect colonies. In these colonies, the private information available to workers is limited to knowledge about stimuli at a local scale, and not at the colony level. Workers rely on interactions with other workers to obtain information which then modulates their behavioural responses (Kocher & Cocroft, 2019; Leonhardt, Menzel, Nehring, & Schmitt, 2016).

One of the most elaborate modes of communication in social insects is the honey bee waggle dance (von Frisch, 1967). Foragers returning from rewarding food sites use the waggle dance to recruit other nest mates to these food sites. The waggle dance is a unique recruitment mechanism amongst social insects because it encodes spatial information about the food source (Jarau & Hrncir, 2009). Each waggle dance consists of multiple waggle runs, and the direction and distance information are encoded in each run. One waggle run typically contains two phases; a straight walking phase in which the dancer waggles its abdomen back and forth (the waggle phase) and a circular walking phase which brings the dancer back towards the point of origin of the first phase (the return phase). The duration of the waggle phase corresponds to the distance to the food source (Seeley, 1995; von Frisch, 1967). In the European honey bee species, *Apis mellifera*, the angle that the waggle phase makes with respect to the vertical (gravity) axis corresponds to the direction of the food source from the hive with respect to the sun's azimuth (von Frisch, 1967). Further, the duration of the return phase corresponds to the reward value of the food source as perceived by the forager (Seeley, Mikheyev, & Pagano, 2000). For food sources very close to the hive, the waggle run becomes nearly circular with a very short waggle phase (Gardner, Seeley, & Calderone, 2008).

Cues and signals from the environment and nest mates can modulate the dance behaviour of foragers (De Marco & Farina, 2001; Seeley, 1989; Seeley et al., 2000). Interactions with nest mates inform nectar foragers about the colony food stores and the nectar influx into the colony (De Marco, 2006; Farina & Núñez, 1991; Grüter & Farina, 2009; Seeley, 1989). In addition, interactions with other foragers can also provide information about predation and overcrowding (Nieh, 2010; Moushumi Sen Sarma, Fuchs, Werber, & Tautz, 2002; Tan et al., 2016; Thom, 2003). An individual forager's dance activity is modulated by information from these interactions along with the perceived reward value of the food source (George & Brockmann, 2019). This in turn drives recruitment to each food source proportional to its relative reward value for the colony (Seeley & Towne, 1992). Thus, the waggle dance acts as the primary regulatory mechanism of the colony's recruitment in addition to its role in navigational information transfer.

Although extensive work has been done on the honey bee waggle dance behaviour, the exact mechanism underlying the spatial information transfer has remained elusive. There are currently two major hypotheses regarding how dance followers obtain meaningful spatial information from the dancer. The first ("tactile hypothesis") posits that dance followers obtain information from cues that a dancer produces. Followers can obtain information from mechanosensory cues associated with the air flow caused by a dancer's vibrating wings (Michelsen, 2003; Michelsen, Kirchner, & Lindauer, 1986). Tactile cues associated with physical contact between the dancer and follower could also be a viable mode of information transfer (Božič & Valentinčič, 1991; Gil & De Marco, 2010). The second hypothesis states that followers obtain information from dancers by physically following behind the dancer (Judd, 1994). Followers thus use information from their own body positions to estimate the direction and distance of the food source being advertised ("follow hypothesis").

So far, there has been limited evidence in support of each of the two hypotheses (Gil & De Marco, 2010; Judd, 1994). The major problem is that, recruits can follow multiple dances before flying out to a food source (Seeley & Towne, 1992). A carefully controlled experimental paradigm is required to distinguish between the two hypotheses. This would involve a limited number of foragers from a naturally foraging colony trained to, and dancing for, multiple novel food source. Under such an experimental condition, a detailed quantification of the dances followed by recruits preceding their first visit to the novel food sources would provide a greater understanding of the mechanism behind the spatial information transfer.

Previous studies have also been mostly limited to observations of *Apis mellifera* and have rarely focussed on the other *Apis* species, even though the dance behaviour is a common recruitment mechanism used by all extant species in the whole genus (Fred C. Dyer, 2002). But there are important differences in some of the cues and signals present in the dances of the various species (Fred C. Dyer, 2002; I'Anson Price & Grüter, 2015; Towne, 1985). The dwarf honey bees (like *A. florea*) dance on a horizontal surface with conspicuous visual cues and no auditory cues associated with the waggle dance (F. C. Dyer, 1985). In contrast, the giant honey bees (like *A. dorsata* and *A. laboriosa*) usually dance on the vertical surface of the bee curtain (Towne, 1985). The dances of *A. dorsata* also contain auditory cues and are more similar to dances by cavity nesting species (Kirchner & Dreller, 1993). Like *A. mellifera*, other cavity nesting species (*A. cerana* and *A. nigoracincta*) also perform waggle dances in the dark which are accompanied by auditory cues to help the dance followers identify dancing bees (Towne, 1985). However, it is very likely that, even though cues and signals involved in the waggle dance differ amongst species', the mechanism of information transfer would likely remain the same (F. C. Dyer & Seeley, 1991; M Sen Sarma, Esch, & Tautz, 2004).

In this study, we propose a comparative approach to understand the mechanism of spatial information transfer in the waggle dance behaviour of honey bees. Comparing the spatial orientations of dance followers across species may provide supporting evidence for either of the two proposed hypotheses. If followers arrange themselves towards the side of the dancer, then it is likely that they use tactile cues (tactile hypothesis) to obtain information about the food source. In contrast, if followers orient themselves behind the dancer, they are more likely using their own body orientation (follow hypothesis) to determine the spatial position of the food source.

Materials and Methods

We observed the behaviour of waggle dance followers as part of a larger study on the effect of varying optic flow environments on dance dialects of different species in the genus *Apis* (Kohl *et al.*, unpublished data).

Colonies

Experiments were done with commercially obtained colonies of *Apis cerana* and wild colonies of *Apis florea* and *Apis dorsata*. The *A. cerana* colony was obtained from Karnataka Apiaries, Bangalore. For *A. florea*, we obtained a colony which was nesting on the National Centre for Biological Sciences (NCBS) campus, Bangalore. For *A. dorsata*, we observed a colony which had made a nest in the Naik Bhavan building adjacent to our experimental location in the University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bangalore. All the colonies we observed were healthy colonies, with an adequate number of brood cells.

Experimental Location

Our experiments were done in the Botanical Garden at GKVK (latitude: 13.07, longitude: 77.57). The garden covers an area of 26 hectares and is home to around 2000 plant species including trees and shrubs. This provided dense vegetation cover and hence good optic flow for the foragers (Fig A2.1 *a*). We obtained a straight line transect of at least 500m from the colony location to train foragers along.

Colony Preparation

Apis florea

Our *A. florea* colony was a wild colony located in a tree on the NCBS campus. We shifted this colony to the first experimental site, the Botanical Garden. First, we carefully cut the branch on which the colony rested. We then transported the colony inside a net to the botanical garden. Finally, at the botanical garden, we transferred it to a wooden box, with metal wires such that the branches could be held by the metal wires (Fig A2.1 *b*). The colony thus was suspended in between the wooden box, which had one opening at the top. This opening was used to record the activity on the crown area of the colony, where most of the recruitment activity happens (F. C. Dyer, 1985).

Figure A2.1

Figure A2.1: *(a)* A photo of the botanical garden in GKVK, Bangalore, where the experiments were performed with all the 3 species. This image is at distance of 200m from the hive in the transect used for both *A. florea* and *A. cerana*. *(b)* The box in which *A. florea* colonies were kept during the feeder training and dance observations. Foragers utilised the crown area of the comb as the dance floor for recruitment of the nest mates. *(c)* The feeder box used to train *A. dorsata* foragers. *(d)* The observation hive used for housing the *A. cerana* colony (picture courtesy Patrick Kohl). The observation hive had a glass wall on the side towards the entrance, which allowed us to record dances happening in the first frame (which acted as the dance floor).

Apis dorsata

We found a naturally nesting *A. dorsata* colony on a building adjacent to the entrance of the botanical garden. We used a feeder box (Fig. A2.1 *c*) to train foragers from the colony to the ground first, and then along the transect.

Apis cerana

We obtained a 4 frame *A. cerana* colony which we then transferred to the botanical garden. The colony was housed in a specially constructed box, with glass walls on one side (Fig. A2.1 *d*). This allowed us to observe the waggle dances and the dance follower behaviour in a non-invasive manner and without having to move the colony into a traditional 2 frame vertical observation hive.

