Freie Universität Berlin

Life history tracking of social communication and navigation behaviors in honeybees (*Apis mellifera L.*)

Inaugural-Dissertation to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)
Submitted to the Department of Biology, Chemistry, Pharmacy
of Freie Universität Berlin

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Berlin, 20th Dec, 2021

This Ph.D thesis was carried out under the supervision of Prof. Dr. Dr. h.c. Randolf Menzel from January 2017 until January 2022 at the Institute of Neurobiology of the Freie Universität Berlin. These years of studies have been supported by China Scholarship Council (CSC).

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Date of defense: 09 January 2022

Acknowledgments

Declaration

I hereby declare that the work presented in the thesis has been conducted independently and without inappropriate support. All sources of information are referenced. I hereby declare that this thesis has not been submitted either in the same or a different form to this or any other university for a degree

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09/02/2022

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1 Summary

The honeybee (*Apis mellifera L.*) is an ideal model for studying social behaviors and navigation. Social activities and navigational flights are two key aspects to regulate the function of social community. These complex processes usually involve dance communication, antennation, trophallaxis (social behaviors), and orientation and foraging flights (navigation). As group-living animals, honeybees are known to rely mainly on social information to help make decisions on whether, how and where to forage for food. However, honeybees may also constantly integrate their own experience with the information from other bees to make a final decision. Therefore, the degree to which bees follow the information from other individuals or apply their own knowledge would be age-dependent and experience-dependent on an individual basis.

Meanwhile, honeybees, in particular living in a colony with small size, may be vulnerable to the external natural environment. There is no knowledge yet about how the development of the indoor and outdoor behaviors is and how the previously mentioned social and non-social factors influence bees' behaviors indoors and outdoors, in particular how social behaviors influence the outdoor activities and vice versa. Therefore, the aim of the current study is to find the answers to these questions. This study combined the advantages of Raspberry Pi with video cameras by aid of infra-red illumination on one side, and harmonic radar on the other side to record the social behaviors inside of the colony without disruption and monitor flight trajectories outdoors in real-time. The social behaviors and flights were recorded over the bees' lifetime within 15 days.

In summary, each individual bee possesses their own rhythms with different levels of variation in responding to both social and non-social factors at both group and individual levels. The age dependence and experience dependence of the indoor and outdoor behaviors were found, however, of which the degrees of such dependence were various for different behaviors among different individuals and within an individual over the lifetime. Within the small community, my results showed that there was a small group of 'elite' bees that outperformed in both social interaction and flights, which in some sense reflect the collective characteristics and exquisite labor division in the eusocial community. Dance communication is known to convey vector information about the food sources that bees discover during foraging flights. Importantly, my studies firstly discovered that dance communication transmit both motivational and

instructive role in the orientation and foraging flights, of which, the influence of information of direction and distance on the orientation and foraging flights in some degree was different. My result firstly discovered that dance communication plays important roles in both motivation and vector roles in bees' orientation and foraging flights. Noise of information transfer is universal in dance communication. However, its influence on the orientation and foraging flights were not similar which depended on the different purposes of orientation and foraging flights. Honeybees could selectively determine to use flight information form dance communication.

For the future, I suggest collecting more datasets about social behaviors to enrich the current conclusions. However, this is critically necessary to rely on an automatically tracking method with high accuracy and fast computing speed.

2 Zusammenfassung

Die Honigbiene (*Apis mellifera L.*) ist ein idealer Modellorganismus zur Untersuchung des Sozialverhaltens und der Navigation. Soziale Aktivitäten und Navigationsflüge sind zwei Schlüsselaspekte, die das Funktionieren der sozialen Gemeinschaft regeln. Zu diesen komplexen Prozessen gehören die Tanzkommunikation, Antennation und Trophallaxis (Sozialverhalten) sowie Orientierungs- und Sammelflüge (Navigation). Als in Gruppen lebende Tiere verlassen sich Honigbienen bekanntermaßen hauptsächlich auf soziale Informationen, um zu entscheiden, ob, wie und wo sie auf Nahrungssuche gehen. Allerdings können Honigbienen auch unentwegt ihre eigenen Erfahrungen mit den Informationen anderer Bienen kombinieren, um eine endgültige Entscheidung zu treffen. Inwieweit Bienen den Informationen anderer Individuen folgen oder ihr eigenes Wissen anwenden, ist daher individuell alters- und erfahrungsabhängig.

In der Zwischenzeit sind Honigbienen, insbesondere wenn sie in einem kleinen Volk leben, anfällig für die äußere natürliche Umgebung sein. Es gibt noch keine Erkenntnisse darüber, wie sich das Verhalten in innerhalb und außerhalb des Volkes entwickelt und wie die zuvor genannten sozialen und nicht-sozialen Faktoren das Verhalten der Bienen innerhalb und außerhalb beeinflussen, insbesondere wie das soziale Verhalten die Aktivitäten im Freien beeinflusst und umgekehrt. Ziel der vorliegenden Studie ist es daher, Antworten auf diese Fragen zu finden.

In dieser Studie wurden die Vorteile des Raspberry Pi mit Videokameras mit Hilfe von Infrarot-Beleuchtung auf der einen Seite und harmonischem Radar auf der anderen Seite kombiniert, um das Sozialverhalten innerhalb der Kolonie ohne Unterbrechung aufzuzeichnen und die Flugbahnen im Freien in Echtzeit zu überwachen. Das Sozialverhalten und die Flüge wurden über die gesamte Lebensdauer der Bienen innerhalb von 15 Tagen aufgezeichnet.

Zusammenfassend hat jede einzelne Biene ihren eigenen Rhythmus, der sowohl auf Gruppen- als auch auf Individualebene unterschiedlich stark auf soziale und nicht-soziale Faktoren reagiert. Es wurde eine Alters- und Erfahrungsabhängigkeit des Innen- und Außenverhaltens festgestellt, wobei das Ausmaß dieser Abhängigkeit für verschiedene Verhaltensweisen bei verschiedenen Individuen und innerhalb eines Individuums im Laufe des Lebens unterschiedlich war. Innerhalb der kleinen Gemeinschaft des Versuchsstockes zeigten meine Ergebnisse, dass es eine kleine Gruppe von "Elite"-Bienen gab, die sowohl bei der sozialen Interaktion als auch bei den Flügen die Leistungen anderer übertrafen, was in gewisser Weise die kollektiven Merkmale und die exquisite Arbeitsteilung in der eusozialen Gemeinschaft widerspiegelt. Weiter ist bekannt, dass die Tanzkommunikation Vektorinformationen über die Nahrungsquellen vermittelt, die die Bienen während ihrer Flüge zur Futtersuche entdecken. Bedeutsam ist, dass meine Studien zunächst zeigen, dass die Tanzkommunikation sowohl eine motivierende als auch eine anweisende Rolle bei der Orientierung und den Futterflügen spielt, wobei der Einfluss von Richtungs- und Entfernungsinformationen auf die Orientierungs- und Sammelflüge zu einem gewissen Maße unterschiedlich war. Meine Ergebnisse zeigen weiterhin, dass die Tanzkommunikation sowohl eine motivierende als auch eine weisende Rolle bei den Orientierungs- und Sammelflügen der Bienen spielt. Ein Rauschen ist universell in der Informationsübertragung der Tanzkommunikation. Der Einfluss auf die Orientierungs- und Suchflüge war jedoch nicht gleich, was von den unterschiedlichen Zielen der Orientierungs- und Sammelflüge abhing. Honigbienen konnten selektiv entscheiden, ob sie Fluginformationen aus der Tanzkommunikation verwenden.

Für zukünftige Studien schlage ich vor, weitere Datensätze über das Sozialverhalten zusammen um die aktuellen Schlussfolgerungen zu ergänzen. Dazu ist es jedoch unbedingt erforderlich, sich auf eine automatische Trackingmethode mit hoher Genauigkeit und schneller Rechengeschwindigkeit zu stützen.

3 Chapter 1 Automatic identification of behaviors inside the colony in the honeybees

3.1 Abstract

Tracking honeybees' behaviors inside the colony precisely over a long-time scale is a fundamental but time-consuming work. Ideally it allows collecting enormous data of social behaviors and provides a powerful tool for revealing various behavioral features and mechanisms in the honeybees. The honeybees occupy in a high-density community, increasing the difficulty and complexity to automatically record and classify various behavioral types. The application of the published methods of automatic behavioral tracking to my study is investigated. The result showed that the current techniques are not applicable in my analyses and only manual tracking can be used to collect real-time behavioral data in the honeybees.

3.2 Introduction

Honeybees with complex and efficient social organization have fascinated neurobiologists, behavioral ecologists, social behavioral scientists and computation scientists. Although there is no central control of how work is allocated for each individual, the honeybees are standing out with self-organization manner, sophisticated labor division and remarkable decision making that are emerging from efficient social interactions, e.g., dance communication, trophallaxis, antennation, defense and cleaning. However, knowledge about how each individual plays a role in their tasks and how they make the social connection to make the colony function remain poorly understood. This is mainly due to major difficulties in collecting quantitative, real-time and precise behavioral data of fast-moving and small sized honeybees in a dense society. To solve the problem, more and more biologists and technical scientists cooperated to develop multiple methods to facilitate the monitoring of behaviors.

Tracking the honeybees automatically within a colony is challenging. Most of the automatic tracking in observational studies of honeybee behaviors were based on videotape and computation estimation. Achieving high accuracy of behavioral tracking automatically required several main difficulties to be overcome. 1) 3D locomotion, 2) tracking of multiple body parts, 3) simultaneous tracking of multiple bees, 4) intermittent occlusions and motion blur, 5) thousands of the honeybees in a dense environment, 6) real-time monitoring of long-duration videos, 7) pose estimation via multiple cameras, 8) processing speed with large images and videos (~3.5 GB/per one), 9) differentiation of a specific behaviors from multiple similar behaviors, 10) differentiation of behaviors that consist of several sequential physical motions, i.e., waggle dances consist of a series of movement: bees arch to right, waggle, arch to left, waggle and so on (von Frisch 1967). Almost all current automatic tracking techniques could only produce trajectories of motions for a single specific behavior, e.g., dance (Blut et al. 2017, Boenisch et al. 2018, Wario et al. 2015), trophallaxis, antennation (Blut et al. 2017, Shen et al. 2015, Wild et al. 2021) or in-and-out behaviors (Tu et al. 2016). Although a few methods could recognize the specific behaviors, most of the automatic tracking techniques could only identify motions (Table 1) which required additional behavioral classifiers like JAABA (Kabra et al. 2013) and Ctrax (Branson et al. 2009) to categorize behaviors. Among these automatic systems, some were developed for tracking bees based on shape, color or

segmentation without marking of number tags. Some were applied to track bees with specific 2D barcodes (Blut et al. 2017, Boenisch et al. 2018, Crall et al. 2015, Wario et al. 2015, Wild et al. 2021). Although a few programs did not required bees marking with specific barcodes (Bozek et al. 2021, Kimura et al. 2014), they were confined to immobile bees, small sizes of videos or small sizes of animal groups with less complicated behaviors. A few programs recognized bees by specific 2D barcodes under natural condition providing fundamental advantage over other techniques. However, they were not open-source and user-friendly. The accuracy of behavioral estimation remained to be improved.

There were a few automatic systems tracking other insects except for honeybees (Table 1). Both bear advantages and limitations. In this study, I will focus on tracking multiple complex social behaviors: dance communication, trophallaxis, antennation, sleep, queen caring and feeding. Although my experimental colony was relatively smaller than normal ones, it still was packed with bees. The number tags attached to the bees' thorax were required to enable the experimenter at the hive exit recognize the focal bees and perform the catch-and-release experiment. However, the barcodes required in the former tracking systems were neither easy for observers to recognize bees at the exit, nor convenient for bees to fly. Videos from two sides of the comb were recorded simultaneously. It required higher robustness of machine learning to label and train the images of videos of two sides to more precisely recognize the focal bees. Therefore, DeepLabCut (Nath et al. 2019) and JAABA were investigated to automatically extract the spatial trajectory dataset of the focal bees and classify behaviors of dance communication, trophallaxis, antennation, sleep, queen caring and feeding within colony, respectively.

3.3 Methods

3.3.1 Video recording

Videos monitoring social behaviors of honeybees were recorded with Raspberry Pi (Raspberry Pi3 Model B, 1.2 GHz QuadCore, 64Bit CPU) and two infrared Camera Modules (v2 Pi NoIR) fixed on each side of the observation hive (More details in 'Materials and Methods', Chapter 3). Each video was 15 fps and 1920 × 1080 pixels. The raw video streams were packaged with H264 codec and converted into mp4 format. 372 videos were recorded over 15 days of experiment. Each video had a duration of 1 hour and a size of 3.56 GB.

3.3.2 Automatic tracking with DeepLabCut

High Performance Computing (HPC) system in the Freie University Berlin was applied to improve the speed of automatic tracking system. Docker environment was built to use GPU to run DeepLabCut. To maximize the tracking performance of DeepLabCut, a neural network of model ResNets-50 (or ResNets-101) was used with 1030000, 1500000, 3000000 and 4000000 iterations, respectively. To improve the tracking accuracy, 8 body parts of each focal bee in each frame and 200 frames for each video were labeled. Two videos were labeled for the training procedure for each focal bee.

3.4 Analyses and Results

It took around 3 hours to label 400 frames with 8 body parts of one focal bee and at least three days to finish the training procedure for two videos (~ 4 GB for each video) (Table 2). Training results with four different iterations showed not obvious improvement in accuracy (Table 2). To compare the training efficiency and accuracy, addition training procedures with compressed videos via ffmpeg were done. Although the time for training with compressed videos was shorter and the training accuracy were similar (not data shown here), it required amounts of time for compression videos. Therefore, the training with original video without compression was determined. Although all training results showed extremely low errors (Table 2), the focal bees recognized in the labeled videos generated after the training procedure were mislabeled. The labeled points on its body parts also jumped to another bee (Figure 1, e.g., focal bee 201).

3.5 Discussion

DeepLabCut was the most promising candidate among the available automatic tracking techniques that would be suitable for our study. It has provided user-friendly application in fruit flies, fish and mosquitoes under both 2D and 3D behavioral space. The current version can track multiple marker-less animals simultaneously. Here its potential use was tested in studying whether the automatic tracking system is more applicable than manual tracking in monitoring social behaviors in the honeybees. However, the automatic tracking performance was not as fast and accurate as what it was expected. Therefore, I preferred to rely on manual tracking rather than on the

automatic tracking method to collect social behavioral data of the honeybees within colony over days for further analyses.

Quantifying behavior is a fundamental procedure to understand behavioral development, consistency, plasticity, diversity and its biological and evolutionary function. Automatic approaches provide high-throughput quantification advantages for advancing behavioral study in animals. To understand how honeybees initiate a behavioral action inside and outside the colony involves procedures of learning, memory, motivation, sensory perception and cognition. For instance, how do different types of social behaviors develop over the life span of individual honeybees? What happens within a social community before bees start an orientation flight or a foraging flight? What is the potential relation between the sociality and flight performance among different bees? how is the interactive influence between bees' social experience inside the colony and the flight experience outside of the colony? Underpinning the mechanisms of these diverse behaviors accurately will benefit from the development of automatic monitoring techniques. However, social behaviors inside the bee colony are complicated and dynamic. Even the criteria to categorize a social behavior via manual observation and measurement, i.e., dance and dance following, would be hard to be applied accurately across different experiments. Most of the current automatic monitoring techniques are only applied to the specific experimental problems. The accuracy of automatic tracking remains to be improved. Meanwhile, a few applicable programs may be less user-friendly and expensive that they require super high investment in hardware and software to improve computation speed. These limitations are likely to impede our comprehensive understanding about diverse behaviors within the bee colony under the natural environment. However, the emergence of these techniques in automatic monitoring of behaviors of honeybees sheds a light on developing more applicable and accessible programs. To accurately map the patterns of diverse behaviors of honeybees and explore how a social group functions, more work requires to be invested in future.