Distance training experiments

Apis florea and *Apis cerana*

We used a similar protocol for distance training in *A. florea* and *A. cerana*. We first placed an artificial feeder (which consisted of a feeder plate and stand) near the colony location and filled it with 1-1.5 M sucrose solution scented with star anise (*Illicium verum*) extract. We then used a stick dipped in this solution and brought it near the colony (towards the crown area for *A. florea* and towards the colony box entrance for *A. cerana*) to attract foragers. Once some foragers climbed on to the stick, we would then transfer them carefully to the artificial feeder. This process was repeated multiple times till 5-10 foragers started coming to the feeder on their own. At this point, the feeder was shifted away from the hive, along the transect, in small steps of 5m. When the feeder was at 25m, we individually paint marked foragers at the feeder using Uni POSCA Paint markers (Uni Mitsubishi Pencil, UK). Once all foragers coming to the feeder were marked, the feeder was shifted further in small steps. At 100m, we recorded dance activity using a Sony HDR CX260V Handycam (Sony Corporation, Tokyo) to cover the dance floor area (crown area in *A. florea* and the frame facing the glass wall in the box containing *A. cerana*). The videos were recorded at 1080p and 50 frames per second. During dance activity recordings, one observer was always present at the feeder, noting down the number of trips made by each marked forager. After an hour of observations, the feeder was shifted to the next distance and recordings were done in a similar manner. We recorded dance activity at 100m, 200m, 300m, 400m and 500m for both species.

The distance training experiments were done during a period of relative food scarcity, such that foragers from both species were attracted to the sucrose solution that we provided. We adjusted the sucrose concentration at our artificial feeder between 1 and 1.5M depending on the number of foragers visiting our food source.

Apis dorsata

The training of *A. dorsata* foragers initially involved a different protocol. Since the colony was on the side of a building, we could not keep an artificial feeder nearby and use a stick dipped in sucrose to train foragers. Instead, we first got foragers to come to a long rod with a piece of comb filled with sucrose solution which was kept next to the bee curtain. We then transferred these foragers carefully into a feeder box. Once foragers got inside the box, it was closed, and the box along with the foragers inside were transported to the ground level. Here, the box was placed in an artificial feeder set up and foragers were released. This process was repeated till foragers came to the feeder on their own. After this, the same protocol of paint marking, feeder shifts, and video recordings were done as described earlier. We recorded the dance activity of *A. dorsata* foragers at distances of 100m, 200m, 300m and 400m. Since we recorded dances for the *A.* dorsata colony during a time of plentiful flowering, we had to use higher concentrations of sucrose solution (up to 2M) to get foragers to come to the feeder. But we could not get foragers to come to a feeder further than 400m from the hive. The transect used for *A. dorsata* feeder training was in the opposite direction of the ones used for *A. florea* and *A. cerana* since the colony had nested on a building at the opposite end of the botanical garden.

Video Analysis

To analyse follower behaviour, individuals were first shortlisted based on whether they were active at the feeder at multiple distances. We then analysed each waggle run in the dances by these individuals to determine the duration of the waggle phase. The videos were observed frame-by-frame in Virtual Dub 1.10.4

(http://www.virtualdub.org). The first frame in which a focal bee clearly moved its abdomen laterally or dorsoventrally was defined as the start of the waggle run in that circuit. The frame in which the bee stopped waggling its abdomen and started turning to the left or right was defined as the end of the waggle run. The time between the above frames is the duration of the waggle phase.

Follower Behaviour

We analysed dance follower behaviour for each waggle run in each waggle dance and did not observe the behaviour of dance attendants. Dance followers were defined as those bees which positioned themselves within one bee length of the dancer while we excluded dance attendants, who were farther away and not following the dancer (Božič & Valentinčič, 1991). In each run, we focussed on 3 phases (time-points); the start, middle and end, based on the waggle run duration we had calculated (Fig A2.2 *a*). This was done to look at whether there was a change in the number of followers in the various zones as the run progressed, which might indicate that followers were shifting to a preferable zone or maintaining their positions (Gil & De Marco, 2010). The number of follower present around the dancing forager was counted in each of the frames. We then divided these followers into 3 groups, based on the position they occupied around the dancer (Gil & De Marco, 2010). Followers positioned around the head region were grouped into the anterior zone, followers occupying positions near the thorax and abdomen on both sides were grouped into the lateral zone and finally followers positioned behind the abdomen were grouped into the posterior zone (Fig A2.2 *b*).

The body angles of the follower in each of the positions with respect to the angle of the dancer were also quantified. We used a free software, OnScreenProtractor v0.3 (http://osprotractor.sourceforge.net), to do this manually. We first used the program to make a vector representing the dancer, pointing in the direction of the dancer's head, away from its abdomen. We then made a similar vector for the follower, but from its head to abdomen. Finally, the angle subtended by the follower vector with respect to the dancer vector in the clockwise direction was quantified (Fig A2.2 *c*).

Figure A2.2

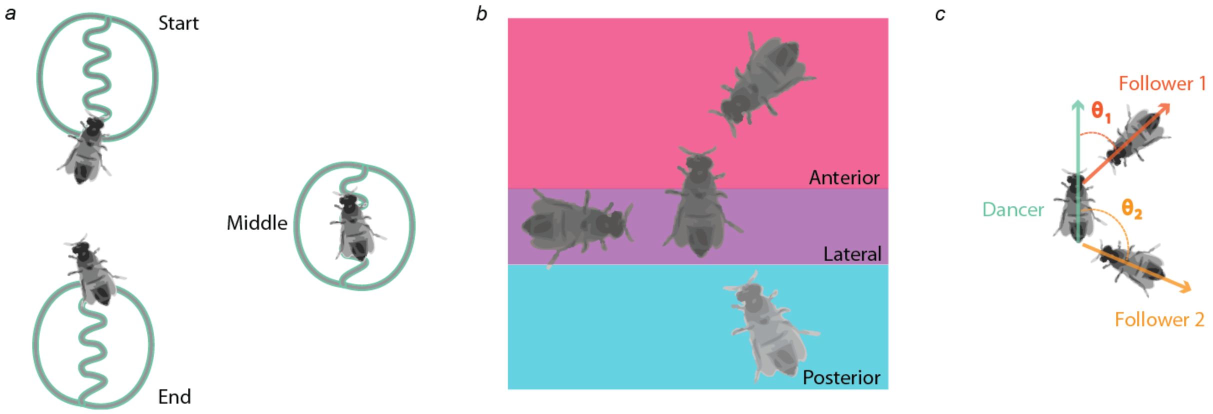

Figure A2.2: Schematic of the analysis done on the dance followers. *(a)* Each waggle run was divided into 3 phases, the start, middle and end and the number of followers around the dancer was quantified. *(b)* Followers around the dancer were grouped into 3 zones (Anterior, Lateral and Posterior) based on the position they occupied around the dancer (area of the zones in the figure are representative). *(c)* The orientations of the followers were then quantified by obtaining the angle made by a vector representing the follower with respect to the vector representing the dancer in the clockwise direction.

In all, we calculated the position and body angles of 1024 followers (from 99 waggle runs from 14 waggle dances) in *A. florea*, 1363 followers (from 126 waggle runs from 7 waggle dances) in *A. dorsata* and 787 followers (from 100 waggle runs from 10 waggle dances) in *A. cerana* (Table A2.1).

Table A2.1

Distance (m)	***Apis florea***			***Apis dorsata***			***Apis cerana***		
	Bees	**Dances**	**Waggle Runs**	**Bees**	**Dances**	**Waggle Runs**	**Bees**	**Dances**	**Waggle Runs**
100	2	3	20	1	1	53	2	3	33
200	1	2	14	2	2	27	1	2	21
300	2	3	19	2	2	28	2	2	14
400	3	3	23	1	2	18	1	1	8
500	2	3	23	-	-	-	2	2	24

Table A2.1: List of bees, dances and waggle runs analysed for each of the 3 *Apis* species in this study.

Statistical Analysis

Number of Followers

We used a model comparison approach to analyse the number of followers. We built generalised linear mixed models with a Poisson error distribution as our response variable (number of followers) was count data. In each model, the bee ID was a random effect. In total, 27 models were built (Table A2.2) with a combination of our 4 predictors: i) zone of dance follower (a categorical variable of 3 levels; Anterior, Lateral and Posterior), ii) phase of waggle run (a categorical variable of 3 levels; Start, Middle and End), iii) species (a categorical variable of 3 levels; *A. florea*, *A. cerana* and *A. dorsata*) and iv) distance (a continuous variable which was scaled). We then compared the models based on their AIC values and shortlisted the models within a cut-off of 0.95 cumulative Akaike (Wagenmakers & Farrell, 2004). We then obtained the list of predictors, effect sizes and confidence intervals from the model. Only those predictors which had confidence intervals not overlapping zero were considered to have a significant effect on the number of followers. We also did multiple comparisons (with Tukey corrections for multiple comparisons) of the estimated mean number of followers. We focussed on: i) comparison between the 3 waggle run phases, ii) comparisons between the different zones within each species and iii) comparisons between the same zones across the 3 species. We did these comparisons as in our shortlisted model, the important predictors were the waggle run phase and an interaction between the zone of the follower and the species.

Table A2.2

Model Number	**Zone**	**Waggle Run Phase**	**Species**	**Distance**
1	+			
2		+		
3			+	
4				+
5	+	+		
6	+		+	
7	+			+
8		+	+	
9		+		+

10			+	+
11	*	*		
12	*		*	
13	*			*
14		*	*	
15		*		*
16			*	*
17	+	+	+	
18	*	*	+	
19	*	+	*	
20	+	*	*	
21	+	+	+	+
22	*	*	+	+
23	*	+	*	+
24	*	+	+	*
25	+	*	*	+
26	+	*	+	*
27	+	+	*	*

Table A2.2: List of models used in the model selection and model averaging. All models had the number of followers as the response and were generalized linear mixed models with the bee ID as a random effect and a Poisson error distribution. The predictors present in each model are either the zone of the follower, the waggle run phase, the species, the distance or a combination thereof. A + indicates that the predictor was encoded in the model without an interaction term, whereas a * indicates that two predictors in the model were encoded with an interaction term between them. Only 2-way interactions were included in the models.

Orientation of followers

We used a projected normal (PN) circular generalized linear model to analyse the data on the angles of the followers (Cremers & Klugkist, 2018). The GLM fits circular data (follower angles) to a PN distribution. We fit 3 predictors in the model: i) phase of waggle run (a categorical variable of 3 levels; Start, Middle and End), ii) species (a categorical variable of 3 levels; *A. florea*, *A. cerana* and *A. dorsata*) and iii) distance (a continuous variable). The PN GLM is a Bayesian model with a Markov Chain Monte Carlo sampler (Gelman et al., 2013). The sampler was run for 10000 iterations with a burn-in phase of 1000 iterations. Estimates obtained from every 3 runs were used to obtain the posterior distribution. We used the 95% Highest Posterior Density (HPD) interval to determine if the circular means of any of the levels of these factors were significantly different from each other.

All the models and the plots were made in R (R Core Team, 2018) using the RStudio IDE (RStudio Team, 2016). The GLMMs were fit using the lme4 package (Bates, Mächler, Bolker, & Walker, 2015), model selection and averaging were done using the MuMIn package (Barton, 2017) and the model assumptions were checked using the DHARMa package (Florian Hartig, 2018). Multiple comparisons were done using the emmeans package (Lenth, 2019). For fitting the PN GLM, the bpnreg package was used (Cremers, 2018).

Results

Number of Followers

Model comparisons between all the GLMMs revealed that there was only one model at the 0.95 cut-off level for cumulative sum of Akaike weights (Table A2.3). In this model, the important predictors were the waggle run phase and an interaction between the species and the zone of the follower (Table A2.4).

Table A2.3

Model Number	**Degrees of Freedom**	**Log Likelihood**	**AICc**	**Delta AICc**	**Weight**
19	*12*	*-3711.613*	*7447.3*	*0.00*	*0.721*
23	13	-3711.594	7449.3	1.98	0.268
12	10	-3718.003	7456.1	8.75	0.009
18	12	-3718.969	7462.0	14.71	0.000

Table A2.3: List of the top four models based on their AICc values for the analysis on the number of followers. For each model, the model number (see Table A2.2 for predictors), the degrees of freedom, log likelihood, AICc, delta AICc and the weight is provided. Only model 19 (highlighted in italics) was present in the final shortlist of models based on a cut-off value of 0.95 of the cumulative sum of weights.

Table A2.4

Predictors			**Effect Size**	**CI**	***p* value**
Zone*	**Waggle Run Phase**	**Species***			
Anterior	*Start*	*A. florea*	*0.833*	*0.715 – 0.952*	*< 0.001*
Lateral	Start	*A. florea*	0.107	-0.051 – -0.051	0.184
Posterior	*Start*	*A. florea*	*0.483*	*0.311 – 0.311*	*< 0.001*
Anterior	*Start*	*A. dorsata*	*0.216*	*0.062 – 0.062*	*0.006*
Anterior	*Start*	*A. cerana*	*-0.192*	*-0.337 – -0.047*	*0.009*
Anterior	*Middle*	*A. florea*	*0.133*	*0.042 – 0.223*	*0.004*
Anterior	*End*	*A. florea*	*0.153*	*0.062 – 0.245*	*0.001*
Lateral	Start	*A. dorsata*	0.176	-0.053 – 0.404	0.131
Posterior	*Start*	*A. dorsata*	*-0.405*	*-0.640 – -0.171*	*0.001*
Lateral	Start	*A. cerana*	0.065	-0.149 – 0.278	0.553
Posterior	*Start*	*A. cerana*	*-0.233*	*-0.459 – -0.006*	*0.044*

Table A2.4: List of predictors in the shortlisted model 19 for the analysis of the number of followers. For each predictor level in the final model, the effect size, confidence interval and p value associated with the effect size are given. The predictors marked with an * were present as an interaction term in the model. Hence the final model contained effect sizes of all combinations of levels of the 2 predictors (3 zones x 3 species = 9 combinations) as well as effect sizes of each level of the waggle run phase (3 levels). Effect sizes with confidence intervals not overlapping zero are highlighted in italics.

Effect of waggle run phase

The waggle run phase had an effect on the number of followers, but this effect was the same across the different zones, species and distance (Table A2.4, Fig. A2.3 and A2.4). There were fewer followers in the start of the waggle run as compared to the middle (Start vs Middle: difference estimate = -0.133, z ratio = -2.872, *p* = 0.011) and end phase (Start vs End: difference estimate = -0.53, z ratio = -3.292, *p* = 0.003). However, the number of followers in the middle and end phase were not significantly different (Middle vs End: difference estimate = -0.021, z ratio = -0.432, *p* = 0.902).

Effect of species and zone

The species and the zone around the dancer had an interactive effect on the number of followers (Table A2.4, Fig. A2.3 and A2.4) and hence their main effects are not considered. Within each species, there were differences in the number of followers in the different zones. In *A. florea*, there were more followers in the posterior zone as compared to both the anterior and the lateral zone (Anterior vs Posterior: difference estimate = -0.483, z ratio = -5.507, $p < 0.001$; Lateral vs Posterior: difference estimate = -0.376, z ratio = -4.188, $p < 0.001$). The number of followers in the anterior and lateral zone were similar (Anterior vs Lateral: difference estimate = -0.107, z ratio = -1.328, $p = 0.379$).

In *A. dorsata*, there were more followers in the lateral zone as compared to both the anterior and the posterior zone (Anterior vs Lateral: difference estimate = -0.283, z ratio = -3.348, $p = 0.002$; Lateral vs Posterior: difference estimate = 0.205, z ratio = 2.379, $p = 0.046$). The number of followers in the anterior and posterior zone were similar (Anterior vs Posterior: difference estimate = -0.078, z ratio = -0.957, $p = 0.604$).

In *A. cerana*, the number of followers were similar between the Anterior and Lateral zone (Anterior vs Lateral: difference estimate = -0.172, z ratio = -2.328, $p = 0.052$) and the Lateral and Posterior zone (Lateral vs Posterior: difference estimate = -0.079, z ratio = -0.998, $p = 0.578$). However, the number of followers in the Posterior zone were higher than in the Anterior zone (Anterior vs Posterior: difference estimate = -0.250, z ratio = -3.321, $p = 0.003$).

In each zone there were differences in the number of followers across the species. In the Anterior zone, *A. dorsata* had higher number of followers than both *A. florea* and *A. cerana* (*A. florea* vs *A. dorsata*: difference estimate = -0.216, z ratio = -2.748, $p = 0.017$; *A. dorsata* vs *A. cerana*: difference estimate = 0.409, z ratio = 5.472, $p < 0.001$). *A. florea* had more followers than *A. cerana* in the Anterior zone (difference estimate = 0.192, z ratio = 2.600, $p = 0.025$).