Table 1. Open sources of computer-based automatic tracking for classify insect behaviors

Name	Validated Animal	Output	Limit	Advantage
Unnamed[Feldman 2003]	honeybee	trajectory, behavior	confined to small training dataset and short video, failed to differentiate similar behaviors, failed to track bees with switching behaviors	track sequential motions of multiple bees
JAABA[Kabra et al. 2013]	honeybee, *drosophila*	behavior	required trajectory input of animals from other programs, offline processing of data	good performance for bees that don't move linearly, higher accuracy than Ctrax
K-Track[Kimura et al. 2014]	honeybee	trajectory	confined to small areas where bees interact	higher accuracy than Ctrax in tracking multiple bees, markerless tracking
LocoTracker[Shen et al. 2015]	honeybee, ant	behavior	confined to immobile bees	could estimate the trajectories of insect body parts even under merge conditions
BEEtag[Crall et al. 2015]	bumble bee	trajectory	confined to bees with 2D barcodes, challenges of data storage and process, tracking performance depending substantially on uneven light, animal posture and tag cleanliness	good performance for complex condition
Unnamed[Boenisch et al. 2018, Wario et al. 2015, Wild et al. 2021]	honeybee	trajectory	confined to bees with 2D barcodes	could automatically detect dance-following and trophallaxis behaviors
BBAS[Blut et al. 2017]	honeybee	trajectory	confined to bees with 2D barcodes, limited to small groups of bees (<100 bees)	relative high accuracy
Unnamed[Bozek et al. 2021]	honeybee	trajectory	confined to low frame rates and small sizes of videos	detected bees in dense group

Table 1. Open sources of computer-based automatic tracking for classify insect behaviors

CADABRA[Dankert et al. 2009]	*drosophila*	behavior	confined to small groups of animals	tracked multiple behaviors simultaneously, easy and cheap to use the system
Ctrax[Branson et al. 2009]	*drosophila*, fish, cockcroach	trajectory, behavior	failed to deal with complex occlusions, lower accuracy than K-Track and JAABA, failed to track complex, various and dis-continuous behaviors, challenges of processing high-throughput data	tracked multiple animals and behaviors, tracked videos with long duration,
Unnamed[Mersch 2013]	ant	trajectory	confined to animals with specific matrix codes, failed to deal with complex occlusions, required high quality of videos	tracked videos with long duration, robust to image loss, high accuracy
IdTracker[Perez-Escudero et al. 2014]	*drosophila*, fish, ant	trajectory	confined to small groups of animals, failed to track videos with long duration	makerless tracking
DeepLabCut[Nath et al. 2019]	*drosophila*, fish, mosquitoe	trajectory	required basic programing skills to build environment for training with GPU	markerless tracking, tracked multiple animals, applied in wide range of species, analyzed multiple videos simultaneously, user-friendly
DeepPoseKit[Graving et al. 2019]	*drosophila*, zebras' fish, locust,	behavior	limited to individual post estimation in each image	easy-to-use, tracked multiple animals, fast and robust animal post estimation

Table 2. Training performance of DeepLabCut with four types of iterations for one video

Neural Network	Training iterations	Train error(px)	Test error(px)	Label number	Number of bodies labeled	Training Duration (Days)
ResNet-101	1030000	2.09	2.87	200	8	4
ResNet-50	1500000	2.05	2.56	200	8	3
ResNet-50	3000000	2.00	2.47	200	8	6
ResNet-50	4000000	1.87	2.40	200	8	8
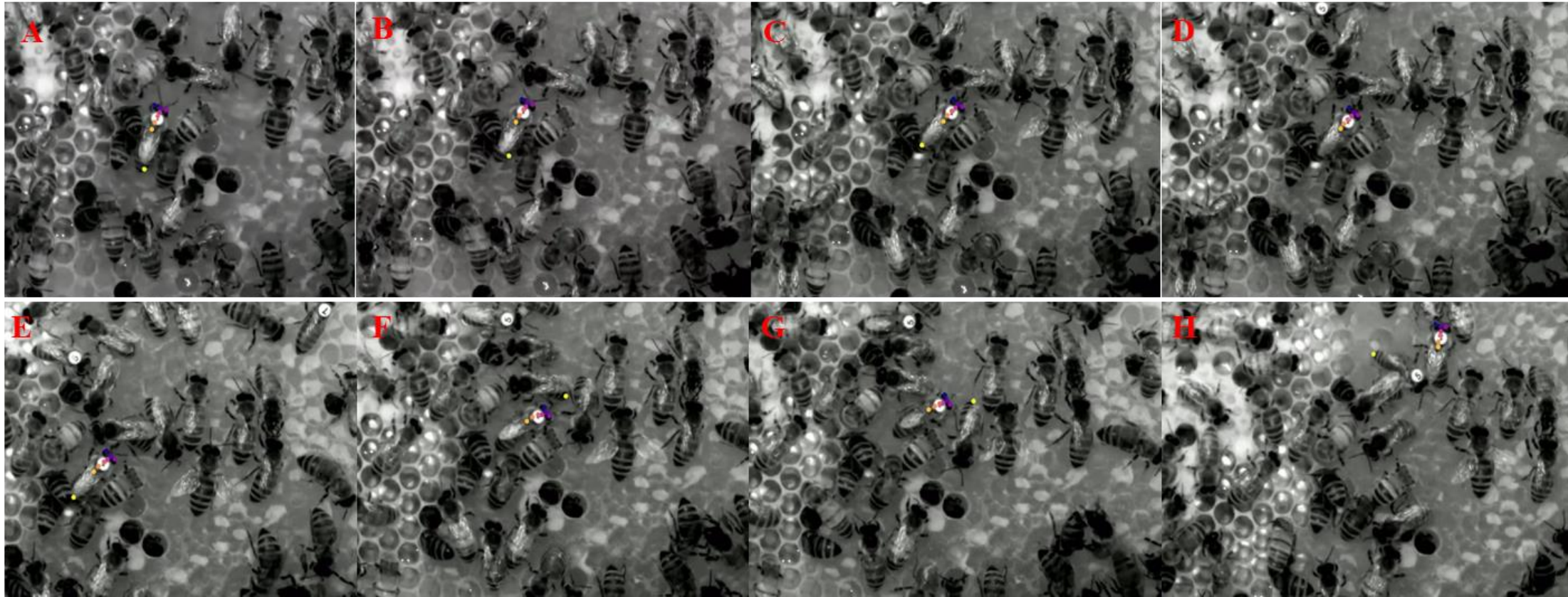
Figure 1. Post estimation of focal bee 201 with DeepLabCut after 4000000 iterations with 200 labels from 8 body parts of focal bee 201. The letters in red present the sequential motions of the bee 201 over time. Dots with colors from pink to yellow indicate the different labeled parts on the body of focal bee 201. The sequential movements showed that some dots which should be on the body of the bee 201 disappeared (Figure 1D) or jump to another bee (Figure 1F, 1G, 1H).

3.6 Reference

Blut, C., Crespi, A., Mersch, D., Keller, L., Zhao, L., Kollmann, M., . . .Beye, M. (2017). Automated computer-based detection of encounter behaviours in groups of honeybees. Sci Rep, 7(1), 17663. doi:10.1038/s41598-017-17863-4.

Boenisch, F., Rosemann, B., Wild, B., Dormagen, D., Wario, F., & Landgraf, T. (2018). Tracking All Members of a Honey Bee Colony Over Their Lifetime Using Learned Models of Correspondence. Frontiers in Robotics and Ai, 5. doi:ARTN 35 10.3389/frobt.2018.00035.

Bozek, K., Hebert, L., Portugal, Y., Mikheyev, A. S., & Stephens, G. J. (2021). Markerless tracking of an entire honey bee colony. Nat Commun, 12(1), 1733. doi:10.1038/s41467-021-21769-1.

Branson, K., Robie, A. A., Bender, J., Perona, P., & Dickinson, M. H. (2009). High-throughput ethomics in large groups of Drosophila. Nat Methods, 6(6), 451-457. doi:10.1038/nmeth.1328.

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), e0136487. doi:10.1371/journal.pone.0136487.

Dankert, H., Wang, L., Hoopfer, E. D., Anderson, D. J., & Perona, P. (2009). Automated monitoring and analysis of social behavior in Drosophila. Nat Methods, 6(4), 297-303. doi:10.1038/nmeth.1310.

Feldman, A., Balch, T. (2003). Automatic identification of bee movement. Paper presented at the 2nd International Workshop on the Mathematics and algorithms of social insects.

Graving, J. M., Chae, D., Naik, H., Li, L., Koger, B., Costelloe, B. R., Couzin, I. D. (2019). DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. Elife, 8, e47994.

Chapter 1

Kabra, M., Robie, A. A., Rivera-Alba, M., Branson, S., & Branson, K. (2013). JAABA: interactive machine learning for automatic annotation of animal behavior. Nat Methods, 10(1), 64-67. doi:10.1038/nmeth.2281.

Kimura, T., Ohashi, M., Crailsheim, K., Schmickl, T., Okada, R., Radspieler, G., & Ikeno, H. (2014). Development of a new method to track multiple honey bees with complex behaviors on a flat laboratory arena. PLoS One, 9(1), e84656. doi:10.1371/journal.pone.0084656.

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.

Nath, T., Mathis, A., Chen, A. C., Patel, A., Bethge, M., & Mathis, M. W. (2019). Using DeepLabCut for 3D markerless pose estimation across species and behaviors. Nature protocols, 14(7), 2152-2176.

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

Shen, M., Szyszka, P., Deussen, O., Galizia, C. G., & Merhof, D. (2015). Automated tracking and analysis of behavior in restrained insects. J Neurosci Methods, 239, 194-205. doi:10.1016/j.jneumeth.2014.10.021.

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. doi:ARTN 103 10.3389/fevo.2015.00103.

Wild, B., Dormagen, D. M., Zachariae, A., Smith, M. L., Traynor, K. S., Brockmann, D., . . . Landgraf, T. (2021). Social networks predict the life and death of honey bees. Nat Commun, 12(1), 1110. doi:10.1038/s41467-021-21212-5.

4 Chapter 2: Non-social factors influence behaviors at the hive exit when bees leave the colony

4.1 Abstract

Trade-off between staying inside the colony and leaving the colony for outside activities of environment exploration (orientation flight) and food collection (foraging flight) is a challenge to each individual bee. Using information sources from personal learning experience and social communication (e.g., waggle dance) are the most well-known and important mechanisms to drive bees' decision-making on outdoor activities. However, abiotic factors such as climate, colony morphology and the availability of food sources in a changing environment as well as other biotic factors, such as a bee's age, time-sense of day and frequencies of behavioral events also affect a bee's outdoor activities. In this chapter, I recorded various outdoor behaviors from 87 bees including 17 bees which performed orientation or foraging flights in a tiny colony. Bees' behaviors at the hive exit ('Out') were categorized into four states: 'LR', 'OW', 'FR' and 'FL'. 'FL' was differentiated into orientation and foraging flights. Multivariate regression mixed models were applied to explore how the above abiotic and biotic factors regulate a bee's outdoor behaviors over life history. The analysis of variability and consistency of behaviors responding to the above non-social factors at inter- and intra-individual levels were analyzed. These behaviors expressed differently to different factors. Age and time only regulated 'Out' rather than drove their flight decision. A bee's decision to fly was not related with the frequency of 'Out'. However, bees flew shorter distances as they aged. When bees flew more trips, they flew longer and faster in the orientation flight, and flew longer in the foraging flight. Bees were more sensitive to weather condition in initiating 'Out' and foraging flight. Space usage of the comb was also essential in regulating bees' outdoor activities. Meanwhile, each individual possessed their own rhythm with different level of variation in response to non-social factors at between- and within-individual levels.

4.2 Introduction

Flight activity is a key component of foraging food to determine a colony's fitness. Honeybees are unique navigators communicating flight information through waggle dances (von Frisch 1967). Successful foragers returning from the visited food sources would communicate information of distance and direction to other bees via dance (Dyer 2002, von Frisch 1967). Bees that followed the dances may leave the colony to forage food. Within a foraging flight, bees can memorize the shape, odor of floral sources and landmarks in the surroundings (Scheiner et al. 2004). With increased number of flights, bees gained more and more individual experience (Klein et al. 2019). Orientation flights performed by naïve bees usually occur before foraging flights. It helps bees to be familiar with sun's azimuth and to remember visual information of the hive's surrounding areas, enabling bees to get back home from foraging flights. When the experienced bees failed or was disrupted in a foraging flight (Degen et al. 2018), they could switch from foraging to orientation flight to update navigation memory.

To make a decision to forage, bees mainly rely on social information about the location and quality of food sources via dance (Couvillon et al. 2012, Dyer 2002, von Frisch 1967) or use private experience to the old food places where they visited before (Al Toufailia et al. 2013, Van Nest & Moore 2012). They also can integrate social information from dances and their own flight experience to make an intermediate decision (Menzel et al. 2011). The odorant cues that bees learning during dance following (Farina et al. 2005, 2007) via trophallaxis or food sources uploaded in the hive may also stimulate bees to collect food (Grüter & Farina 2009). Bees can also make use of other social signals, such as the stop signal (Kietzman & Visscher 2015, Seeley et al. 2012) or the trembling dance (Kirchner 1993, Seeley 1992) to stop foraging activity. Several studies have suggested that orientation flight can be also initiated by odor learnt via trophallaxis (Chaffiol et al. 2005, Ikeno et al. 2014). However, the threshold to food-associated stimuli (e.g., specific location, smell, visual cues) can be different among bees (Scheiner et al. 2004), the decision-making to perform a flight would thus be different.

Despite intrinsic factors including social learning and response threshold mainly determine a bee's decision on whether to leave the colony and perform a flight, bees also adjust their behaviors flexibly in response to a changing environment (Hamilton et al. 2019). This adaptive process usually accompanies with biotic factors including

age-dependent physiological adaptation (e.g., development of flight muscle) (Roberts & Elekonich 2005) and activity bouts. The external environmental abiotic factors include colony size, usage of hive space and the availability of food storage (Hamilton et al. 2019, Robinson et al. 1992), the quality and quantity of food sources (Grüter & Farina 2007, Polatto et al. 2014, Seeley 1992), climate factors (e.g., temperature, cloudiness and wind speed) (Clarke & Robert 2018, Polatto et al. 2014). Honeybees' time sense is highly adaptive, allowing bees to synchronize the foraging activity with the daily floral rhythms (Moore 2001, Moore et al. 1989). The circadian rhythms always depend on age (Toma et al. 2000), biotic and abiotic environmental factors (Hut & Beersma 2011), which may regulate bees' outdoor activities. Variations of behavioral phenotypes across species, across populations of the same species, across individuals of the same population and across repeated observations of the same individual are the most striking features in the natural world (Sih et al. 2004). Within a social community, phenotypic variability among individual bees gives rise to labor division. Unlike queens and drones specializing in producing offsprings, workers take part in non-productive tasks (e.g., nursing, cleaning, building and defending, scouting and foraging). Labor division is a colony feature whereas task specialization is an individual characteristic (Pasquaretta & Jeanson 2018). Workers show within-caste and within-individual variations in behavioral phenotypes which is termed as *polytheism* (Evans & Wheeler 2001, Whitman & Agrawal 2009). That is individuals specialise in subsets of tasks based on physiological changes that is correlated with age (Jones et al. 2020, Khamis et al. 2015, Robinson 1992), morphology difference (Wang et al. 2010), colony need and external environmental change. For a long time, intra- and inter-individual variations have been recognized as 'noise' rather than meaningful phenotypic variation (Guscelli et al. 2019). Heterogeneity, both among- and within-individual could provide new insights into how individual animals evolve and how the behavioral patterns develop in an environment with fluctuating challenges. Between-individual variation concerns with individual animals' average *behavioral expression* while within-individual variation refers to *behavioral plasticity* in response to biotic and abiotic environment changes (Hertel et al. 2020). Questions about within- and between-individual variation require that phenotypic (co)variation be partitioned into variance components (Lynch & Walsh 1998).

Biological and ecological datasets are often highly structured and hierarchical in nature (Harrison et al. 2018) that fall outside the scope of basic statistics (Bolker et al. 2009). They are always binary and non-normally distributed or count data. Where basic statistics quantifies effects of each predictor, it often involves random effects that exists across temporal and spatial observation studies. To handle these limits, mixed models provide a good tool to flexibly estimate the biological effects of interest. It incorporated components of both fixed factors which are used to define systematic influence and random effects, which account for variability among individuals around the systematic relationships captured by the fixed effects (Bolker et al. 2009) provide an efficient tool for examining between- and within-individual differences. On the individual level, individuals' average phenotypic *behavioral types* can be interpreted by *repeatability* (R) (the random-intercept variation of reaction norm) (Hertel et al. 2020). Within-individual variation under specific context or temporal can be explained by random slope, explaining individual animal's response to changes in specific environment (Hertel et al. 2020, Nakayama et al. 2016).