Figure A2.3

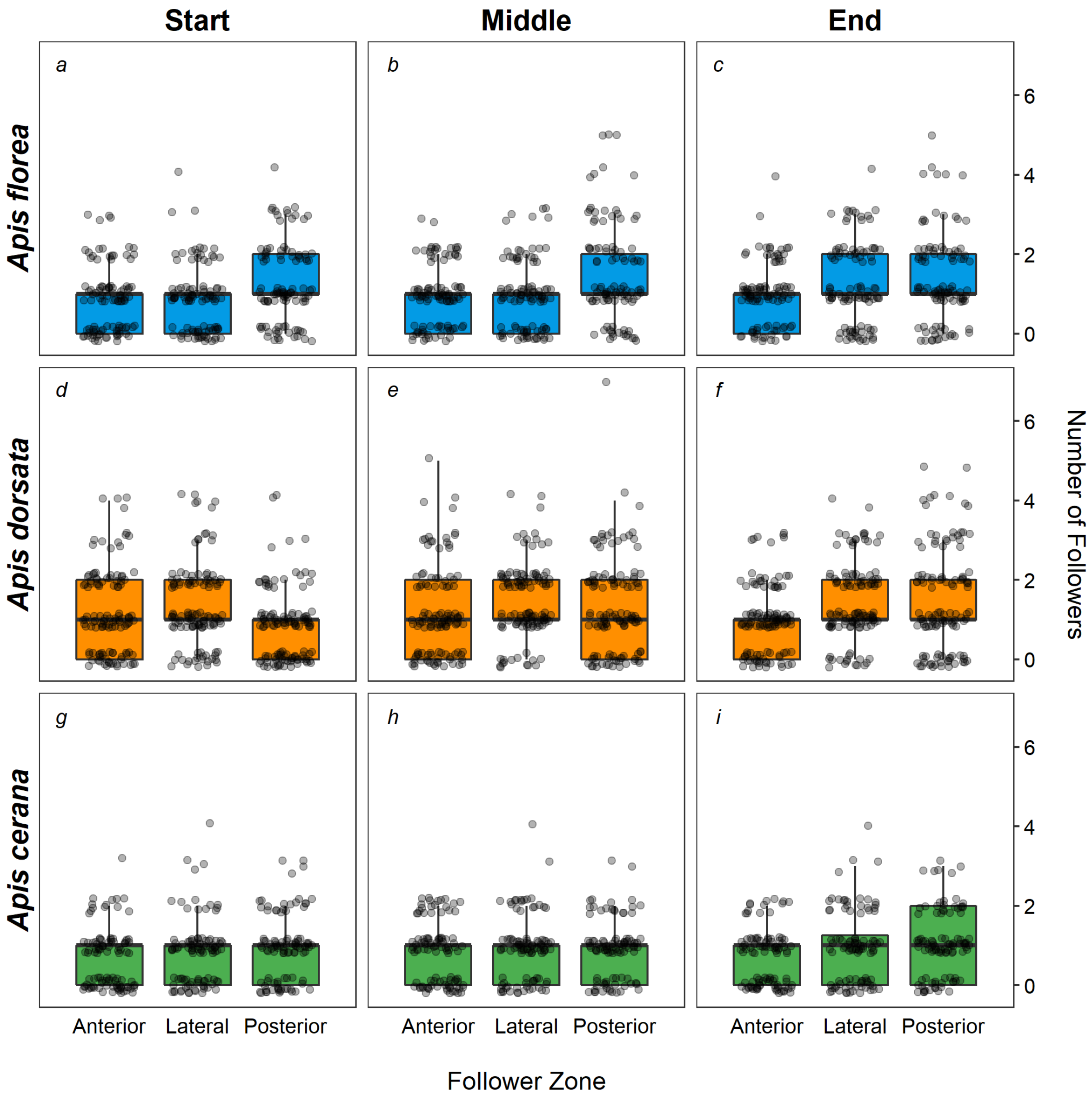

Figure A2.3: Boxplots (with the median and the quartile ranges) representing the number of followers present in each zone over the 3 phases of the waggle run for all 3 species. Each circle represents the number of followers present in one waggle run. The boxplots are filled according to the species, with blue representing *A. florea*, orange representing *A. dorsata* and green representing *A. cerana*.

Figure A2.4

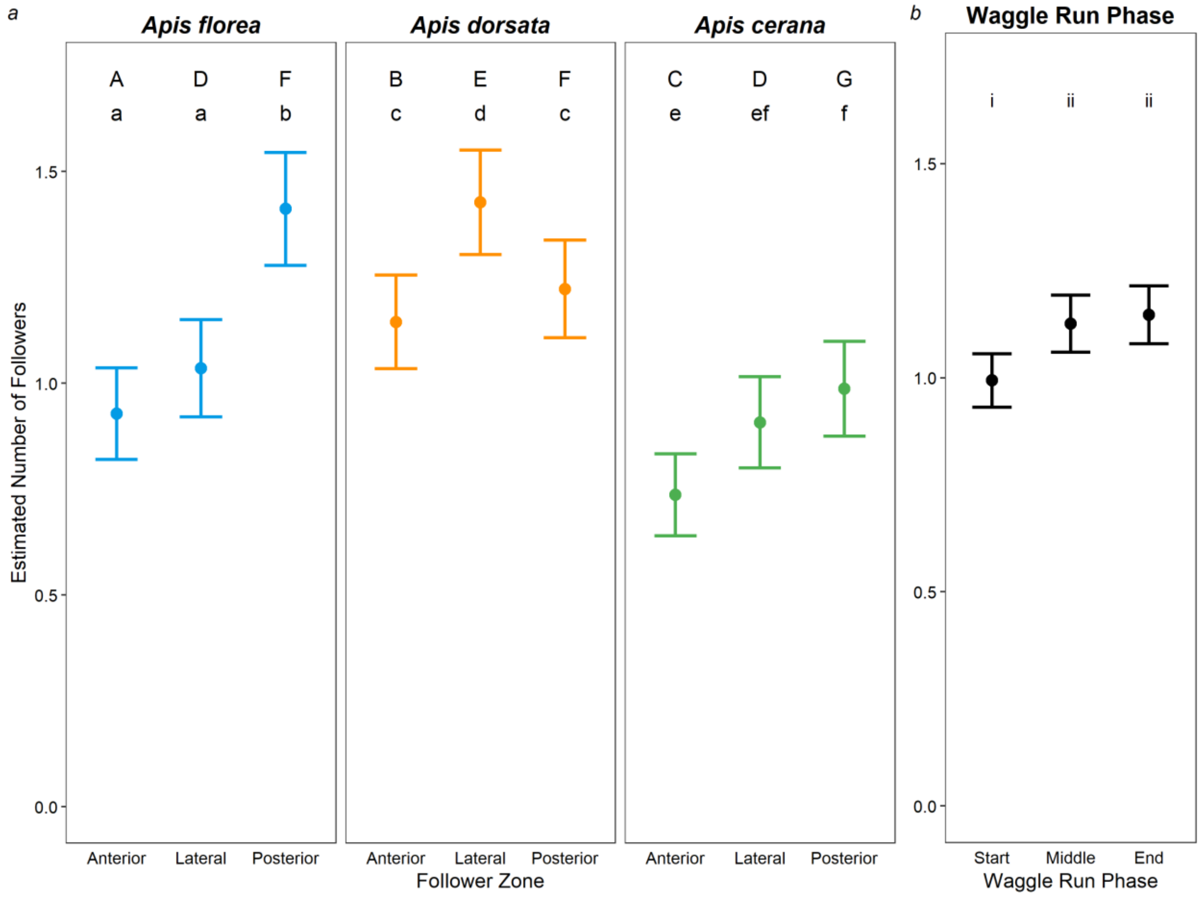

Figure A2.4: Estimated number of followers and the 95% confidence intervals (circles and error bars) for the different predictors present in the final shortlisted model (model 19, see Table A2.2 and A2.3). *(a)* Species and the zone of the follower had an interactive effect on the number of followers. The general pattern of increasing number of followers from Anterior to Posterior of the dancer was the seen in both *A. florea* (blue) and *A. cerana* (green). However, in *A. dorsata* (orange) more followers were present in the lateral zone. The alphabets above each circle represents results from the multiple comparisons done (estimates with different alphabets were significantly different from each other at the $p < 0.05$ level). Upper case alphabets represent differences in the number of followers present in the same zone across species. Lower case alphabets represent differences in the number of followers present in the different zones within each species. *(b)* The waggle run phase had an effect on the number of followers. The number of followers initially increased but then saturated as the waggle run progressed (different roman numerals above the estimates represent significant differences at the $p < 0.05$ level).

In the Lateral zone also, *A. dorsata* had higher number of followers than both *A. florea* and *A. cerana* (*A. florea* vs *A. dorsata*: difference estimate = -0.392, z ratio = -4.563, $p <$

0.001; *A. dorsata* vs *A. cerana*: difference estimate = 0.520, z ratio = 6.226, $p < 0.001$). However, both *A. florea* and *A. cerana* had similar number of followers in this zone (difference estimate = 0.128, z ratio = 1,591, $p = 0.249$).

In the Posterior zone, *A. dorsata* had similar number of followers as *A. florea* (difference estimate = 0.189, z ratio = 2.100, $p = 0.090$) and but more followers than *A. cerana* (difference estimate = 0.236, z ratio = 2.882, $p = 0.011$). *A. florea* had more followers than *A. cerana* in this zone (difference estimate = 0.425, z ratio = 4.775, $p < 0.001$).