These early studies (Clarke & Robert 2018, Grüter & Farina 2007, Hamilton et al. 2019, Polatto et al. 2014, Robinson 1992, Seeley 1992) on the influences of non-social factors of abiotic and biotic factors on bees' behaviors did provide evidences to understand better about bees' adaptability to external environment, but these studies viewed bees' behavioral response as a single group unit. Recently, several studies in the bumble bees (Klein et al. 2017) and one study in the honeybees (Walton & Toth 2016) have shed light on bees' behavioral variation on consistent inter- and intra-individual levels. However, these studies did not focus on exploring how non-social factors regulate individual bees' leaving the colony and approaching the hive exit. The honeybees' behavioral response to environment changes differs depending on the colony size (Beekman et al. 2004, Bonoan et al. 2020, Smith et al. 2017). My observation bee colony equipped with one comb (~ 800 bees) to observe social behaviors inside colony. Flight activity is a cost-consuming, gain and risk-coexist process in a highly variable environment that requires bees to flexibly take strategies to minimize costs and optimize energetic efficiency (Stabentheiner & Kovac 2014, 2016). Therefore, this chapter will ask how the non-social factors, such as weather condition, age, colony demography i) bees' leaving the colony to the hive exit, 2) how orientation and foraging flights are performed in response to these factors, 3) how these outdoor behaviors vary among and between individual bees.

4.3 Methods

4.3.1 Colony

The bee colony was kept in a tiny observation hive equipped with a frame. Compared to a full standard frame of 'Zander' type as 2*20*40 cm with 2*1000 bees and 2*3200 cells and 'German normal' type as 2*20*35 cm with 2*900 bees and 2*2800 cells, the observation frame with 20*14.5 cm was fully composed of a queen, 1160*2 cells (including brood and food) and 370*2 adult workers. Eighty-seven newly emerged bees aged 'day 1' attached with number tags on the bees' thorax in order to recognize bees' identities were introduced into the colony. These 87 bees with number from 201 to 297, excluding the numbers that could be confused when experimenter read them upside-down, were the focal bees for behavioral study. Over 200 bees without knowing ages (with number among 1 ~ 197, 301 ~ 397 and 401 ~ 497 depending on the orientation of the number tag) were marked in order to induce dances during training procedure. Daily behaviors of the honeybees inside the colony were recorded with Raspberry Pi 3. The video recording was started at around 9:00 am and ran till 9:00 pm. Although the colony was tiny, it remained a great difficulty to count the number of bees for the whole colony. Currently, there is not a 100% precise way to measure the colony size. Therefore, I referred to 'Liebefeld Method' (Dainat 2020) and the official Agroscope website: https://www.agroscope.admin.ch/agroscope/en/home/topics/livestock/bees/biologie/volksentwicklung/bienen-schaetzmethode.html to estimate the colony strength. The relative strength of the colony with brood cells, honey cells, empty cells and adult workers over each day were subjectively measured.

4.3.2 Monitoring of behaviors outside the colony

Focal bees' behaviors at the hive exit were recorded. When bees appeared at the inside of the hive exit, the time and additionally note whether they immediately return into the colony or continue to go out to the outside of the hive exit were recorded. When bees appeared at the outside of the hive exit and tended to rush out for flights, they were caught immediately and released after a transponder was attached to the bee's thorax. After that, the status of the released bees was noted down. Here, 'Out' described status of the honeybees leaving the colony. This behavior was differentiated into 4 subclasses: 'LR', 'OW', 'FR' and 'FL'. 'LR' indicated bees appeared at the inside of hive exit but soon returned into the colony. 'OW' represented bees that left the colony were caught and released with a transponder

attached but did not fly up. 'FR' described the bees returned to the hive exit after flying up close to the hive exit in the immediate surroundings. 'FL' characterized the bees performing successful flights with the radar transponder. 'FL' was discriminated between flight to explore the surrounding environment around the hive ('Orientation') and flight to collect food ('Foraging'). 'OW' and 'FR' with status of 'Can't' and 'Won't' were also noted. 'Can't' characterized those bees were physically unable to fly because they were visibly too weak to fly. 'Won't' characterized bees did not fly up because the number tag or transponder interfered with their fight motivation. When there were too many bees appearing at the hive exit, they were demotivated by placing each respective bee in a separate device under a black cover for around 5 minutes bees and let them walk back into the hive. A few bees returned from an extended flight without transponder and some bees did not return to colony at all. All the above cases were reliably recorded with a complete protocol (Table 3)

4.3.3 Weather recording

The climate conditions of temperature, wind speed and weather (clear, partial cloudy, most cloudy and overcast) from 9:00 am to 21:00 over a day between 24th July and 7th August, 2018 for the local Marburger Ring, Kirchhain, Hesse, Germany was referred on the website: https://darksky.net/forecast/40.7127,-74.0059/us12/en.

4.3.4 Data preparation and cleaning

According to the protocol about different cases of behaviors at the hive exit, the cases of bees that were physically unable to fly or got arbitrary demotivated were excluded. Successful flight, i) the bees did not return back to colony, ii) the bees performed successful flights while the transponders was lost during the flight or iii) they escaped to fly before a transponder for radar tracks was attached were excluded. Data cleaning was shown in the Table 3.

4.4 Data statistical analyses

R packages including 'lme4', 'lmerTest', 'optimx', 'car', 'MuMIn', 'plm', 'DHARM', 'mlogit', 'performance' and 'sjPlot' were applied to perform statistical analyses in R v4.0.2 (R Core Team 2020). The packages 'lme4' and 'lmerTest' were used to build linear models (LMs), linear mixed models (LMMs), generalized linear mixed models (GLMMs) and generalized linear model (GLMs). Step regression method and manual establishment were combined to construct models. Types and structures of models were designed based on the questions to be addressed and the distribution of response variables. The package 'optimx' was applied to optimize the regression

models accompanied with the iterative algorithm optimizer 'BOBYQA'. The package 'car' was applied to calculate type-II or type-III analyses-of-variance for models depending on whether interactive factors were established. The package 'MuMIn' was used for calculating marginal and conditional R^2 to explain the proportion of variance by the fixed effects and by both the fixed and random effects respectively in the mixed models. The package 'plm' was conducted for determining whether panel variables can be fixed effect or random effect in the panel dataset. AIC (Akaike information criterion) and BIC (Bayesian information criterion) were mainly used to select fitted model. To further confirm the model fit, diagnostic plots to detect the linearity of data, normality of residuals, homogeneity of residuals variance and independence of residuals variance error terms were checked for LMs. glm.diag.plots() function in the package 'boot' was applied to evaluate GLMs. Marginal R^2 [variance explained by the fixed factors and covariates, R^2(G)LMM (m)], conditional R^2 [variance explained by the fixed and random factors, R^2(G)LMM (c)] and visual inspection of model diagnostic were combined to evaluate the fitted models of GLMMs and LMMs. Overdispersion of (G)LMMs caused by zero-inflation, aggregation was detected via the package 'DHARMA'. The vif() function in the package 'car' was used to test multicollinearity among the predicted variables. Some predicted variables were necessarily excluded when correlation between two predicted variables was extremely high. When the residuals showed bad normal distribution and the models were over-fitted, the response variables were log-transformed, and the data was re-modeled.

To analyze a single model, deviance analyses was performed by Chisq test with Anova() function in the package 'car' in the GLM(M)s while F test for variance analyses with anova() function in the package 'stats' in LM(M)s. LRT (Likelihood Ratio Test) was applied to compare two nested models. The package 'mlogit' was used to establish multinomial logistic regression models when the response variables were more than two levels.

To explore the influences of the non-social factors (i.e., daytime, bee age, frequency of activity event, temperature, wind speed, weather, colony condition including temporal distribution of capped brood, empty cell, honey cell and adult workers) on bees' outdoor activities, the following stepwise questions were addressed: whether these non-social factors influenced bees': step 1) leaving the colony to the hive exit ('Out'), step 2) preference on 'LR', 'OW', 'FR' and 'FL', step 3) frequency of the

orientation flight ('O') or foraging flight ('F'), step 4) flight performance on the orientation and the foraging flights including the parameters of flight speed, duration, range and loops.

All the above model analyses firstly included the variable of individual bees ('BeeID') as the random effect for accounting the non-independence of measurements on the same individual through time. When 'BeeID' showed no significant variance in the random effects, it was excluded from the random effect in the GLMMs and LMMs and LM and GLM were considered. When necessary, the influences of age, daytime and frequency of behavioral events nested in the random effect along with 'BeeID'.

On the basis of the best-fit models, how the between- and within-individual variances varied across multiple behaviors was discussed. Multivariate mixed models provided powerful tools to decompose the observed behavioral variation into components of the fixed effects and random effects (Araya-Ajoy et al. 2014). 'BeeID' as random intercept and context-specific variables as random slope and their correlation allowed to estimate whether the individual bees differed in i) behavioral type based on the random intercept and ii) behavioral plasticity across specific contexts based on random slope. To assess the inter- and intra-individual difference, I tested for the significance of random intercept and random slope effects by applying a likelihood ratio test (LRT), comparing the models with random intercept or slope with models without random effects. To quantify inter- and intra-individual variability, adjusted intra class correlation (ICC) and behavioral *repeatability* (R) were calculated to examine the consistency of different behaviors among individual bees.

4.5 Results

4.5.1 Data cleaning

Data for each step of analyze before starting model building was cleaned. The purposes for these four steps are listed (Table 3). In the first step, two focal bees never appeared at the hive exit in their whole life ('Bee not out'). Hence, 85 focal bees were included into model building. In the second step, two out of 87 focal bees appeared at the hive exit were manually demotivated due to a high activity at the exit and long waiting time which lead to a delay in releasing these bees ('Bee demotivated'). In the third step, four focal bees rushed out too quickly to be caught from the hive exit ('Bee escaped without transponder') without flight trajectories recorded from the radar. Four bees flew out with transponder but did not return back

to the colony ('Bee not back with transponder'). One bee flew out with transponder but the transponder dropped and no flight trajectory was recorded from the radar ('Bee with transponder lost'). Two bees flew out without any information from the radar track protocol ('Unsure'). Eight bees appeared at the hive exit but did not carry out a flight in their life ('Bee without flight').

Chapter 2 was focused on investigating bees' outdoor activities at the hive exit while the next chapter 3 was focused on investigating the relation between the indoor social behaviors and the outdoor flights. However, manual tracking of the social behaviors over the focal bees' life history was a marathon job. It required a large amount of time to collect the data of 87 bees' social behaviors over their life span. To compare the frequency and age when bees performed the orientation and foraging flights and the patterns of activities at the hive exit (e.g., some bees appearing at the hive exit frequently, but did not perform any successful flight), 17 bees were studied.

Table 3. Clean data for the analyses of the behaviors at the hive exit

Step 1_'Out': to examine whether the non-social factors influence bees' decision whether to leave from colony to hive exit	**Numbers**
Total marked focal bee	87
Bee not out	2
Bee for model	85

Step 2_'LR/OW-FR-FL': to examine whether the non-social factors affect bees' behavioral switch among 'LR', 'OW', 'FR' and 'FL'	**Numbers**
Total marked focal bee	87
Bee demotivated	2
Bee for model	85

Step 3_'O-F': to examine whether the non-social factors affect bees' behavior to perform orientation flight or foraging flight	**Numbers**
Total marked focal bee	87
Bee escaped without transponder	4
Bee not back with transponder	4
Bee with transponder lost	1
Unsure	2
Bee without flight	8
Bee with orientation flight	75
Bee with foraging flight	5
Bee for models	17

Note: number of the bees excluded from each step and the bees remained for further model analyses are listed.

Table 3. Clean data for the analyses of the behaviors at the hive exit

Step 4_Flight performance: to examine whether the non-social factors affect bees' performance in orientation and foraging flights	**Numbers**
Total marked focal bee	87
Bee escaped without transponder	4
Bee not back with transponder	4
Bee with transponder lost	1
Unsure	2
Bee without flight	8
Bee with orientation flight	75
Bee with foraging flight	5
Bee for models	17

Note: number of the bees excluded from each step and the bees remained for further model analyses are listed.

4.5.2 Overall variation in behaviors outside colony among individual bees over life history

Out of the 87 focal bees, 85 bees appeared at the hive exit after leaving the colony. Of which, 75% appeared at the hive exit for 1 ~ 5 times, while only 9% appeared at the hive exit 10 ~ 38 times within 15 days, including bees 216, 221, 244, 257, 267, 271, 275 and 282 (Figure 2). The first time when bees left the colony to the hive exit were mostly at the age between 6th ~ 7th days. However, the early age of day to leave the colony could also at 3rd day after bees' emergence (Figure 3a). Most of the 85 focal bees started to leave the colony in the midafternoon and reduced the outdoor activities by the nightfall (Figure 3b).

When the bees appeared at the hive exit, they behaved differently which were differentiated into 4 patterns. A few bees returned into the colony immediately after appearing at the hive exit ('LR'). 'OW' was when the bees rushed out from the inside hive exit but did not fly up after release. Although a few bees flew up close to the hive exit, they immediately returned to the colony. This case was called as 'FR'. 'FL' meant the focal bees performed successful a flight.

When the 85 focal bees appeared for the first time at the hive exit, 55 bees flew up ('FL'), 19 bees walked around the hive exit without flying up ('OW') and 11 bees returned to the colony immediately ('LR'). Nine bees leaving the colony did not perform flights within 15 days (Figure 4a). In particular, bees 257 (12 'Out': 12 'OW'), 275 (12 'Out': 8 'LR', 4 'OW') and 282 (10 'Out': 10 'OW') appeared at the hive exit over 10 times without flights. As for the 76 bees performed successful flights, a few bees performed the first flights in the early age of 4 days old, while most were at the age of 6 ~ 7 days old. The daytime when the bees initiated 'LR', 'OW', 'FR' or 'FL' was not in a sequential order. Bees could perform 'LR', 'OW' or 'FL' in the early time of a day and returned to the hive exit immediately after flying up ('FR') in the mid-afternoon even though they have performed multiplel flights before (Figure 4b). Of them, 83 bees performed flights after leaving the colony. Ninety-three percent of them only performed the orientation flights and only 5 bees (i.e., bees 216, 244, 262, 267, 271 and 276) performed the foraging flights (Figure 5a). Bees typically performed orientation and foraging flights after the age of 6 and 9 days old, respectively. However, they could also start to perform these two types of flights as early as at the age of 4 and 6 days old, respectively (Figure 5a). Bees could switch between orientation and foraging flights. Interestingly, all the other bees started the first foraging flight only after orientation flights, except that bee 263 initiated foraging flight before the first orientation flight (Figure 5a). Within different time periods of a day, bees performed orientation and foraging flights randomly without a specific sequence (Figure 5b).
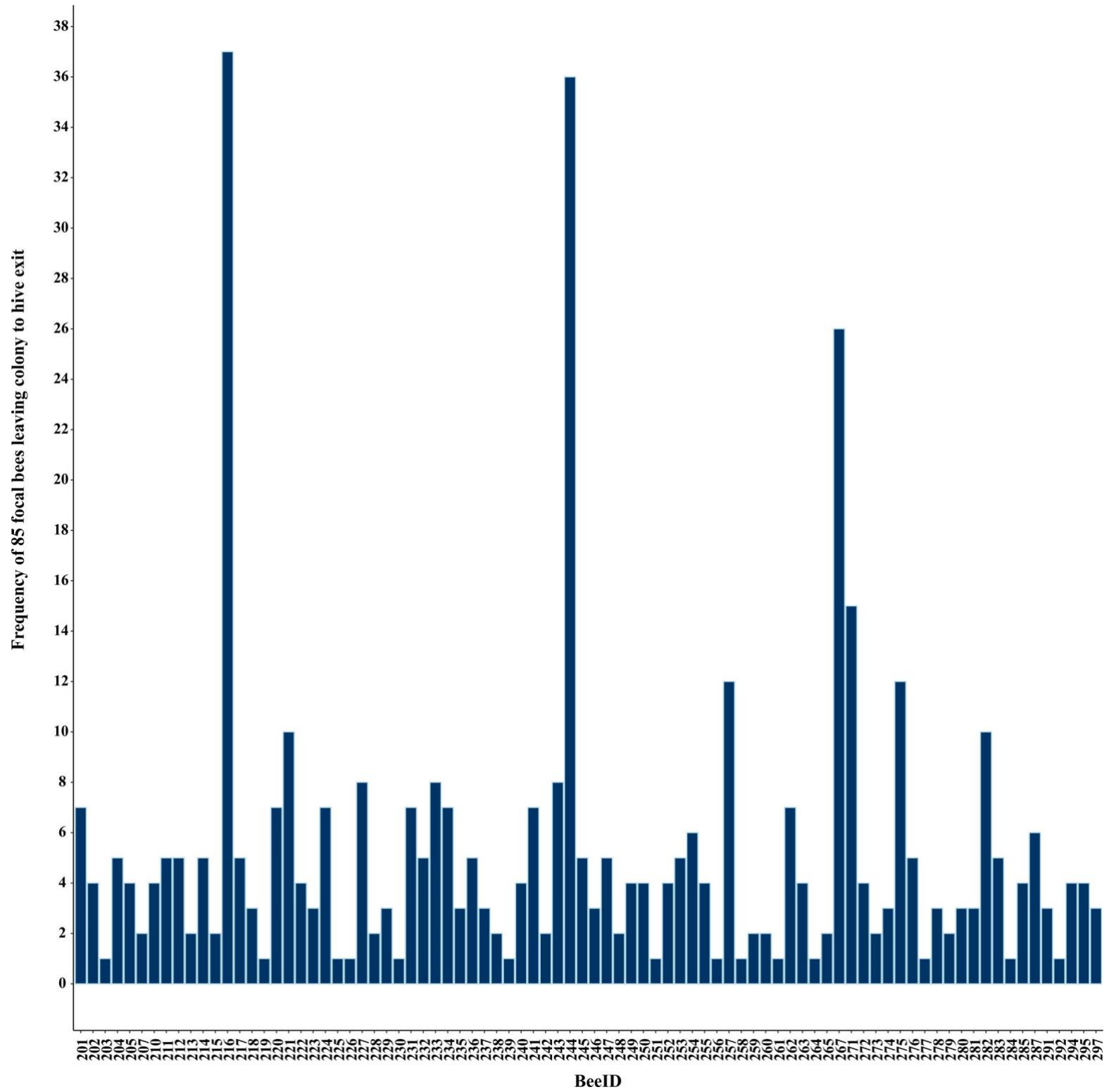
Figure 2. Frequency of the 85 focal bees' leaving the colony to the hive exit
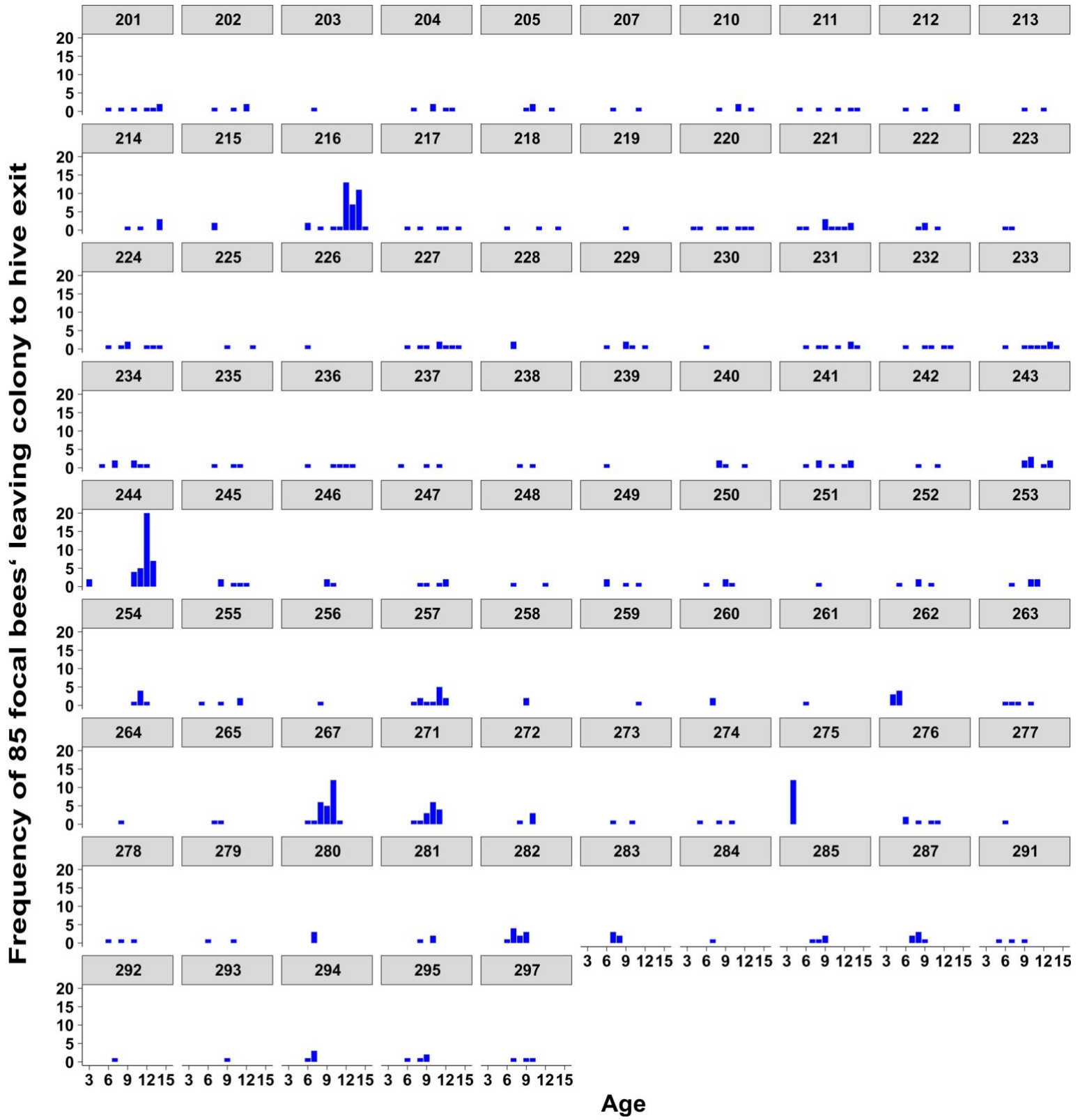
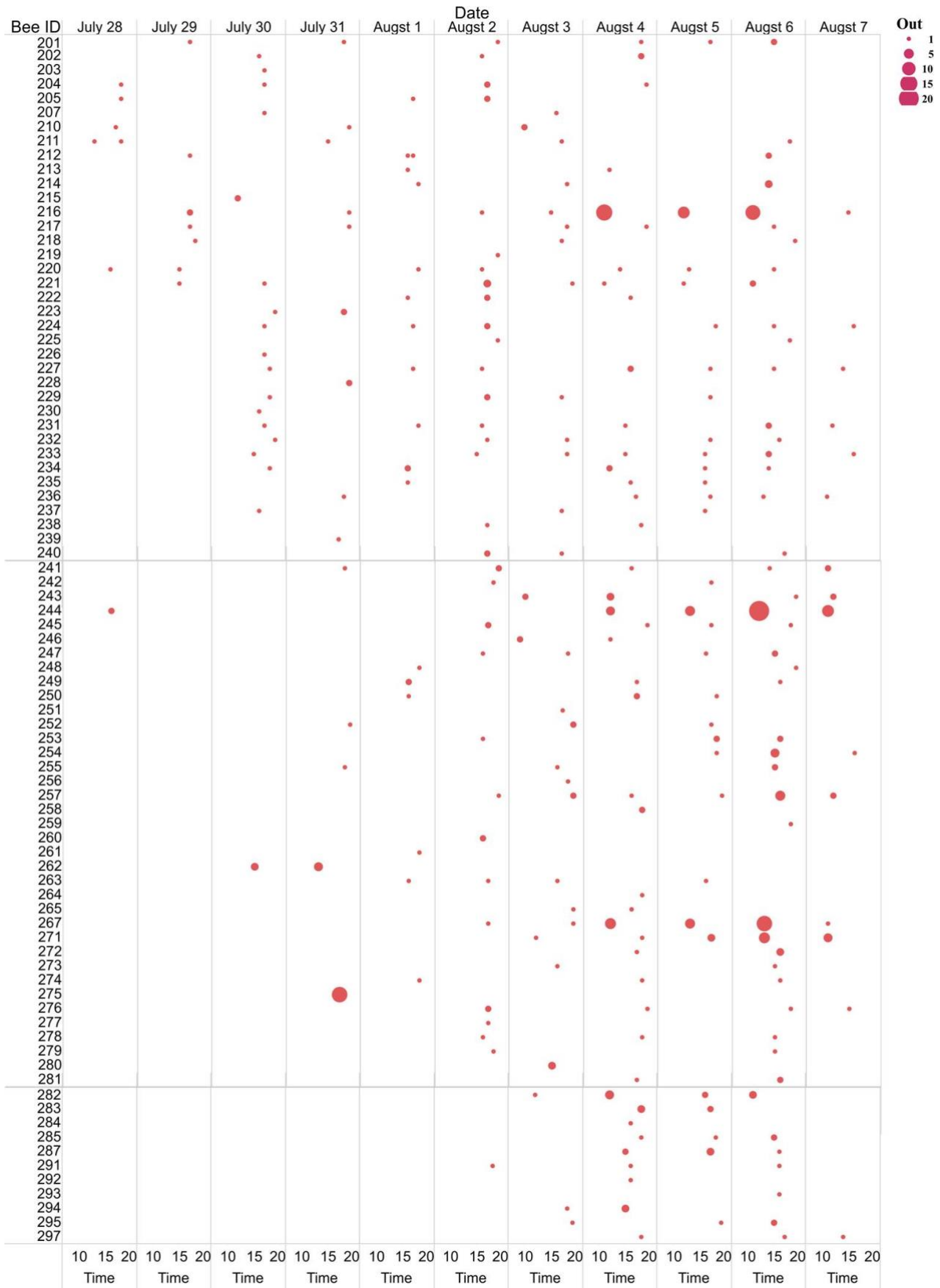Figure 3a. Frequency of the 85 focal bees' leaving the colony to the hive exit over ages

Figure 3b. Events of the 85 focal bees' leaving the colony to the hive exit during daytime.

The size of red circle indicates the frequency for each bee at different daytime over dates. Number ranging from 201 to 297 represents the identity of the 85 focal bees

Figure 4a. Frequency of 'LR', 'OW', 'FR' and 'FL' of the 85 focal bees over ages.

'LR', bees appear at the hive exit, but soon return into colony; 'OW', bees rush out to the outside of the hive exit, but do not fly up after being released with transponder; 'FR', bees fly up close to the hive exit with transponder, but soon return to the hive; 'FL', bees perform a complete flight with transponder.
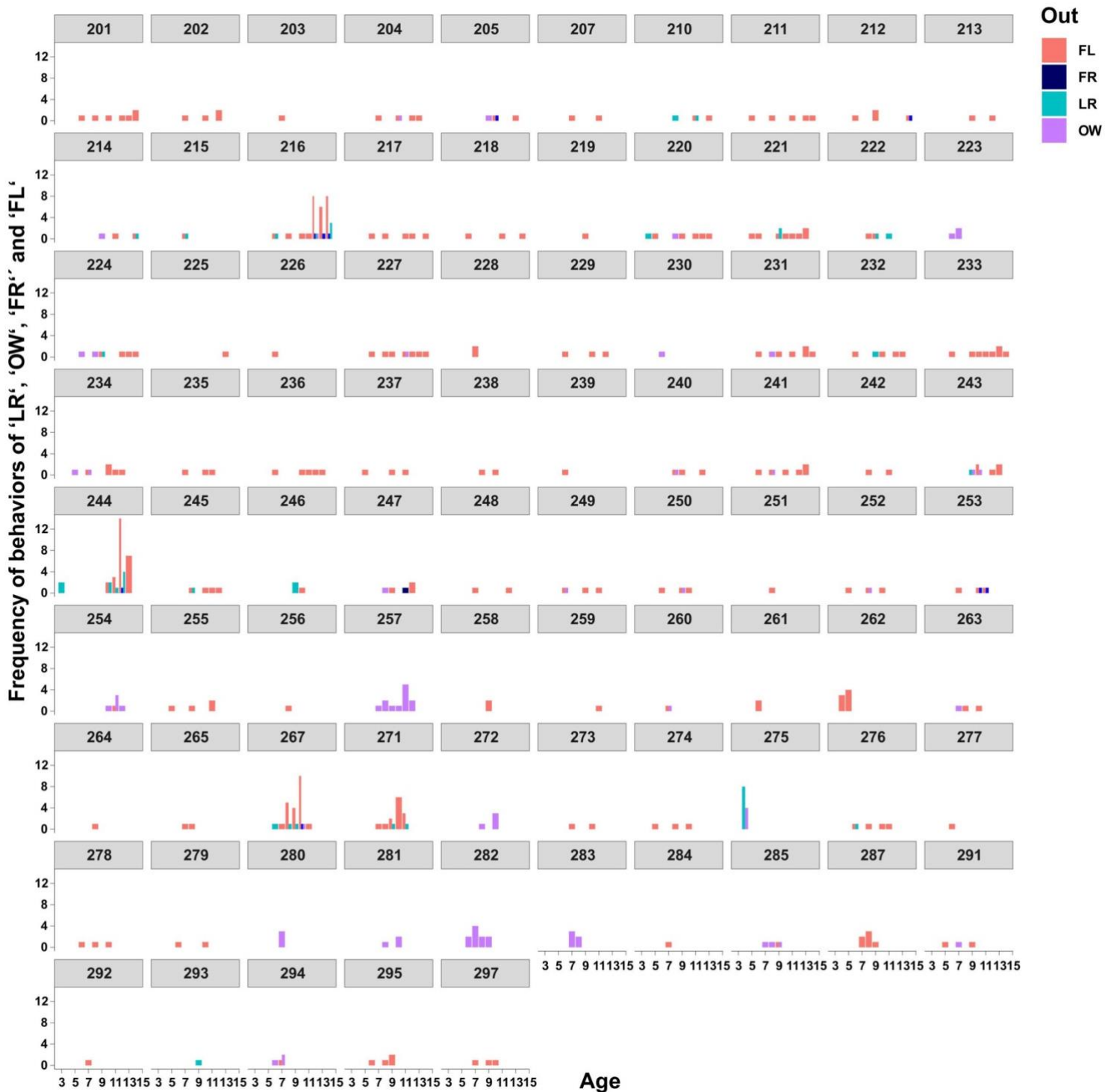
Figure 4b. Events of 'LR', 'OW', 'FR' and 'FL' of the 85 focal bees in the daytime.

'LR', bees appear at the hive exit, but soon return into the colony; 'OW', bees rush out to the outside

of hive exit, but do not fly up after being released with transponder; 'FR', bees fly up close to the hive

exit with transponder, but soon return to the hive; 'FL', bees with transponder flw away from the hive exit and perform successful flights. The size of circle indicates the frequency of behaviors.
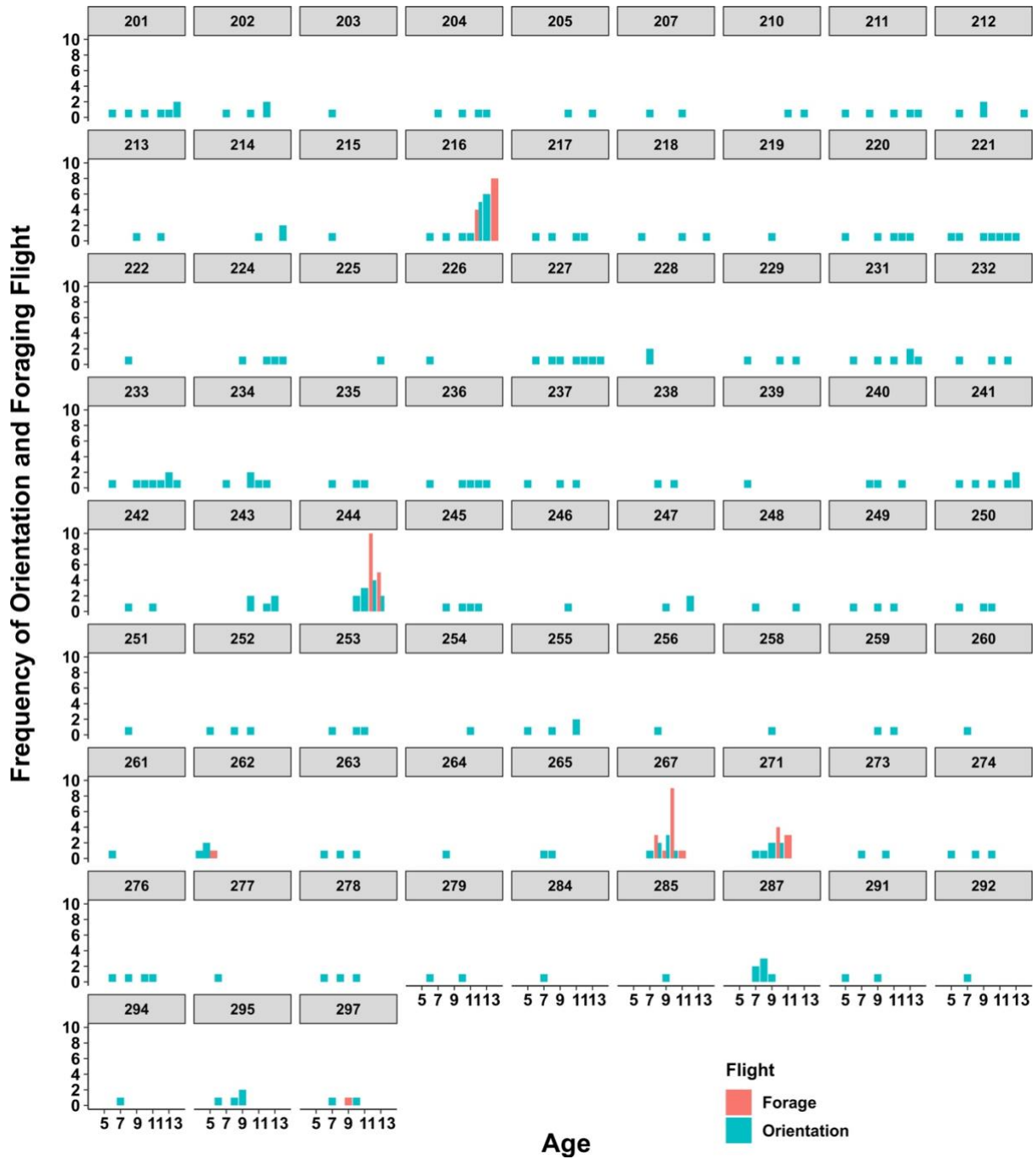