Effect of distance

Distance had no effect on the number of followers (Fig. A2.5). Distance was not a predictor that was present in the short-listed models. Thus, the main effects of the waggle run phase and the interactive effects of zone and species were similar across all distances.

Orientation of followers

The circular means of the orientation of the followers were not affected by either of our 3 predictors (Table A2.5, Fig. A2.6 and A2.7). The HPD intervals of the circular means were similar across all phases of the waggle run (Table A2.5 and Fig. A2.7). The HPD intervals of the circular means overlapped for all 3 species (Table A2.5 and Fig. A2.7).

Table A2.5

Predictors		**Mean**	**Mode**	**SD**	**LB HPD**	**UB HPD**
Waggle Run Phase	**Species**					
Start	*A. florea*	65.3991	72.29328	95.45285	-115.486	211.0482
Middle	*A. florea*	39.2624	45.82657	76.07739	-107.925	200.3821
End	*A. florea*	55.64611	56.72581	60.74465	-81.4719	190.5595
Start	*A. dorsata*	136.8125	139.1147	29.44819	75.82887	193.3142

Start	*A. cerana*	-30.7599	-42.0799	86.10036	-172.861	131.9149
Middle	*A. dorsata*	126.8412	126.8954	32.46732	59.05223	192.6481
Middle	*A. cerana*	-14.5129	-20.2491	67.69122	-169.829	121.4971
End	*A. dorsata*	118.7927	113.1987	26.07451	66.15804	169.251
End	*A. cerana*	13.27277	28.43403	66.18634	-135.017	152.2358

Table A2.5: Results of the categorical predictors in projected normal GLM model with the follower angles as the response variable. Posterior estimates of the circular means of the phase difference are provided, along with the mode, standard deviation and the lower and upper bound of the 95% highest posterior density interval. None of the circular means were significantly different from each other, as the bounds around the mean overlapped in all 9 combinations of the 2 categorical predictors.

The intervals around the slope of the relationship between distance and the projected normal distribution of the angles also overlapped 0, indicating that there was no change in the circular means of the followers with distance (mean of the average slope posterior samples of the circular regression = 7.859, 95% HPD interval = -5.998 – 8.064).

Discussion

The results from our study revealed that there were differences between how the dance followers positioned themselves amongst the 3 species of *Apis*. In *A. florea* more followers positioned themselves behind the dancer, whereas in *A. dorsata*, more followers positioned themselves to the sides of the dancer (see Fig. A2.4). In *A. cerana* there were no significant differences between the number of followers who positioned themselves to the side and those who positioned themselves behind the dancer. This trend was the same across all phases of the waggle run and across all distances. There were also less followers in the beginning and more followers towards the middle and the end of the waggle run across all species. But the mean orientation of the dance followers was not different across species, waggle run phase or distance.

Figure A2.5

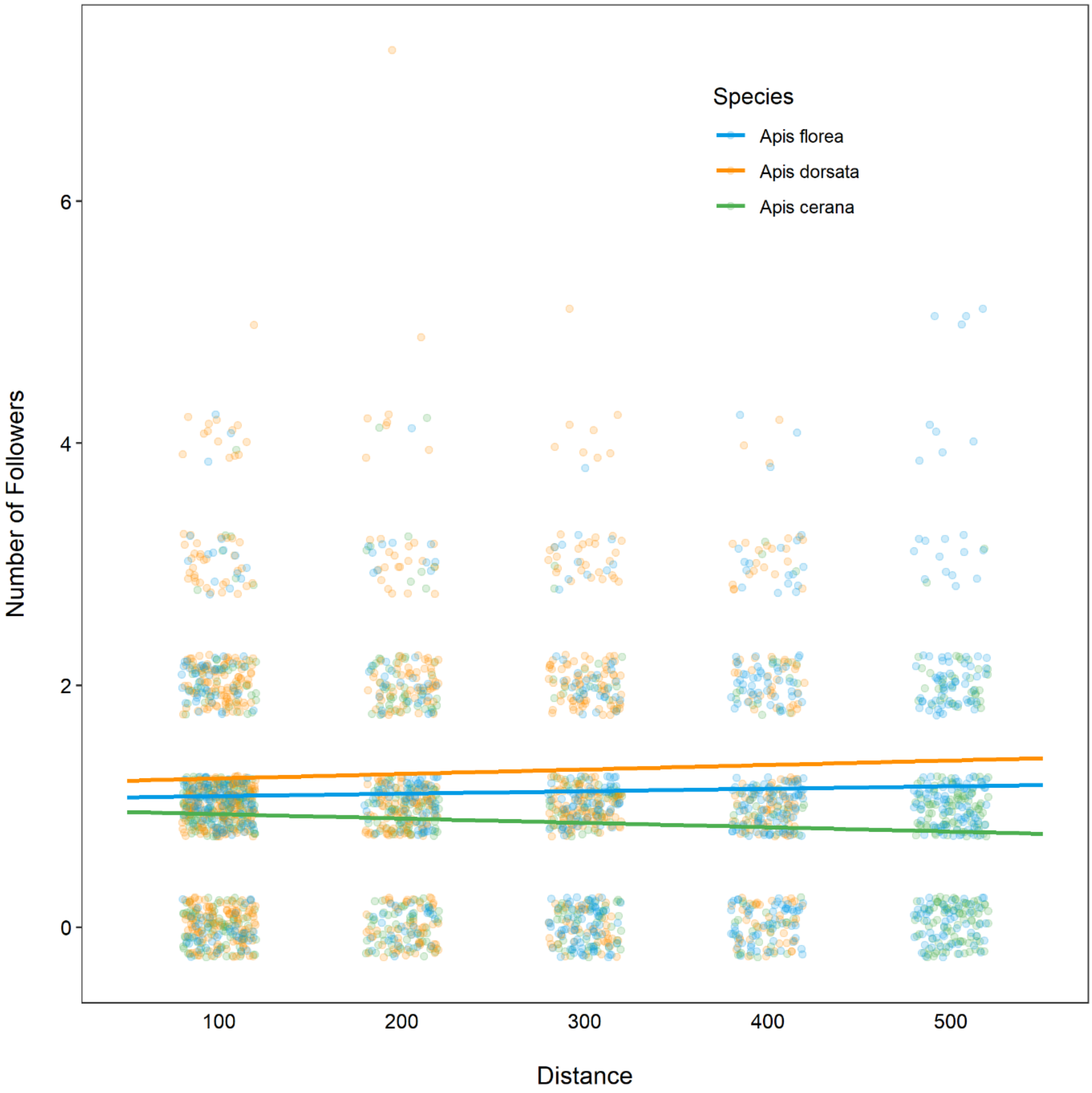

Figure A2.5: Correlation between distance and the number of followers in all 3 species. The line represents the best fitting linear correlation with each circle representing data from one run. The lines and circles are coloured according to the species, with blue for *A. florea*, orange for *A. dorsata* and green for *A. cerana*. There was no effect of distance on the number of followers in a waggle run in either of the 3 species, as can be seen from the horizontal lines.

Studies on the honey bee waggle dance, and specifically on the mechanism of information, transfer have focused on *A. mellifera* so far (Gil & De Marco, 2010; Landgraf et al., 2018; Seeley, 1995; von Frisch, 1967). However, various aspects of waggle dance differs amongst the various species in the genus *Apis* (F. C. Dyer & Seeley, 1991; Fred C. Dyer, 2002; I'Anson Price & Grüter, 2015; Towne, 1985). The dwarf honey bees (like *A. florea*) and the giant honey bees (like *A. dorsata*) dance in the open and have access to celestial cues while dancing, whereas the cavity nesting species (like *A. cerana* and *A. mellifera*) dance in the dark (Fred C. Dyer, 2002). However, the dwarf honey bees dance on a horizontal or sloped surface, whereas the giant honey bees and the cavity nesting bees dance on a vertical surface (Fred C. Dyer, 2002). The cues and signals associated with the dance are also different between the various species. For example, in *A. mellifera*, the dancer vibrates its wings during the waggle run, producing an air flow (Michelsen, 2003) which can be sensed by followers (Wachtler, Ikeno, Kumaraswamy, Ai, & Kai, 2017). In contrast, *A. florea* foragers do not vibrate their wings during the waggle phase (Towne, 1985) and hence these cues are absent in the dance.

Our results, taken together with previous work on *A. mellifera* (Gil & De Marco, 2010; Rohrseitz & Tautz, 1999; Tanner & Visscher, 2008) point to the importance of tactile cues experienced by foragers, who positioned themselves laterally with respect to the dancer, for the spatial information transfer in both the giant and cavity nesting honey bees. Dance followers who are laterally positioned experience a regular pattern of antennal deflections which correlate strongly with the number of abdomen waggles (Gil & De Marco, 2010; Michelsen, 2012; Rohrseitz & Tautz, 1999). Since the frequency of waggling of the abdomen is physically constrained to be similar amongst bees of the same species (Hrncir, Maia-Silva, Mc Cabe, & Farina, 2011), followers can use this to estimate the waggle phase duration (Kohl *et al.*, unpublished data). At the same time, they can obtain the orientation of the waggle run by using their own body position with respect to gravity as a reference. The Johnston's organ may play a vital role in extracting information about the direction of the waggle phase and hence the direction of the food source (Brockmann & Robinson, 2007).

Figure A2.6

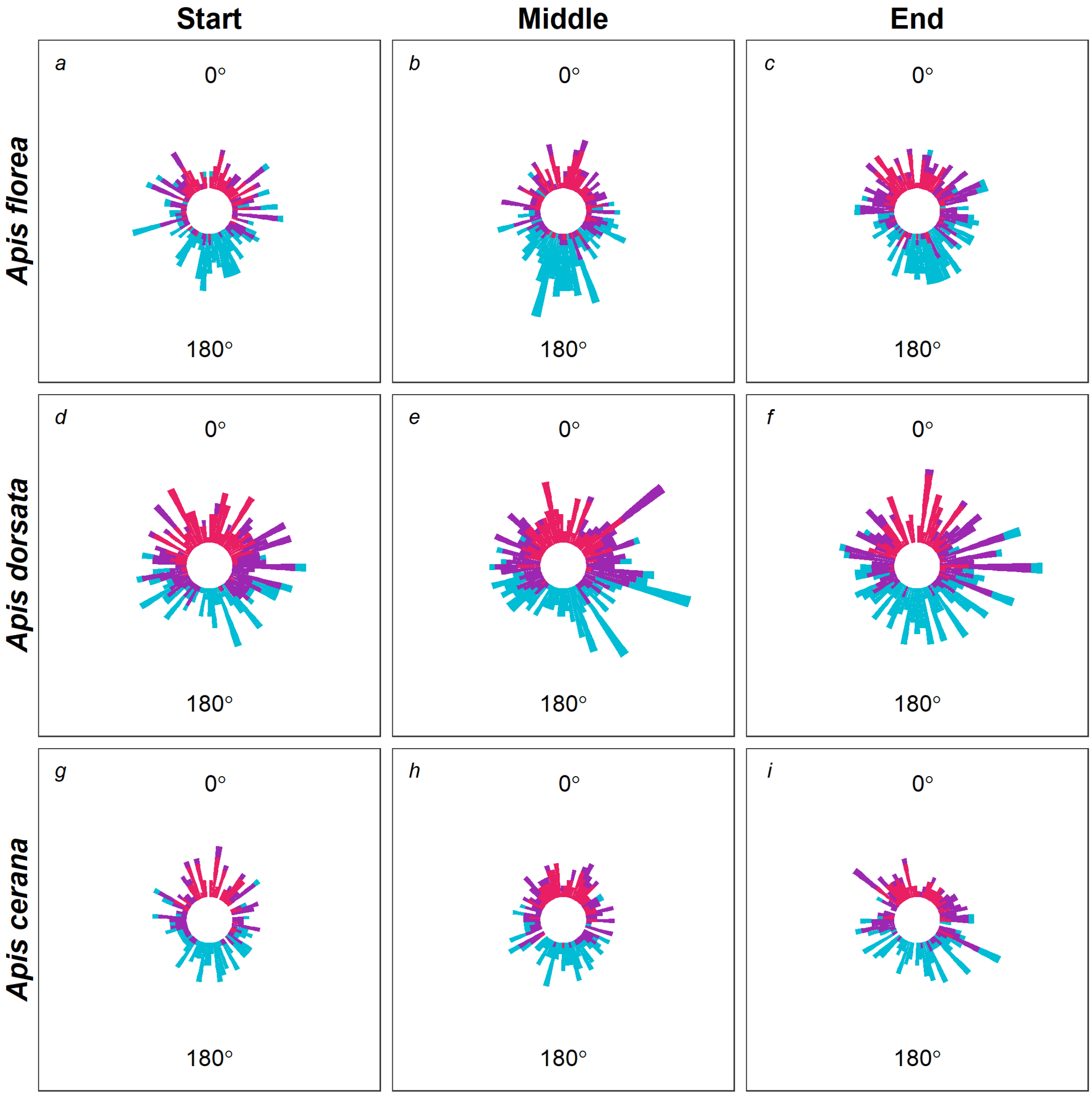

Figure A2.6: A circular histogram of the body orientation of the followers with respect to the dancer. Each bar represents the number of followers in a bin width of 5°. A follower at 0° is facing the dancer and a follower at 180° is exactly behind the dancer. The bars are coloured based on the zones occupied by the follower with pink for followers in the Anterior zone, purple for followers in the Lateral zone and cyan for followers in the Posterior zone.

Figure A2.7

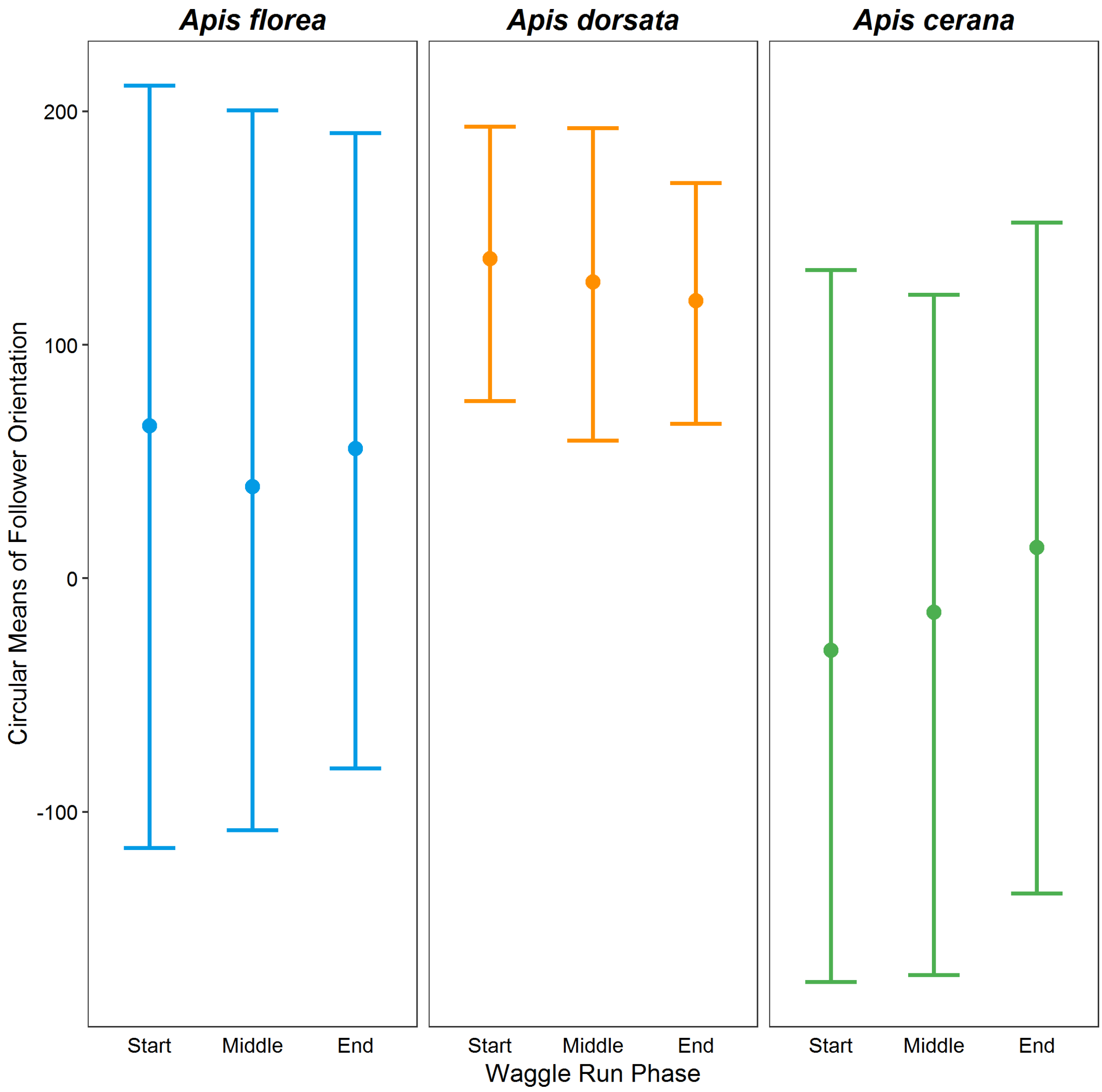

Figure A2.7: The circular means of the follower orientation in the different waggle run phases in each of the 3 species. The means are provided along with the 95% highest posterior density (HPD) interval (circles and error bars). The upper and lower bounds of the 95% HPD interval overlapped for all conditions, indicating that there were no differences in the circular means in the different phases or the different species.