Figure 5a. Frequency of orientation and foraging flights among the 83 focal bees over ages.
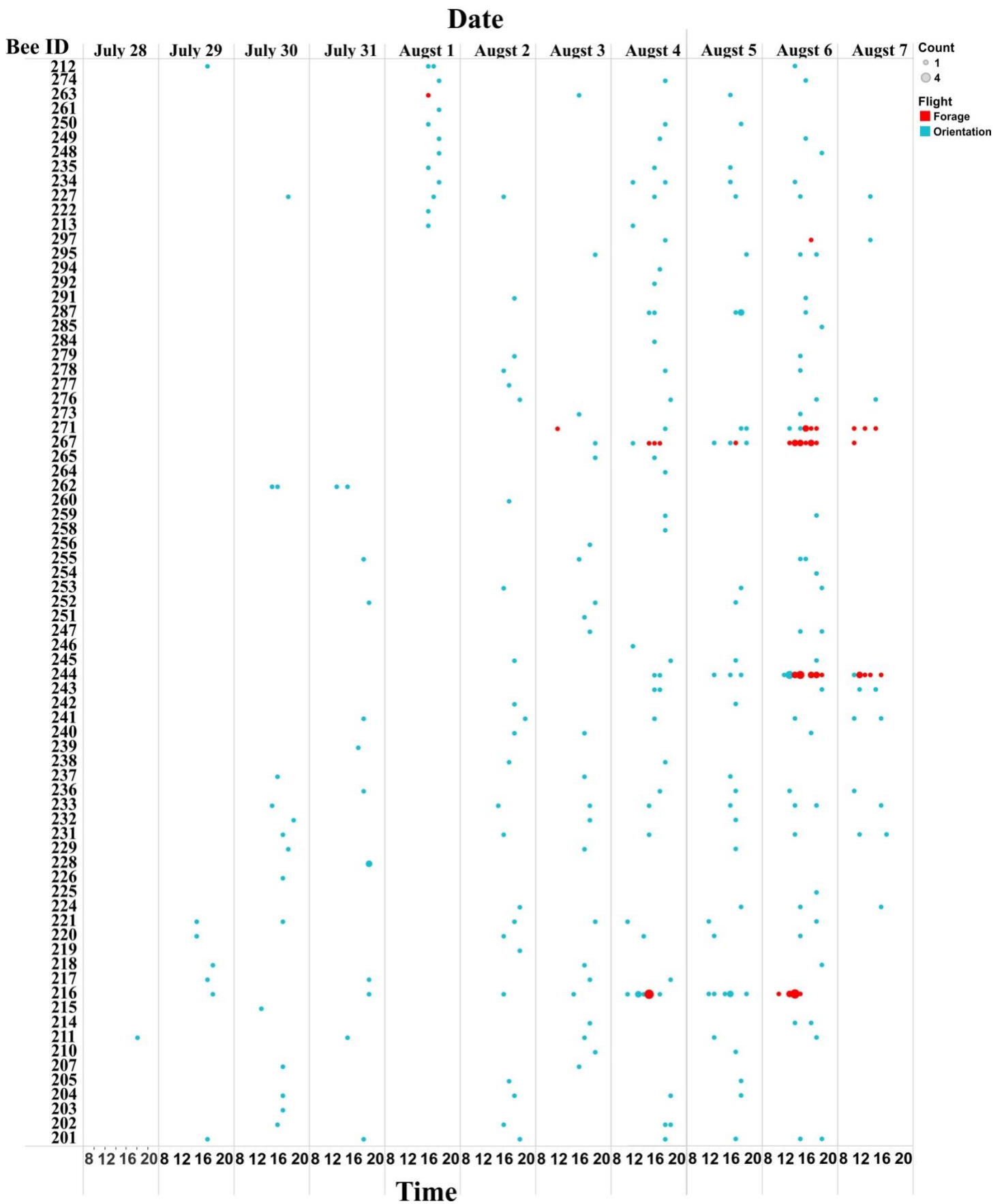
Figure 5b. Events of orientation and foraging flights among the 83 focal bees during daytime. The size of circle indicates the frequency of flights.

4.5.3 Influence of non-social factors on bees' outdoor behaviors

Given the variation in the different behavioral expression over daytime and age (Fig 2 ~ 5b), whether individual bees differed in the way they shifted between different behaviors were tested by fitting random slopes of daytime and age and nested them into the random factors along with bee identities ('BeeID'). Whether non-social factors influenced bees' behaviors at the hive exit was investigated. These non-social factors were: age, 'Age'; frequency of bees' leaving the colony to the hive exit: 'NoO'; frequency of the flights: 'NoF'; the daytime when a behavior occurred: 'Time'; temperature: 'Temp' (°); wind speed: 'Wind' (mph); weather condition: 'Weather' (clear, partial cloudy, most cloudy and overcast), colony constitution: brood: 'Brood'; empty cells: 'Empty cell'; honey cells: 'Honey cell' and adult workers: 'Worker'.

4.5.3.1 Bees left the colony to the hive exit

The dataset of 'Out' were binary, therefore, GLMM was applied to test whether the aforementioned non-social factors (i.e., 'Age', 'Time', 'Temp', 'Wind', 'Weather', 'Brood', 'Honey cell', 'Empty cell' and 'Worker') motivated the bees to leave the colony to the hive exit ('Out'). The most common concern for applying GLMMs is over-dispersion, under-dispersion and zero-inflation, therefore, performance of the best fitted model was checked (Table S1). 'Time' nested in the random effect of a model that failed to converge (Table 4, model H2) was excluded from the random effects. After a better model (H5) was fitted (Table 4, in grey), a more complicated model with interaction factors between fixed effects were investigated ('Temp*Worker' in the model H6) to see whether temperature co-influence with colony's temporal distribution on 'Out'. Although the interaction factor improved model fitting with lower AIC and BIC compared to the model H5, no significant differences were found for influence of the interaction on 'Out' ('Temp*Worker' in the model H6: Chisq = 3.53, df = 1, $p = 0.06$). Therefore, model H5 was the best fitted model. Within model H5, marginal R^2_{GLMM} (R_GLMM (m^2)) indicated 86% of the variance can be explained by the fixed effects (Table 4). 'Out' differed significantly in response to 'Age' (Chisq = 23.9, df = 1, $p < 0.01$), 'Temp' (Chisq = 9.7, df = 1, $p < 0.01$), 'Wind' (Chisq = 26.1, df = 1, $p < 0.01$), 'Empty cell' (Chisq = 8.2, df = 1, $p < 0.01$), Worker' (Chisq =13.6, df = 1, $p < 0.01$) and 'Time' (Chisq = 187.5, df = 12, $p < 0.01$). As expected, frequency of 'Out' increased as bees aged (Figure 6A) while higher temperature and stronger wind would reduce the possibility of 'Out' (Figure 6B, C). With more comb space available and lower number of workers, bees were

more likely to leave the colony to the hive exit (Figure 6D, E). A quadratic relationship between time and 'Out' was also found (Figure 6F). Focal bees started to increase outdoor activities in the midday, reach a peak in the late afternoon and gradually decrease activity of 'Out' in the early evening.

Table 4. Influence of age, colony population, and daytime on 'Out' of the 83 focal bees' leaving the colony to the hive exit

Tested Models	AIC	BIC	R_GLMM $(m)^2$	R_GLMM $(c)^2$	Random effect		
					Group	Variance	Std.Dev.
Null: Out ~ (1|BeeID)	3717.7	3732.8	0	0.14	BeeID	0.52	0.72
H1: Out ~ (Age|BeeID)	3491.1	3521.4	0	0.22	BeeID	1.02	1.02
					Age	0.04	0.21
H2: Out ~ (Time|BeeID)	3540.3	4235.6	/	/	BeeID	/	/
					Time	/	/
H3: Out ~ Age + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (Age|BeeID)	2929.8	3126.3	0.86	0.89	BeeID	1.16	1.08
					Age	0.03	0.19
H4: Out ~ Age + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (1|BeeID)	2997.1	3178.5	0.85	0.88	BeeID	0.77	0.88
H5: Out ~ Age + Temp + Wind + Empty cell + Worker + Time + (Age|BeeID)	2944.9	3103.6	0.86	0.89	BeeID	0.73	0.85
					Age	0.02	0.15
H6: Out ~ Age + Temp*Worker + Wind + Empty cell + Time + (Age|BeeID)	2943.2	3109.5	0.86	0.89	BeeID	0.74	0.86
					Age	0.02	0.15

Note: R_GLMM(m^2) and R_GLMM(c^2) provide the variance explained only by fixed effects and by the entire model, respectively. AIC and BIC are used for model selection, with lowest index is selected. Variance of random effect indicates the how much variability is between individuals across all treatments
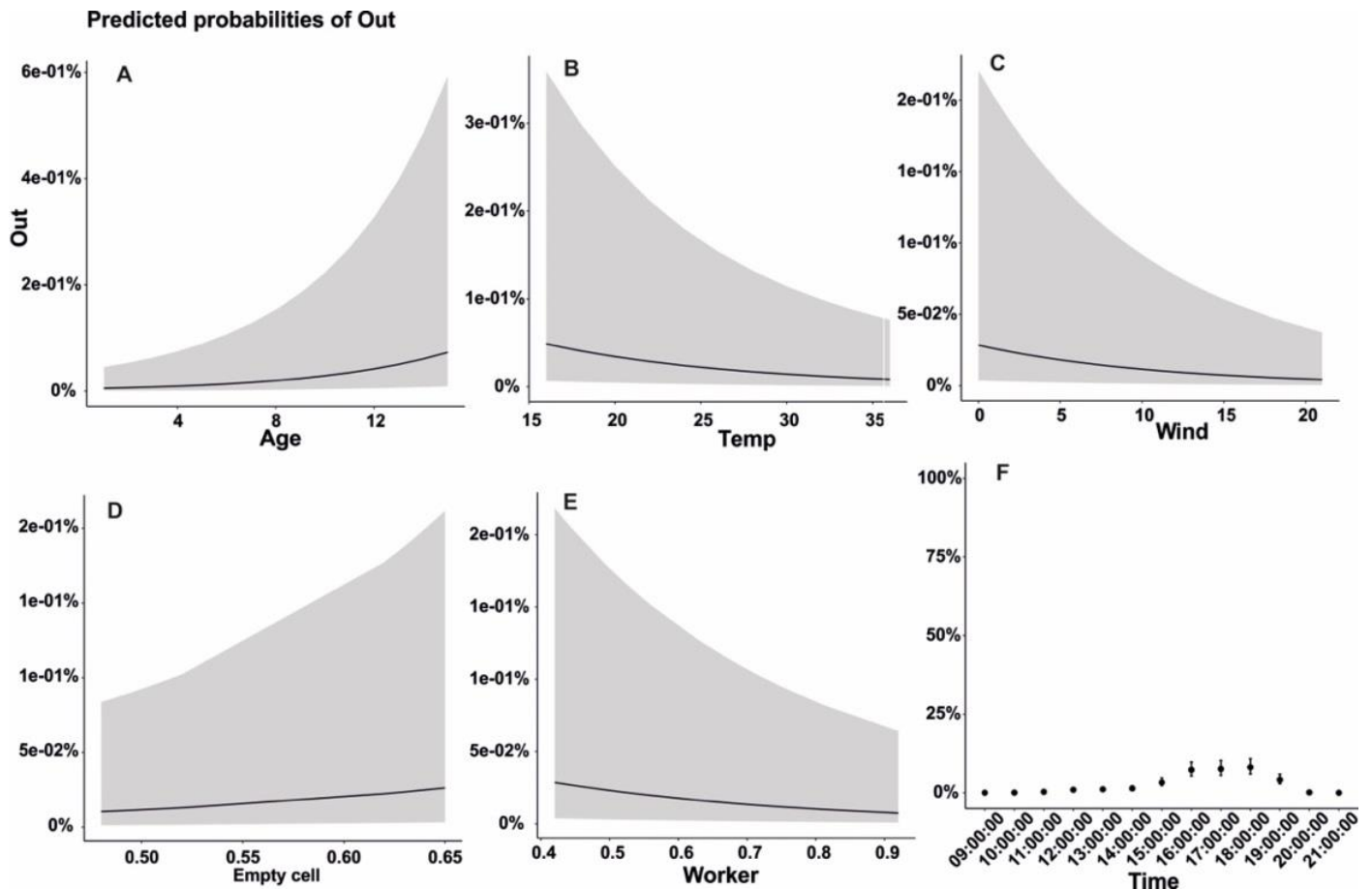
Figure 6. Predicted probabilities of bees' leaving the colony to hive exit plotted against temperature, wind speed, distribution of empty cells, number of workers and daytime

4.5.3.2 Preference of bees to perform 'LR', 'OW', 'FR' and 'FL'

When bees appeared at the hive exit ('Out'), they would behave differently. A few bees returned into the colony immediately after appearing at the hive exit ('LR'). When bees rushed out from the inside hive exit but did not fly up after being released, this behavior called 'OW'. Though a few bees flew up, they flew close to the hive exit and would immediately return back to the hive exit. This behavior was 'FR'. 'FL' indicated focal bees performed a flight. Next, the influence of a series of non-social variables on bees' preference to behave 'LR', 'OW', 'FR' or 'FL' was investigated. These variables were: bee age ('Age'), frequency of leaving the colony ('NoOut'), daytime ('Time'), temperature ((°), 'Temp'), wind speed ((mph), 'Wind'), weather (clear, partial cloudy, most cloudy and overcast), distribution of brood cells ('Brood'), empty cells ('Empty cell'), honey cells ('Honey cell') and adult workers ('Worker') within the hive. Frequency of 'FR' was extremely low (2%) compared to

that of 'FL' (69%), 'OW' (19%) and 'LR' (10%). Therefore, 'FR' was excluded from regression analyses and focused on comparing analysis between 'LR' and 'OW' (Table 5), between 'OW' and 'FL' (Table 6). Age and NoOut were tested in the random effect along with BeeID. Age as random factor in LR/OW and OW/FL analyses induced extremely high multicollinearity (model H1 in the Table 5 and 6) and caused zero variance in Age:BeeID as a random intercept (model H2 in the Table 5 and 6). NoOut produced zero variance in NoOut:BeeID as random intercept (model H4 in Table 5 and 6) and showed no significant effect as random slope among all the models. Therefore, 'Age' and 'NoOut' were excluded from the random effect. After model diagnostics (Table S2, S3), model H7 in 'LR'/'OW' (Table 5, in grey) and model H6 in 'OW'/'FL' analyses (Table 6, in grey) were the best fitted models, respectively. Marginal R^2_{GLMM} (R_GLMM (m^2)) showed that 34% of variance could be explained by fixed effects in the model H7 (Table 5) for 'LR'-'OW' analyses. Compared to 'OW', the frequency of 'LR' was significantly higher when temperature increased (Chisq = 7.49, df = 1, $p < 0.01$) and more adult bees were inside the colony (Chisq = 5.56, df = 1, $p =0.02$) (Figure 7A, B). Bees' preference to behave 'LR' or 'OW' was not influenced by the frequency of 'Out' (Chisq = 2.24, df = 1, $p = 0.13$). Marginal R^2_{GLMM} (R_GLMM (m^2)) in the analyses of 'OW'-'FL' suggested that 10% of the variance was explained by the fixed effects (Table 6, H6). Though higher wind speed (Chisq = 2.25, df = 1, $p = 0.13$) and more workers (Chisq = 3.23, df = 1, $p = 0.07$) reduced bees' tendency to carry out successful flights, compared to 'OW', the impact was not significant. As expected, when bees were older (Chisq = 14.8, df = 1, $p <0.01$), they performed more successful flight (Figure 8).

Table 5. Influence of bee age, external and internal colony condition and daytime on individual bees' preference to perform 'LR' or 'OW'

Tested Models	AIC	BIC	R_GLMM (m)²	R_GLMM (c)²	Random effect		
					Group	Variance	Std.Dev.
Null: Out$_{LR/OW}$~(1|BeeID)	119.4	125.0	0	0.76	BeeID	10.66	3.27
H1: Out$_{LR/OW}$~(Age|BeeID)	123.2	134.5	0	0.76	BeeID	6.31	2.51
					Age	0.007	0.08
H2: Out$_{LR/OW}$~(1|BeeID/Age)	121.4	129.8	0	0.76	BeeID	1.07	3.27
					Age:BeeID	<0.0001	<0.0001
H3: Out$_{LR/OW}$~(NoOut|BeeID)	116.8	128.0	0	0.95	BeeID	11.42	3.38
					NoO	0.99	0.99
H4: Out$_{LR/OW}$~(1|BeeID/NoOut)	121.4	129.8	0	0.76	BeeID	10.66	3.27
					NoO:BeeID	0	0
H5: Out$_{LR/OW}$~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + Time + (1|BeeID)	99.1	157.9	0.63	0.98	BeeID	60.43	7.77
H6: Out$_{LR/OW}$~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + (1|BeeID)	101.7	138.1	0.4	0.99	BeeID	170.1	13.04
H7: Out$_{LR/OW}$~Temp + Worker + NoOut + (1|BeeID)	103.5	117.5	0.22	0.84	BeeID	13.24	3.64

Table 6. Influence of age, external and internal colony condition and daytime on individual bees' preference to perform 'OW' or 'FL'

| Tested Models	AIC	BIC	R_GLMM $(m)^2$	R_GLMM $(c)^2$	Random effect		
					Group	Variance	Std.Dev.
Null: Out_ OW/FL~(1|BeeID)	278.3	286.2	0	0.68	BeeID	7.02	2.65
H1: Out_ OW/FL~(Age|BeeID)	264.6	280.4	0	0.79	BeeID	78.05	8.83
					Age	0.34	0.59
H2: Out_ OW/FL~(1|BeeID/Age)	280.3	292.1	0	0.68	BeeID	7	2.64
					Age:BeeID	0	0
H3: Out_OW/FL~(NoOut|BeeID)	276.7	292.5	0	0.78	BeeID	19.50	4.42
					NoO	0.05	0.21
H4: Out_ OW/FL~(1|BeeID/NoOut)	280.3	292.1	0	0.68	BeeID	7.02	2.65
					NoO:BeeID	0	0
H5: Out_ OW/FL~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + Time + (1|BeeID)	280.7	363.4	0.35	0.82	BeeID	8.29	2.88
H6: Out_ OW/FL~Wind + Worker + Age + (1|BeeID)	262.6	282.3	0.10	0.70	BeeID	6.7	2.59
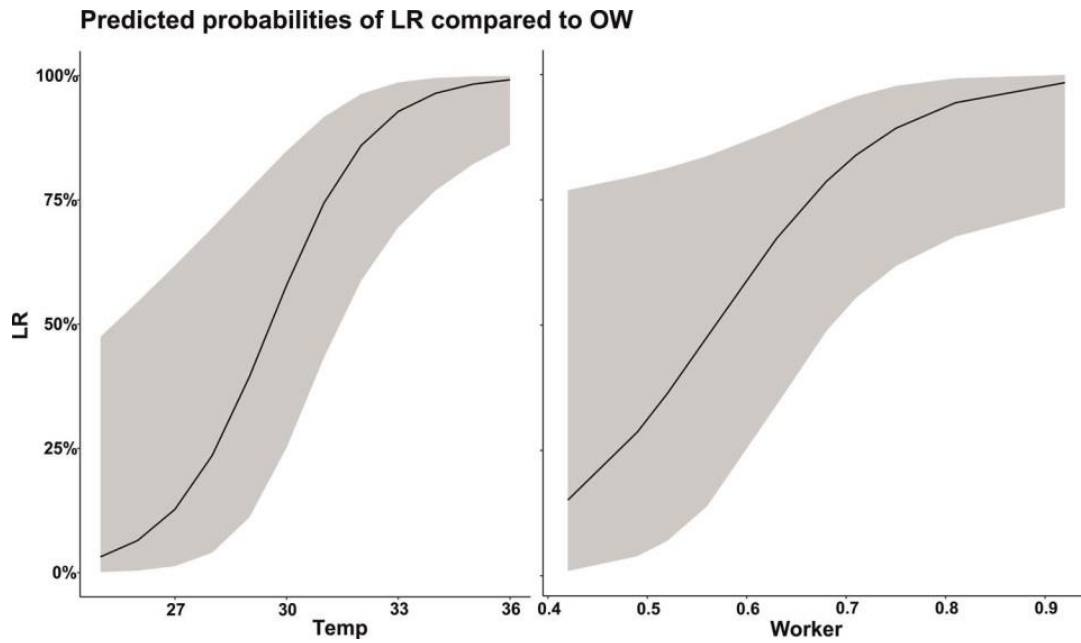
Figure 7. Predicted probabilities of LR compared to OW against temperature and number of workers in the colony. 'LR', when bees left the colony to the hive exit, but soon returned back into colony. 'OW', bees did not fly up after they rushed out from colony to the outside of hive exit, were caught and attached the transponder on the thorax.
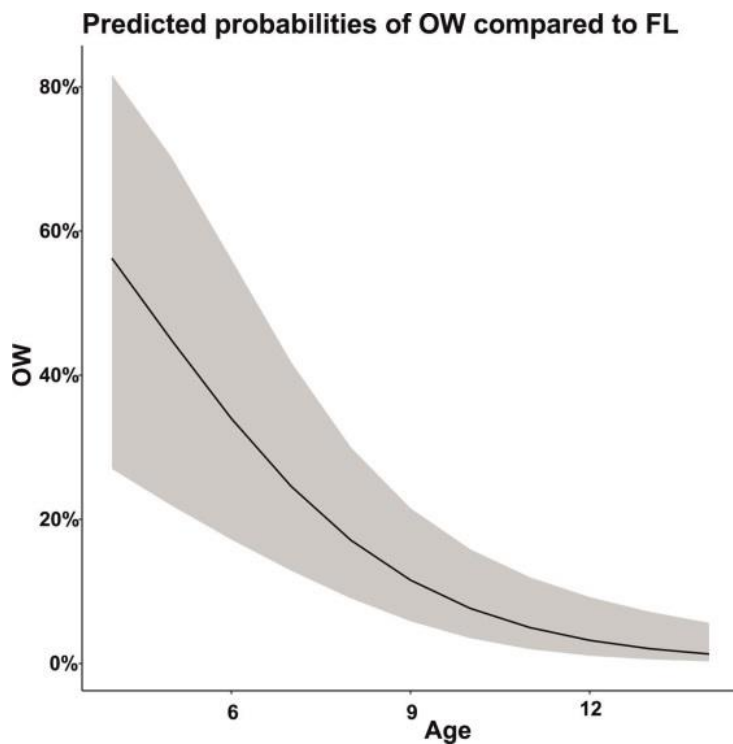

Figure 8. Predicted probabilities of OW compared to FL against age. 'FL', when bees perform flights after being caught, attached the transponders on their thorax and released at the hive exit

4.5.3.3 Bees' preference to perform orientation or foraging flights

Eleventeen focal bees with flights were compared to analyze whether a set of non-social variables would impact bees' preference to perform orientation or foraging flights. These variables included: temperature ((°), 'Temp'), wind speed ((mph), 'Wind'), weather (clear, partial cloudy, most cloudy and overcast), percentage of brood cells ('Brood'), empty cells ('Empty cell'), honey cells ('Honey cell') and workers ('Worker') within the colony, time ('Time'), age ('Age') and frequency of flights ('NoF'). General linear mixed models (GLMM) were applied to analyses. Three bees 257, 272 and 282 did not perform any flight and only 5 bees 216, 244, 262, 267 and 271 performed both orientation and foraging flights over ages (Figure 5a). Therefore, whether 'Age' and 'NoF' should be nested in the random effect with 'BeeID' was explored. Although 'Age' nested in the random slope increased model fit with lower AIC, it produced extremely high multicollinearity (Table 7, model H1). 'NoF' caused 'BeeID' to produce zero variance in the random effect (Table 7, model H2). Nesting 'Age' and 'NoF' in the random intercepts with 'BeeID' was studied. Results showed that 'NoF:BeeID' produced zero variance (Table 7, model H4). Hence, 'NoF' from the random effect with 'Age' in the random intercept nested within 'BeeID' was excluded. Goodness-of-fit test without significant dispersion, zero-inflation and outlier (Table S4) were checked. Model H6 was the best fitted (Table 7, H6). Marginal R^2_{GLMM} (R_GLMM (m^2)) showed 65% of variance could be explained by fixed effects. As bees gained more flight experience, they tended to perform foraging flight (Chisq = 19.8, df = 1, $p < 0.01$) (Figure 10A). When number of workers in the colony increased, the workers reduced foraging flight and switched to orientation flight (Chisq = 4.8, df = 1, $p = 0.03$) (Figure 10B). This result suggested that workers could adjust foraging activity by following the colony's morphology.

Table 7. Influence of age, flight frequency, external and internal colony condition on bees' preference to perform orientation and foraging flights

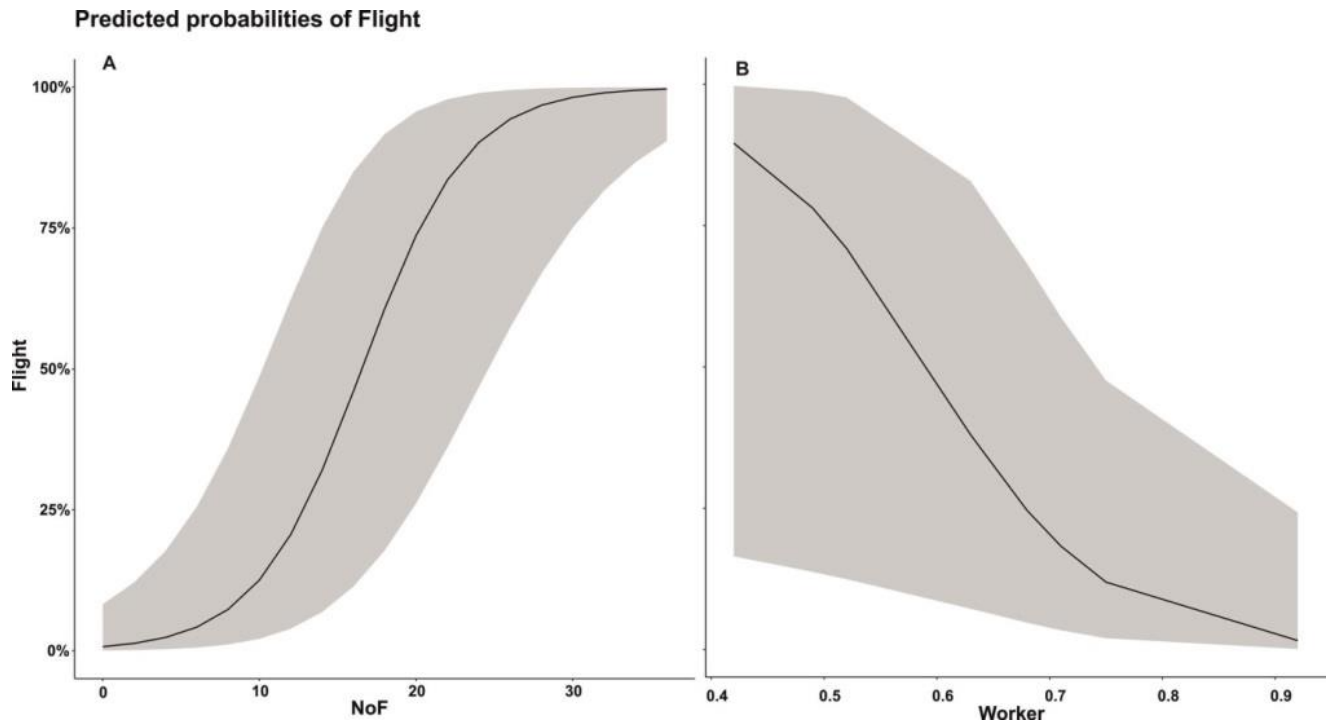| Tested Models	AIC	BIC	R²_GLMM (m)	R²_GLMM (c)	Random effect		
					Group	Variance	Std.Dev.
Null: Flight ~ (1 | BeeID)	148	153	0	0.59	BeeID	4.53	2.13
H1: Flight ~ (Age | BeeID)	133	144	0	0.95	BeeID	4.06	2.01
					Age	0.88	0.94
H2: Flight ~ (NoF | BeeID)	113	124	0	0.87	BeeID	0.00	0.00
					NoF	0.09	0.31
H3: Flight ~ (1| BeeID/Age)	131	139	0	0.78	BeeID	6.20	2.49
					Age:BeeID	5.55	2.36
H4: Flight ~ (1| BeeID/NoF)	150	158	0	0.78	BeeID	4.72	2.17
					NoF:BeeID	0.00	0.00
H5: Flight ~ NoF + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (1 | BeeID/Age)	100	155	0.97	0.99	BeeID	6.00	2.45
					Age:BeeID	0.49	0.70
H6: Flight ~ NoF + Temp + Honey cell + Worker + (1 | BeeID/Age)	89	107	0.65	0.83	BeeID	3.22	1.80
					Age:BeeID	0.40	0.63

Predicted probabilities of Flight

Figure 10. Predicted probabilities of foraging flight compared to orientation flight against the number of flight and workers. 'NoF', number of flight; 'Worker', number of workers.

4.5.3.4 Performance of orientation and foraging flights

How non-social factors influenced bees' flight performance were studied during orientation and foraging flights with parameters of 'NoF', 'Loops', 'Duration', 'MaxDist', 'MedianDist', 'AccuDist' and 'AvrgSpeed' (Figure S1). These factors were: temperature ((°), 'Temp'), wind speed ((mph), 'Wind'), weather (clear, partial cloudy, most cloudy and overcast), percentage of brood cells ('Brood'), empty cells ('Empty cell'), honey cells ('Honey cell') and workers ('Worker'), time ('Time'), age ('Age') and frequency of flights ('NoF'). Flight patterns in orientation and foraging flights were different, therefore, regression analyses for orientation and foraging flights were separately performed. Types of models (GLMM, LMM, LM, GLM) were selected based on the distribution of dataset of different parameters (Figure S1). Predictors of 'Empty cell', 'Honey cell', 'Brood' and 'Worker' were highly correlated. The high correlation indicated a redundancy between predicted variables which would induce a problem of multicollinearity. Hence, some of these predicted variables were removed when necessary. Models were built for orientation (Table 8) and foraging flights (Table 9). Since there were only 5 bees performed foraging flight. BeeID in the

random effect produced negative coefficient variation which was therefore excluded from the random effect.

Orientation flight (Table 8) was not significantly influenced by weather compared to foraging flight (Table 9). During the orientation flights, individual bees performed more flight trips ('NoF') when temperature was higher ('Temp': 0.08 ± 0.02 (estimate±SE, same as followed), $F_{(1, 56)}$ = 9.91, $p < 0.01$) and area of brood cells decreased ('Brood': -38.31 ± 9.44, $F_{(1, 62)}$ = 16.46, $p < 0.01$). 'NoF' was not correlated with bees' age ('Age': 0.12 ± 0.06, $F_{(1, 61)}$ = 3.73, $p = 0.06$). As expected, with more flights, bees flew further ('MedianDist': 0.07 ± 0.02, $F_{(1, 53)}$ = 11.30, $p < 0.01$, 'AccuDist': 0.06±0.02, $F_{(1, 60)}$ = 11.56, $p < 0.01$, 'MaxDist': 0.06±0.02, $F_{(1, 59)}$ = 6.43, $p < 0.05$) and faster ('AvrgSpeed': 0.04 ± 0.02, $\chi2$ = 5.83, $p = 0.02$). As bees were older, they flew in shorter distance ('AccuDist': -0.24 ± 0.04, $F_{(1, 39)}$ = 35.35, $p < 0.01$; 'MaxDist': -0.27 ± 0.08, $F_{(1, 23)}$ = 11.05, $p < 0.01$), less loops ('Loops': -0.08 ± 0.04, $\chi2$ = 4.52, $p = 0.03$) and shorter time ('Duration': -0.17 ± 0.03, $\chi2$ = 48.11, $p < 0.01$). When there were more empty cells in the colony, bees extended flight range ('MaxDist': 7.77 ± 3.48, $F_{(1, 35)}$ = 4.99, $p = 0.03$).