In contrast, in *A. florea*, following the dance is the most prominent behavioural pattern of potential recruits. The nature of the waggle dance of *A. florea* might provide an explanation for this. *A. florea* foragers dance on a horizontal surface and more importantly, dance in the direction of the food source (Beekman, Makinson, Couvillon, Preece, & Schaerf, 2015; F. C. Dyer, 1985). In addition, *A. florea* foragers present a strong visual cue by lifting their abdomen and shaking it vertically during the waggle run (Towne, 1985). Since *A. florea* is open nesting, this has been hypothesised to attract recruits to a dancing forager, similar to the sound produced by the vibrating wings of dancers in cavity nesting species (I'Anson Price & Grüter, 2015; Towne, 1985). This strong cue might cause potential recruits to make a direct approach towards the abdomen of the dancer. Thus, by positioning themselves behind the dancer, followers directly get to know the direction to fly out to and there is no need to transpose any direction information.

Across all 3 species, the number of dance followers initially increased and then remained similar as the phase of the waggle run changed. This indicates that potential recruits initiated the process of following the waggle run near the beginning of the run. This is expected given that the whole run duration encodes the correct distance information. We did not find any interaction between the waggle run phase and the zone of the follower, implying that the number of followers in the different zones were similar across the phases of the waggle run. In addition, we found no difference in the circular means of the follower orientation between the different phases of the waggle run, suggesting that there was very little turnover of followers across the different zones as the waggle run progressed. These results are in contrast with an earlier study by Gil and De Marco (2010) which showed that the percentage of followers in the different zones changed from the beginning to the end of the waggle run. However, their study did not look at whether the number of followers increased as the waggle run progresses. We believe that an increase in the number of followers (as seen in our results) would explain the change in proportions seen in their study.

Gil and De Marco (2010) further showed that the number of followers in *A. mellifera* increased with distance, although this was only apparent at the highest distance. In contrast, distance had no effect on the number of followers present in a waggle run across all 3 species in our study. We also found that the circular means of the follower orientation did not change with distance. The discrepancy between the two studies might be due to a statistical issue as the previous study relied on an ANOVA to test differences, whereas we used a more appropriate model with a Poisson error distribution for the count data (McCullagh & Nelder, 1989; O'Hara & Kotze, 2010).

The behavioural responses of *A. cerana* followers however does not fit into the pattern of expected responses for either of the two hypotheses. We are unsure why the cavity nesting *A. cerana* followers positioned themselves equally behind and to the side of the dancer, whereas followers in the closely related *A.* mellifera positioned themselves to the side (Gil & De Marco, 2010; Tanner & Visscher, 2008). It could be possible that followers could obtain some relevant information from orienting themselves to the side as well as behind the dancer. This indicates that the body orientation of the dance followers might not matter as much as the tactile cues, like antennal contacts that they experience (Tanner & Visscher, 2008). We could not record and analyse the antennal contacts between the dancers and the followers. Further, our study does not answer whether the spatial information is transferred to the dance follower (Judd, 1994; Tanner & Visscher, 2008). Combining detailed observations of the follower behaviour using a high-speed camera with tracking of their foraging trips (Menzel et al., 2011) is needed to gain a more detailed understanding of the mechanism underlying spatial information transfer in the waggle dance.

In conclusion, our study provides the first comparative observations of the dance followers across 3 species in the *Apis* genus. Our results suggest that the information transfer in the genus *Apis* is dependent on the 'complexity' of the information content present in the dance itself. In the simpler dances in *A. florea*, where no transposition of the direction information to another reference was present, followers positioned themselves behind the dancer. This could possibly help them to directly obtain the direction to fly out to. In the more complex dances of *A. dorsata* and *A. mellifera*, where the direction information is transpositioned with reference to the gravity axis in the dance, followers positioned themselves to the side of the dancer. This would presumably help the followers obtain direction and distance information from antennal contacts with the dancers abdomen (Gil & De Marco, 2010; Rohrseitz & Tautz, 1999). However, even in the species with the more 'complex' dance, followers could possibly obtain some information from positioning themselves behind the dancer. Further comparative experiments on the Asian honey bees are needed to understand the differences in the mechanism of spatial information transfer between the various species.

Future Directions

The most important next step would be to obtain data from other colonies of the 3 species used. Currently, the experimental design and analysis in this chapter closely follows earlier work in A. mellifera (Gil & De Marco, 2010; Judd, 1994; Rohrseitz & Tautz, 1999). In all these studies, only dancers and followers from one colony were observed. The underlying assumption was that the highly stereotypical nature of the waggle dance would mean that dancers and followers from any colony of *A. mellifera* would behave similarly (at a qualitative level). However, direct comparisons between followers from multiple colonies would be needed to conclusively establish the veracity of this assumption in the Asian honey bees. This would be relatively easy for both *A. florea* and *A. cerana*, as colonies from these species can be transported and observed in the same location. However, a repeat of these experiments would be more difficult in the case of *A. dorsata* and would rely on a colony arriving back to the same building.

The next step would be to determine which followers actually obtain the spatial information from the dancer. This would require multiple trap feeders set up in an arc around the feeder that is being advertised (Tanner & Visscher, 2008). In addition, most of the potential recruits in the colony should be marked to correlate the position of the follower around the dancer with the feeder it flew to. This could help provide more conclusive evidence of the mechanism of the spatial information transfer in the different Asian honey bee species.

References

Barton, K. (2017). MuMIn: Multi-Model Inference. Retrieved from https://cran.r-project.org/package=MuMIn

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using {lme4}. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Beekman, M., Makinson, J. C., Couvillon, M. J., Preece, K., & Schaerf, T. M. (2015). Honeybee linguistics - a comparative analysis of the waggle dance among species of Apis. *Frontiers in Ecology and Evolution*, *3*(February), 1–11. https://doi.org/10.3389/fevo.2015.00011

Božič, J., & Valentinčič, T. (1991). Attendants and followers of honey bee waggle dances. *Journal of Apicultural Research*, *30*(3–4), 125–131. https://doi.org/10.1080/00218839.1991.11101246

Brockmann, A., & Robinson, G. E. (2007). Central projections of sensory systems involved in honey bee dance language communication. *Brain, Behavior and Evolution*, *70*(2), 125–136. https://doi.org/10.1159/000102974

Chittka, L., & Leadbeater, E. (2005). Social learning: Public information in insects. *Current Biology*. https://doi.org/10.1016/j.cub.2005.10.018

Conradt, L., & Roper, T. J. (2003). Group decision-making in animals. *Nature*, *421*, 155–158. https://doi.org/10.1038/nature01294

Cremers, J. (2018). bpnreg: Bayesian Projected Normal Regression Models for Circular Data. Retrieved from https://cran.r-project.org/package=bpnreg

Cremers, J., & Klugkist, I. (2018). One Direction? A Tutorial for Circular Data Analysis Using R With Examples in Cognitive Psychology. *Frontiers in Psychology*, *9*(October), 1–13. https://doi.org/10.3389/fpsyg.2018.02040

De Marco, R. J. (2006). How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' `eagerness'. *Journal of Experimental Biology*, *209*(3), 421–432. https://doi.org/10.1242/jeb.02025

De Marco, R. J., & Farina, W. M. (2001). Changes in food source profitability affect the trophallactic and dance behavior of forager honeybees (Apis mellifera L.). *Behavioral Ecology and Sociobiology*, *50*(5), 441–449. https://doi.org/10.1007/s002650100382

Duboscq, J., Romano, V., MacIntosh, A., & Sueur, C. (2016). Social information transmission in animals: Lessons from studies of diffusion. *Frontiers in Psychology*. https://doi.org/10.3389/fpsyg.2016.01147

Dyer, F. C. (1985). Mechanisms of dance orientation in the Asian honey bee Apis florea L. *Journal of Comparative Physiology A*, 183–198. Retrieved from http://www.springerlink.com/index/KG584734M403TRM1.pdf

Dyer, F. C., & Seeley, T. D. (1991). Dance Dialects and Foraging Range in 3 Asian Honey-Bee Species. *Behavioral Ecology and Sociobiology*, *28*(4), 227–233. https://doi.org/10.1007/BF00175094

Dyer, Fred C. (2002). The biology of the dance language. *Annual Review of Entomology*, *47*(1), 917–949. https://doi.org/10.1146/annurev.ento.47.091201.145306

Farina, W. M., & Núñez, J. A. (1991). Trophallaxis in the honeybee, Apis mellifera (L.) as related to the profitability of food sources. *Animal Behaviour*, *42*(3), 389–394. https://doi.org/10.1016/S0003-3472(05)80037-5