Within foraging flights, as bees were older, they performed more flights (NoF: 0.19 ± 0.03, $\chi2$ = 55.89, $p < 0.01$) and narrowed flight distances (MedianDist: -0.26 ± 0.10, $\chi2$ = 6.41, $p = 0.01$; AccuDist: -201.10 ± 68.61, $\chi2$ = 8.59, $p < 0.01$, MaxDist: -0.12 ± 0.03, $\chi2$ = 22.07, $p < 0.01$). When bees gained more experience via more flight trips (NoF), they explored more remote areas by increasing flight distances in 'MedianDist' (0.06 ± 0.03, $\chi2$ = 4.64, $p = 0.03$) and 'AccuDist'(54.61 ± 17.56, $\chi2$ = 9.67, $p < 0.01$). More empty cells and honey cells encouraged bees to performed more forging flights ('Empty cell': 49.18 ± 19.00, $\chi2$ = 6.7, $p < 0.01$, 'Honey cell': 59.49 ± 16.51, $\chi2$ = 12.99, $p < 0.01$) and longer in Duration ('Empty cell': 2.44 ± 1.10, $\chi2$ = 4.93, $p = 0.03$). It was reasonable that more honey cells stimulated bees to perform more foraging flights and fly with longer duration as the base distribution level of honey cells was relatively low to satisfy the colony need (Figure S2). When wind was stronger, bees would narrow flight areas ('AccuDist': -220.36 ± 61.25, $\chi2$ = 12.94, $p < 0.01$; 'MaxDist': -0.11 ± 0.04, $\chi2$ = 9.29, $p < 0.01$) and decrease flight duration ('Duration': -0.007 ± 0.002, $\chi2$ = 9.89, $p < 0.01$). Instead, bees flew longer ('MedianDist': 0.55 ± 0.25, $\chi2$ = 4.76, $p = 0.03$), explored more ('AccuDist': 523.35±166.58, $\chi2$ = 9.87, $p < 0.01$) and flew faster ('AvrgSpeed': 1.11 ± 0.31, $\chi2$ = 12.87, $p < 0.01$) during weather that was partially cloudy.

Table 8. Influence of bee age, frequency of flight, external and internal colony condition on bees' orientation flight

Model	Method	Family	AIC	BIC	R²_GLMM(m)	R²_GLMM(c)
NoF ($\log_{10}$) ~ Age + Temp** + Wind + Brood** + (1|BeeID)	LMM	\	104.9	119.8	0.52	0.86
MedianDist ($\log_{10}$) ~ Temp + NoF** + (1|BeeID)	LMM	\	176.8	187.4	0.15	0.60
AccuDist ($\log_{10}$) ~ Age** + NoF** + Time + (1|BeeID)	LMM	\	137.6	163.1	0.41	0.56
MaxDist ($\log_{10}$) ~ Age** + Wind* + NoF* + Empty cell* + Time+ (1|BeeID)	LMM	\	172.6	189.7	0.27	0.41
Loops ~ Age*	GLM	poisson (link='log')	161.7	165.9	\	\
Duration ~ Age**	GLM	Gamma (link='log')	346.1	352.5	\	\
AvrSpeed ~ NoF*	GLM	Gamma (link='log')	142.7	149.0	\	\

Note: marginal R^2 and conditional R^2 are only calculated for GLMM and LMM with random effects.

'*' ($p < 0.05$) and '**' ($p < 0.01$) showed significant difference.

Table 9. Influence of bee age, frequency of flights, external and internal colony condition on bees' foraging flight

Model	Method	Family	AIC	BIC
NoF ~Age* + Temp + Weather_partialcloudy + Wind + Empty cell* + Honey cell* + Worker	GLM	poisson(link='log')	211.2	220.3
MedianDist ~Age* + Weather_partialcloudy* + Wind + NoF*	GLM	gaussian(link='identity')	112.9	123.8
AccuDist ~Age** + Weather_partialcloudy** + Wind** + NoF**	GLM	gaussian(link='identity')	710.2	721.2
MaxDist ~Age** + Wind**	GLM	Gamma(link='log')	609.1	616.4
Duration ~Wind** + Empty cell*	GLM	Gamma(link='log')	316.4	323.7
AvrgSpeed ~Temp* + Weather_partialcloudy**	GLM	gaussian(link='identity')	137.2	144.5

Note: '*' ($p < 0.05$) and '**' ($p < 0.01$) show significant difference.

4.5.4 Between- and within-individual variation and repeatability across honeybees over life history

On the basis of the best fitted models built in the above results for different behaviors, I used the likelihood ratio test (LRT) to assess inter- and intra-individual differences. Random effects were tested by comparing between the best fitted and the models without random effects (Table 10). Calculated by dividing between-individual variance relative to the total phenotypic variance (Nakagawa & Schielzeth 2010), *repeatability* (R) measures the proportion of total variance accounted for by difference among groups (Rohlf 1995). Intraclass correlation coefficients (ICCs) calculated by dividing random effect variance by the total variance can be interpreted as proportion of variance explained by grouping structure in a population (Nakagawa et al. 2017). The ICC includes both conditional and adjusted ICCs. Of which, the former takes the fixed effects variances into account while the latter only takes all random effects into account (Nakagawa et al. 2017). Here I reported on behavioral variation between- and within-individual bees. Behavioral index studied were listed in the Table 11, those were: 'Out': whether bees left the colony to the hive exit; 'LR/OW': when bees appeared at the inside of the hive entrance, whether bees returned into the colony soon or rushed out from the exit but without flying up after release; 'OW/FL': whether bees only walked at the hive exit without flying up or performed successful flights; 'Orientation/Foraging': whether bees performed orientation or foraging flights. A few parameters of orientation flights were calculated. These parameters were: 'NoF': the frequency of flight; 'MedianDist': median distance of a flight; 'AccuDist': accumulated distance of a flight; 'MaxDist': maximum distance of a flight. Adjusted ICC, between- and within-individual variances, *repeatability* (R), *marginal* R^2 and *conditional* R^2 were calculated (Table 11).

With the exception of analysis on 'MaxDist', other analyses on 'Out', 'LR/OW', 'OW/FL', 'Orientation/Foraging', 'NoF', 'MedianDist' and 'AccuDist' were significantly explained by inter-individual difference (Table 10). Although 'MaxDist' with and without random effect did not show significant difference, it was wore fitted using linear model (LM) rather than linear mixed model (LMM), therefore, the random effect was more supportive. These results meant that bees showed consistent difference in the average level of outdoor behaviors. Bees also differed in the level of intra-individual variability (Table 10, 'Out' with random slope and intercept: (Age|BeeID)).

A relatively low adjusted ICC estimate and repeatability in 'Out' among individual bees (ICC = 0.17, R = 0.19) were observed after accounting for 86% of the variance by fixed effects. Although this result indicated the random effect explained less variance for behavioral characteristic, 85 individual bees still displayed their own rhythms of leaving the colony to the hive exit with 18% of interspecific variance accounted for the partitioned variance. Eighty-one percent of the variance of random effect within individual bee accounted for the behavioral variability. This behavioral plasticity associated with individual bees' age (Table 11, Figure 12). Instead, the variability of bees' behavioral preference to perform 'LR' or 'OW' (ICC = 0.76, R = 0.78, Figure 13) and to perform 'OW' or 'FL' (ICC = 0.67, R = 0.69, Figure 14) were highly accounted for by random effects. This result could be supported with the low *marginal* R² and high *conditional* R² (Table 11). The variation of bees' preference between LR & OW (LR/OW), and between OW & FL (OW/FL) were highly consistent within bees and various among individual bees, with inter-bee variation of 71% and 63% mainly explaining the random variance, respectively. These results were also confirmed by large standard deviation and variance of intercepts (LR/OW: sd = 2.019, variance = 4.077, Figure 13; OW/FL: sd = 1.599, variance = 2.556, Figure 14). With moderate random effects (ICC = 0.52, R = 0.51), 51% of the inter-individual difference accounted for the variability of bees' preference between performing orientation or foraging flights. Of which, 46% of the inter-individual variation was accounted for across individual bees (BeeID: sd = 0.992, variance = 0.984, Figure 15B). Bees also differed in levels of age and this difference caused 5% of the intra-individual variability (Age:BeeID: sd = 0.135, variance = 0.018, Figure 15A). Within orientation flights, frequency of flights at the within-individual level was highly homogenous with only 28% of the variance, while at the between-individual was with 72% of the variance. This result supported the existence of high inter-variability in the frequency of flights (ICC = 0.71, R = 0.71), with moderate standard deviation and variance from intercept (BeeID: sd = 0.59, variance = 0.348, Figure 16A). I also found moderate variance for ''MedianDist' of flights among individual bees (ICC = 0.53; R = 0.53) with 53% between-individual of the variance (BeeID: sd = 0.748, variance = 0.559, Figure 16B). Conversely, with low random effect ('AccuDist': ICC = 0.25, R = 0.25; 'MaxDist': ICC = 0.2, R = 0.2), 'AccuDist' and 'MaxDist' of orientation flights varied highly within individual bee rather than between bees, with intra-individual variance of 76% and 81%, respectively (Table 11). Correspondingly, the standard

deviation and variance of the intercepts were relatively low (AccuDist: sd = 0.252, variance = 0.064, Figure 16C; MaxDist: sd = 0.271, variance = 0.073, Figure 16D). Although ICCs among a few behaviors and behavioral parameters ('Out', 'AccuDist' and 'MaxDist' parameters in the orientation flights) may be considered to low, previous study has been interpreted the ICC values as low as 0.168 to be suggestive of repeatability (Petelle, 2013). These results provided evidences that all the behaviors and behavioral parameters listed in Table 11 were consistent and distinct within and among individual bees.

Table 10. Log-likelihood ratio test to estimate inter- and intra-individual variability on the outdoor behaviors.

Random effect	Method	df	AIC	Loglik	L.Ratio	P
H0: Out ~ Age + Temp + Wind + Empty cell + Worker + Time	GLM	18	3223.9	-1594		
Out ~ Age + Temp + Wind + Empty cell + Worker + Time + (Age|BeeID)	GLMM	21	2944.9	-1451	285	< 0.01**
H0: LR/OW ~ Temp + Worker + NoOut	GLM	5	116.7	-53.4		
LR/OW ~Temp + Worker + NoOut + (1|BeeID)	GLMM	6	103.4	-45.7	15.3	< 0.01**
H0: OW/FL ~ Wind + Worker + Age	GLM	4	354.6	-173.3		
OW/FL ~ Wind + Worker + Age + (1|BeeID)	GLMM	5	262.6	-126.3	93.9	< 0.01*
H0: Flight ~ NoF + Temp + Honey cell + Worker	GLM	5	96.6	-43.3		
Flight ~ NoF + Temp + Honey cell + Worker + (1 | BeeID/Age)	GLMM	7	89.3	-37.6	11.3	< 0.01**
H0: NoF (log$_{10}$) ~ Age + Temp** + Wind + Brood**	LM	6	137.1	-62.6		
NoF (log$_{10}$) ~ Age + Temp** + Wind + Brood** + (1|BeeID)	LMM	7	104.9	-45.5	34.2	< 0.01**
H0: MedianDist (log$_{10}$) ~ Temp + NoF**	LM	4	185.6	-88.8		
MedianDist (log$_{10}$) ~ Temp + NoF** + (1|BeeID)	LMM	5	176.8	-83.4	10.8	< 0.01**
H0: AccuDist (log$_{10}$) ~ Age** + NoF** + Time	LM	6	135.2	-62.6		
AccuDist (log$_{10}$) ~ Age** + NoF** + Time + (1|BeeID)	LMM	5	132.4	-60.2	4.9	0.03*
H0: MaxDist (log$_{10}$) ~ Age** + Wind* + NoF* + Empty cell* + Time	LM	7	172.9	-79.4		
MaxDist (log$_{10}$) ~ Age** + Wind* + NoF* + Empty cell* + Time+ (1|BeeID)	LMM	8	172.6	-78.3	2.2	0.14

Note: the best fitted models (in grey) are compared with models (H0) without random slope and random intercept. Degree of freedom (df), Akaike Information Criterion (AIC), Log-likelihood values (Loglik) and Log-likelihood ratio test (L.Ratio) are presented with the corresponding *p*-values. '*' and '**' indicate significant   effect. 'NoF', 'MedianDist', 'AccuDist' and 'MaxDist' were the parameters of orientation flights.

Table 11. Summary of adjusted ICC, variance of outdoor behaviors within and between individuals, and the corresponding *repeatability*, *marginal* R² and *conditional* R²

Random effect	ICC adjusted	Variance within individual	Variance between individual	R	R²_GLMM(m)	R²_GLMM(c)
Out ~ Age** + Temp** + Wind** + Empty cell** + Worker** + Time** + (Age|BeeID)	0.17	0.81	0.18	0.19[0.02, 0.32]	0.86	0.89
LR/OW~ Temp** + Worker* + NoOut + (1|BeeID)	0.76	0.23	0.71	0.78[0.42, 0.78]	0.34	0.85
OW/FL~ Wind + Worker + Age** + (1|BeeID)	0.67	0.31	0.63	0.69[0.18, 0.95]	0.10	0.70
Orientation/Foraging ~ NoF** + Temp + Honey cell + Worker* + (1|BeeID/Age)	0.52	0.49	0.51	0.51[0.00, 0.79]	0.65	0.83
NoF(log$_{10}$) ~ Age + Temp** + Wind + Brood** + (1|BeeID)	0.71	0.28	0.72	0.71[0.59, 0.81]	0.52	0.86
MedianDist(log$_{10}$) ~ Temp + NoF** + (1|BeeID)	0.53	0.47	0.53	0.53[0.32, 0.68]	0.15	0.60
AccuDist(log$_{10}$) ~ Age** + NoF** + Time + (1|BeeID)	0.25	0.76	0.24	0.25[0.04, 0.45]	0.41	0.56
MaxDist(log$_{10}$) ~ Age** + Wind* + NoF* + Empty cell* + Time+ (1|BeeID)	0.20	0.81	0.19	0.20[0.00, 0.41]	0.27	0.41

Note: The '*' and '**' indicated significant difference ($p < 0.05$ and $p < 0.01$, respectively).