Florian Hartig. (2018). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. Retrieved from https://cran.r-project.org/package=DHARMa

Gardner, K. E., Seeley, T. D., & Calderone, N. W. (2008). Do honeybees have two discrete dances to advertise food sources? *Animal Behaviour*, *75*(4), 1291–1300. https://doi.org/10.1016/j.anbehav.2007.09.032

Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., & Rubin, D. B. (2013). *Bayesian data analysis*. *Chapman & Hall/CRC* (3rd ed.). Boca Raton, FL. https://doi.org/10.1017/9781316216491.030

George, E. A., & Brockmann, A. (2019). Social modulation of individual differences in dance communication in honey bees. *Behavioral Ecology and Sociobiology*, *73*(4), 41. https://doi.org/10.1007/s00265-019-2649-0

Gil, M., & De Marco, R. J. (2010). Decoding information in the honeybee dance: Revisiting the tactile hypothesis. *Animal Behaviour*, *80*(5), 887–894. https://doi.org/10.1016/j.anbehav.2010.08.012

Grüter, C., & Farina, W. M. (2009). Past experiences affect interaction patterns among foragers and hive-mates in honeybees. *Ethology*, *115*(8), 790–797. https://doi.org/10.1111/j.1439-0310.2009.01670.x

Hrncir, M., Maia-Silva, C., Mc Cabe, S. I., & Farina, W. M. (2011). The recruiter's excitement - features of thoracic vibrations during the honey bee's waggle dance related to food source profitability. *Journal of Experimental Biology*, *214*(23), 4055–4064. https://doi.org/10.1242/jeb.063149

I'Anson Price, R., & Grüter, C. (2015). Why, when and where did honey bee dance communication evolve? *Frontiers in Ecology and Evolution*, *3*(November), 1–7. https://doi.org/10.3389/fevo.2015.00125

Jarau, S., & Hrncir, M. (2009). *Food Exploitation by Social Insects : Ecological, Behavioral, and Theoretical Approaches*. Taylor & Francis.

Judd, T. M. (1994). The waggle dance of the honey bee: which bees following a dancer successfully acquire the information? *Journal of Insect Behavior*, *8*(3), 343–354. https://doi.org/10.1007/BF01989363

Kao, A. B., Miller, N., Torney, C., Hartnett, A., & Couzin, I. D. (2014). Collective Learning and Optimal Consensus Decisions in Social Animal Groups. *PLoS Computational Biology*, *10*(8), 35–37. https://doi.org/10.1371/journal.pcbi.1003762

King, A. J., & Cowlishaw, G. (2007). When to use social information: The advantage of large group size in individual decision making. *Biology Letters*, *3*(2), 137–139. https://doi.org/10.1098/rsbl.2007.0017

Kirchner, W. H., & Dreller, C. (1993). Acoustical Signals in the Dance Language of the Giant Honeybee , Apis dorsata. *Behavioral Ecology and Sociobiology*, *33*(2), 67–72.

Kocher, S. D., & Cocroft, R. B. (2019). Signals in Insect Social Organization. *Encyclopedia of Animal Behavior*, 558–567. https://doi.org/10.1016/B978-0-12-809633-8.90781-7

Landgraf, T., Bierbach, D., Kirbach, A., Cusing, R., Oertel, M., & Mar, R. O. (2018). Dancing Honey bee Robot Elicits Dance-Following and Recruits Foragers. *ArXiv*, 1–25.

Lenth, R. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. Retrieved from https://cran.r-project.org/package=emmeans

Leonhardt, S. D., Menzel, F., Nehring, V., & Schmitt, T. (2016). Ecology and Evolution of Communication in Social Insects. *Cell*, *164*(6), 1277–1287. https://doi.org/10.1016/j.cell.2016.01.035

McCullagh, P., & Nelder, J. A. (1989). *Generalized Linear Models*. Chapman and Hall.

Menzel, R., Kirbach, A., Haass, W., Fischer, B., Fuchs, J., Koblofsky, M., … Wilhelmshaven, O. (2011). A Common Frame of Reference for Learned and Communicated Vectors in Honeybee Navigation. *Current Biology*, *21*(8), 645–650. https://doi.org/10.1016/j.cub.2011.02.039

Michelsen, A. (2003). Signals and flexibility in the dance communication of honeybees. *Journal of Comparative Physiology A*, *189*(3), 165–174. https://doi.org/10.1007/s00359-003-0398-y

Michelsen, A. (2012). How Do Honey Bees Obtain Information About Direction by Following Dances? In *Honeybee Neurobiology and Behavior* (pp. 65–76). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-2099-2_6

Michelsen, A., Kirchner, W. H., & Lindauer, M. (1986). Sound and vibrational signals in the dance language of the honeybee, Apis mellifera. *Behavioral Ecology and Sociobiology*, 207–212. Retrieved from http://link.springer.com/article/10.1007/BF00290824

Morand-Ferron, J., Doligez, B., Dall, S. R. X., & Reader, S. M. (2010). Social Information Use. *Encyclopedia of Animal Behavior*, 394–402. https://doi.org/10.1016/B978-0-12-813251-7.00281-9

Nieh, J. C. (2010). A negative feedback signal that is triggered by peril curbs honey bee recruitment. *Current Biology*, *20*(4), 310–315. https://doi.org/10.1016/j.cub.2009.12.060

O'Hara, R. B., & Kotze, D. J. (2010). Do not log-transform count data. *Methods in Ecology and Evolution*, *1*(2), 118–122. https://doi.org/10.1111/j.2041-210x.2010.00021.x

Pruitt, J. N., & Goodnight, C. J. (2014). Site-specific group selection drives locally adapted group compositions. *Nature*, *514*(7522), 359–362. https://doi.org/10.1038/nature13811

R Core Team. (2018). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rohrseitz, K., & Tautz, J. (1999). Honey bee dance communication: waggle run direction coded in antennal contacts? *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *184*(4), 463–470. https://doi.org/10.1007/s003590050346

RStudio Team. (2016). RStudio: Integrated Development Environment for R. Boston, MA. Retrieved from http://www.rstudio.com/

Sarma, Moushumi Sen, Fuchs, S., Werber, C., & Tautz, J. (2002). Worker piping triggers hissing for coordinated colony defence in the dwarf honeybee Apis florea. *Zoology*, *105*(2002), 215–223.

Seeley, T. D. (1989). Social foraging in honey bees: how nectar foragers assess their colony’s nutritional status. *Behavioral Ecology and Sociobiology*, *24*(3), 181–199. https://doi.org/10.1007/BF00292101

Seeley, T. D. (1995). *The Wisdom of the Hive*. *Harvard University Press*.

Seeley, T. D., Mikheyev, A. S., & Pagano, G. J. (2000). Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, *186*(9), 813–819. https://doi.org/10.1007/s003590000134

Seeley, T. D., & Towne, W. F. (1992). Tactics of dance choice in honey bees: do foragers compare dances? *Behavioral Ecology and Sociobiology*, *30*(1), 59–69. https://doi.org/10.1007/BF00168595

Sen Sarma, M, Esch, H. E., & Tautz, J. (2004). A comparison of the dance language in Apis mellifera carnica and Apis florea reveals striking similarities. *Journal of Comparative Physiology A*, *190*(1), 49–53. https://doi.org/10.1007/s00359-003-0470-7

Tan, K., Dong, S., Li, X., Liu, X., Wang, C., Li, J., & Nieh, J. C. (2016). Honey Bee Inhibitory Signaling Is Tuned to Threat Severity and Can Act as a Colony Alarm Signal. *PLOS Biology*, *14*(3), e1002423. https://doi.org/10.1371/journal.pbio.1002423

Tanner, D., & Visscher, K. (2008). Does the body orientation of waggle dance followers affect the accuracy of recruitment? *Apidologie*, *40*(1), 55–62. https://doi.org/10.1051/apido:2008074

Thom, C. (2003). The tremble dance of honey bees can be caused by hive-external foraging experience. *Journal of Experimental Biology*, *206*(13), 2111–2116. https://doi.org/10.1242/jeb.00398

Towne, W. F. (1985). Acoustic and visual cues in the dances of four honey bee species. *Behavioral Ecology and Sociobiology*, *16*(2), 185–187. https://doi.org/10.1007/BF00295154

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

Wachtler, T., Ikeno, H., Kumaraswamy, A., Ai, H., & Kai, K. (2017). Interneurons in the Honeybee Primary Auditory Center Responding to Waggle Dance-Like Vibration Pulses. *The Journal of Neuroscience*, *37*(44), 10624–10635. https://doi.org/10.1523/jneurosci.0044-17.2017

Wagenmakers, E.-J., & Farrell, S. (2004). AIC model selection using Akaike weights. *Psychonomic Bulletin and Review*, *11*, 192–196. https://doi.org/10.3758/BF03206482

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