Repeatability (R) is given with 97.5%CI. Random effects are specified differently: (1|BeeID) containing intercept term, account for the intercepts differed among individual bees (BeeID); (Age|BeeID) containing both random intercept and slope terms, account for the fact that effect of age (Age) differs with individual bees (BeeID). (1|BeeID/Age) is equivalent to (1|BeeID) + (1|Age:BeeID), explaining the intercept differed among individual bees (BeeID) and among age (Age) within an individual bee (BeeID). *Marginal* R² considers only the variance of the fixed effects and *conditional* R² takes both the fixed and random effects into account.
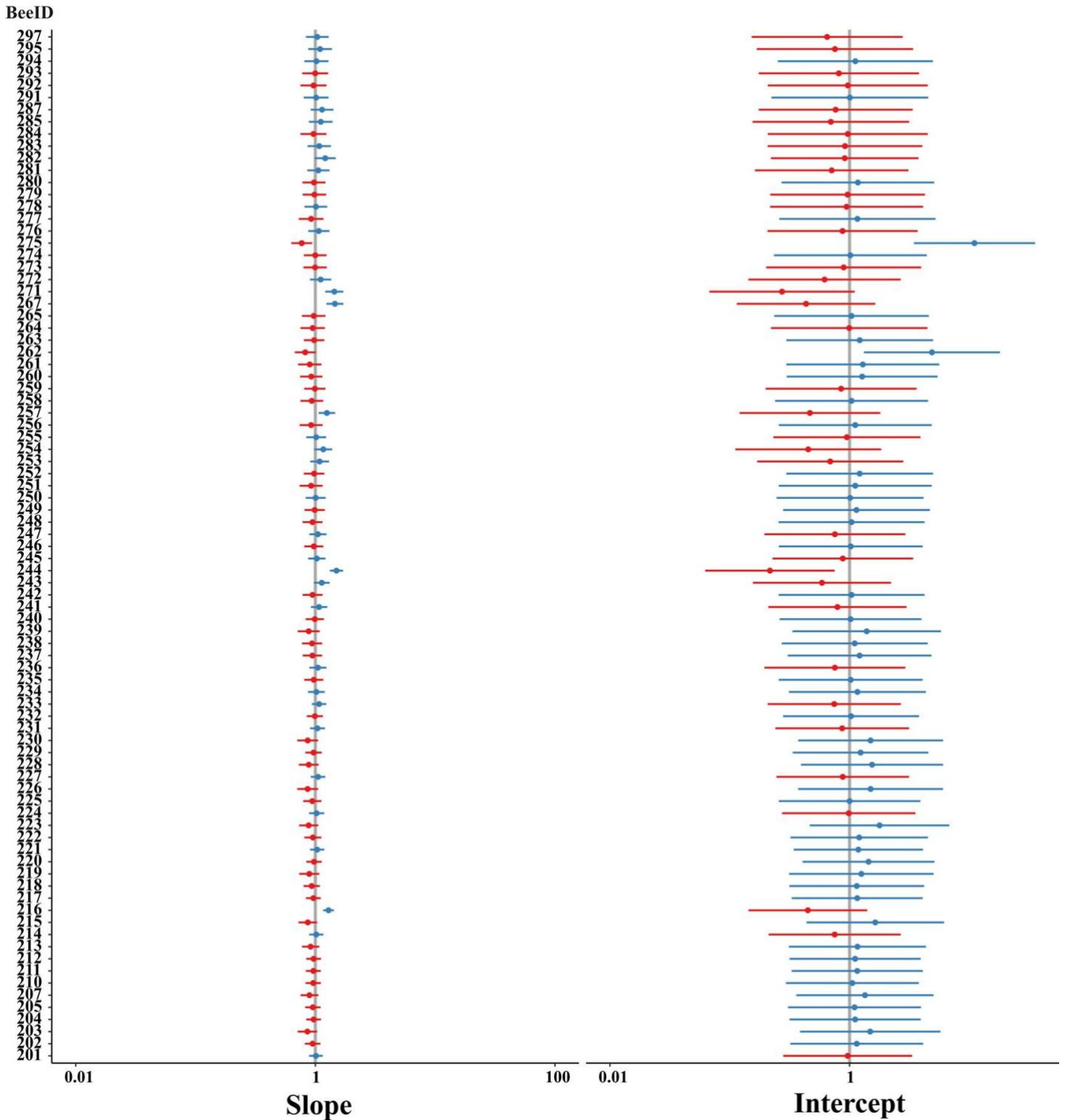
Figure 12. Random variance within individual bee and between individual bees demonstrated within- and between-individual variation of leaving the colony to the hive exit for potential outdoor behaviors. 'BeeID' represented bee identity. The variance in the slope and intercept suggested that the variation of the behavior that bees leave the colony to hive exit ('Out') could be attributed by the variance of the age ('Age') as random slope and individual bee ('BeeID') as random intercept.

Figure 13. Random intercepts among individual bees demonstrated the variation of bees' behavioral switching between 'LR' or 'OW'
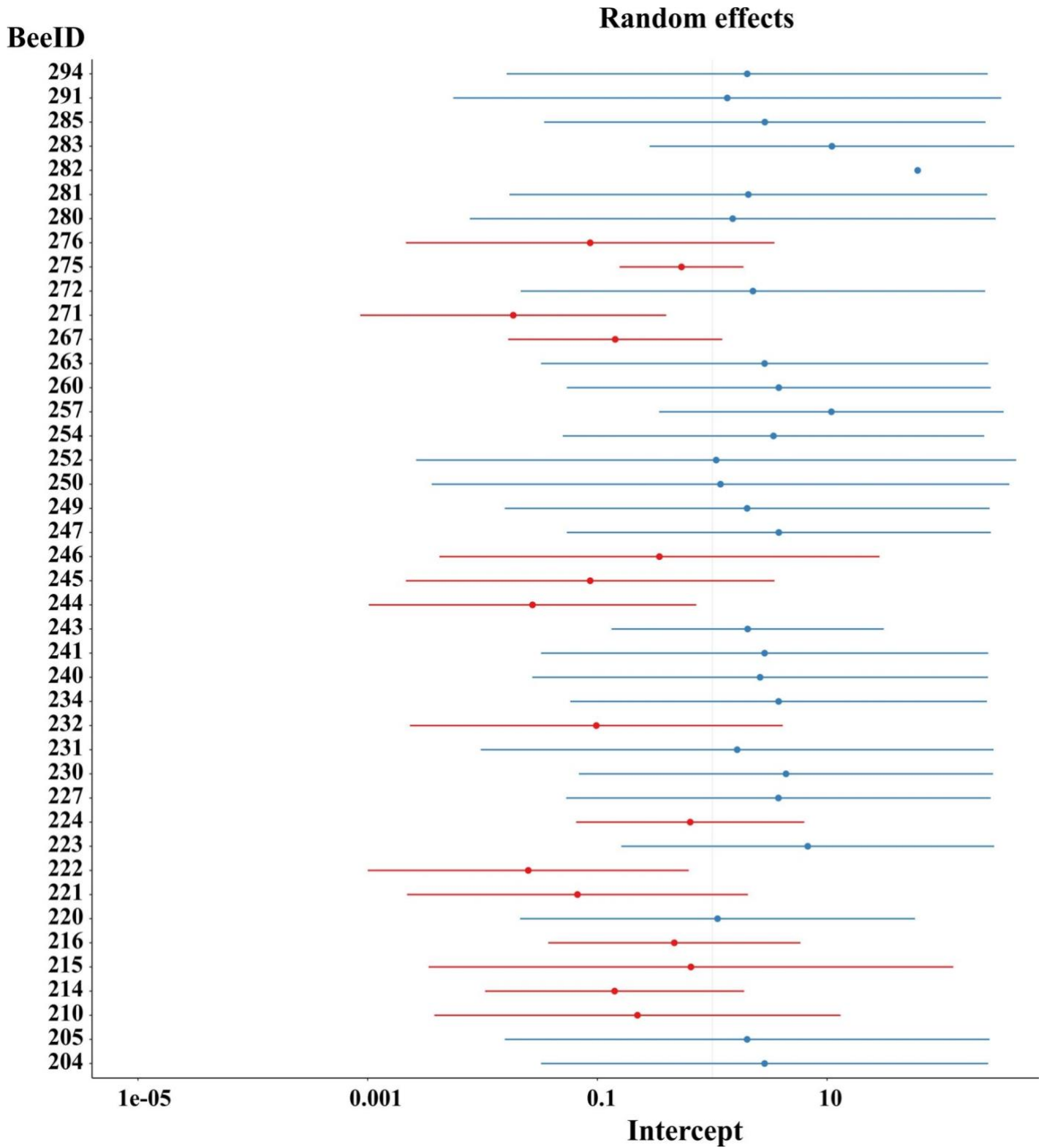
Figure 14. Random intercept among bees demonstrated the between-individual variation of bees' behavioral switch between 'OW' or 'FL'.
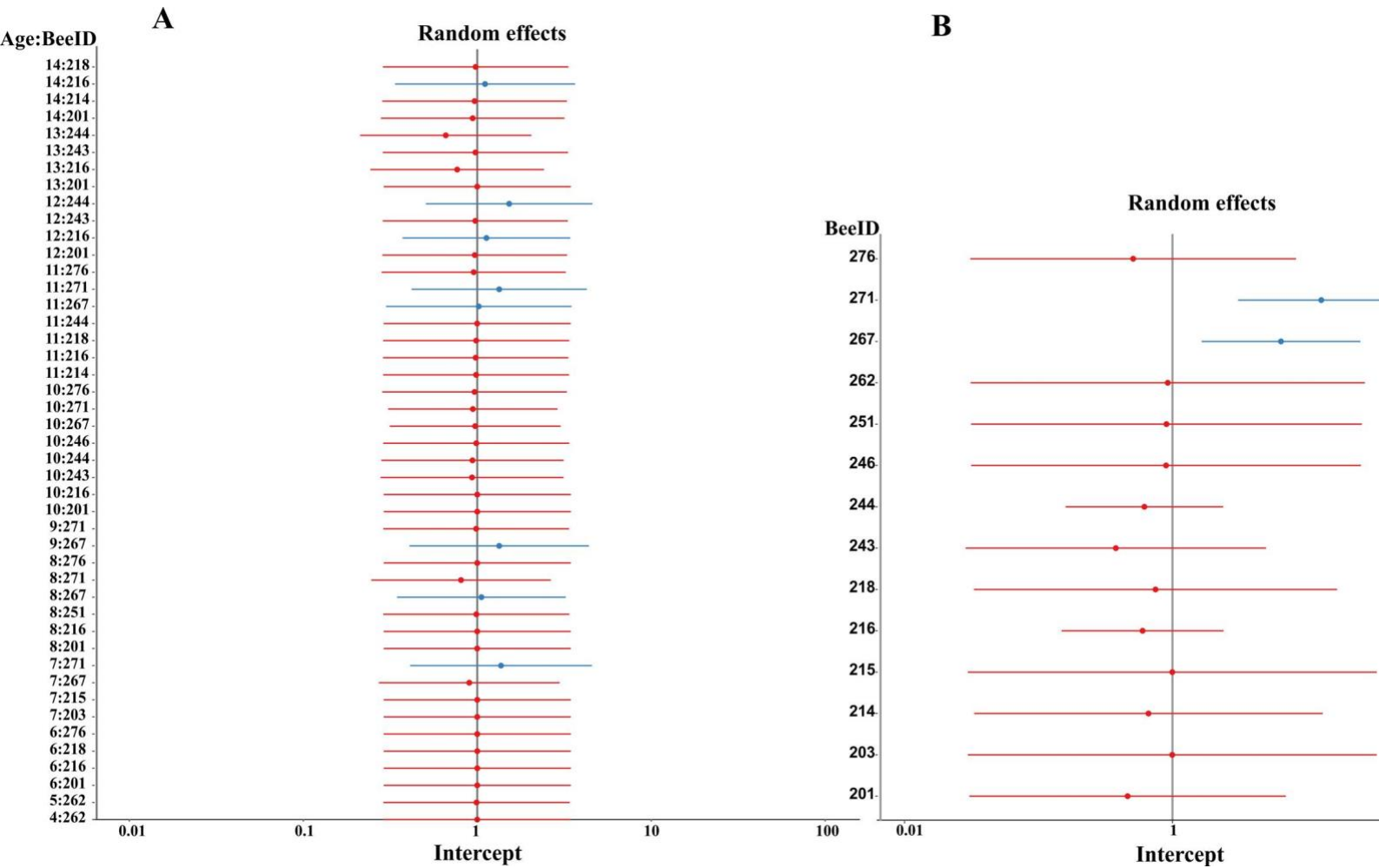
Figure 15. Random intercepts between individual bees and between ages within individual bee demonstrated variation of bees' behavioral switching between orientation or foraging flight. The variances in the intercept Age:BeeID and BeeID were suggested to explain the variance of bees' switch to perform orientation or foraging flight. Random intercept Age:BeeID also suggested the effect of age on the variance was different across different bees.
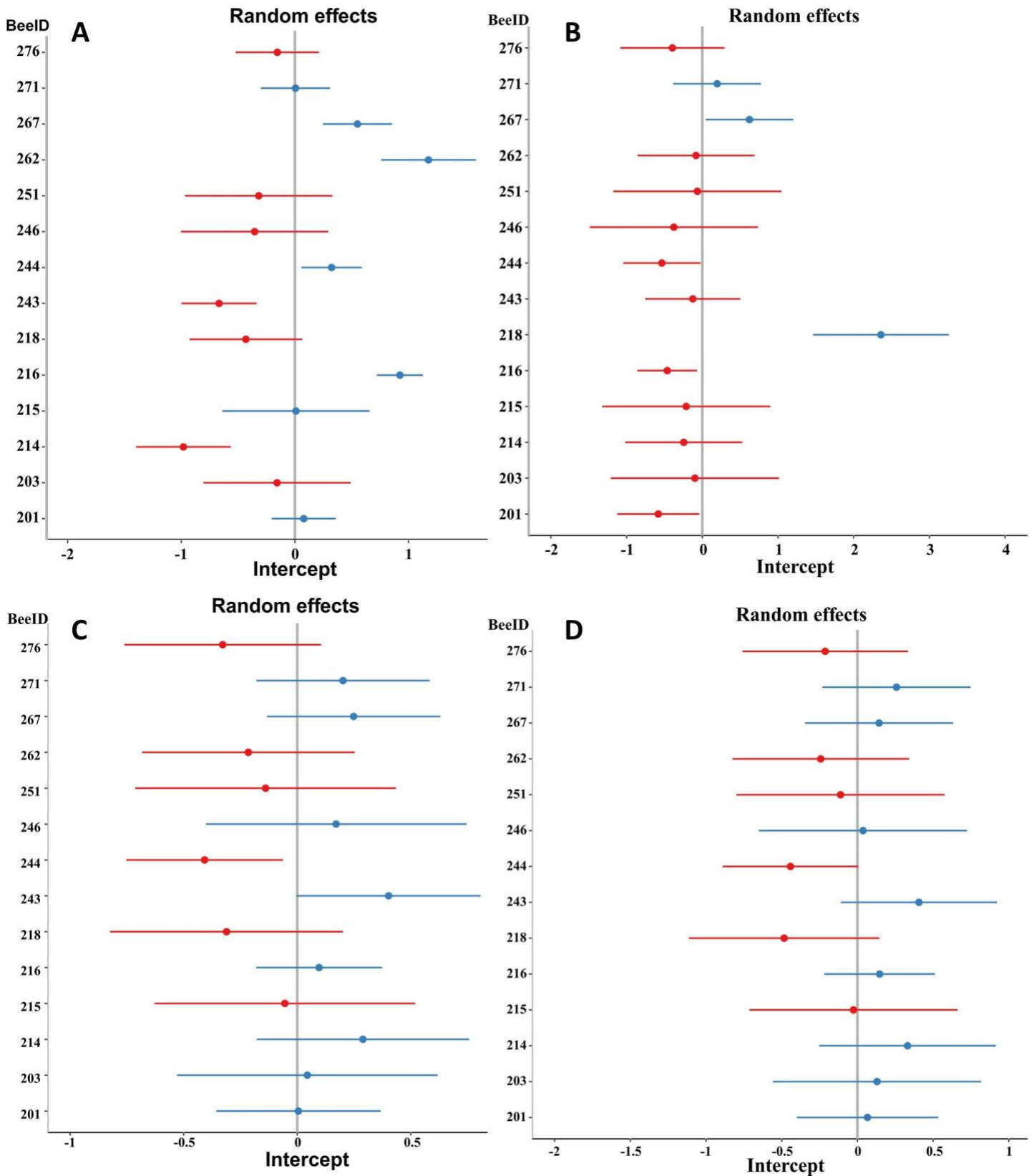
Figure 16. Random intercepts between individual bees demonstrated the between-individual variation of parameters of orientation flight. Plot A, B, C, D represented the random effect of BeeID on shaping bees' orientation performance in NoF, MedianDist, AccuDist and MaxDist, respectively.

4.6 Summary and Discussion

Although intrinsic factors such as social learning and bees' response to stimuli play a key role in determining the honeybees' outdoor activities, there are a series of biotic and abiotic factors challenge bees, making them consequently adaptively adjust their behaviors differently. Therefore, uncovering correlation between these non-social factors and bees' outdoor behaviors and investigating how they response to these factors differently at the inter- and intra-individual levels may provide insights in fully understanding the mechanism of how behavioral phenotypes evolve and context-specific behavioral plasticity develops.

Here, the influences of non-social factors including the biotic and abiotic factors (i.e., bee age, time, sequence of behavioral event, climate, colony morphology and food storage) on bees' outdoor behaviors with 7 categories were studied. Outdoor behavioral patterns that were repeatable within the life history and consistent across life stages were further demonstrated.

4.6.1 Influence of non-social factors on outdoor behaviors

After bees left the colony to the hive exit, 'Out' was differentiated with 'LR', 'OW', 'FR' and 'FL'. 'LR' indicated the bees appeared at the hive exit and returned into the colony soon. 'OW' meant that bees appeared at the hive exit, did not fly up and only walked around the hive exit after being released with a transponder on the thorax. 'FR' described that bees flew up after release, but turned back to the hive exit without performing flights. Flights 'FL' were differentiated into orientation and foraging flights. The frequency of 'FR' was extremely low. Therefore, I did not discuss it. To reveal how the non-social factors influence bees' outdoor behaviors, multivariate regression methods were applied.

Age neither influenced bees' initiating 'LR' or 'OW' nor to perform orientation or foraging flights. It also was not critical to improve frequency of orientation flight. However, during orientation flights, bees flew in shorter distances, with less loops and shorter time as they aged. Within foraging flights, older bees flew more trips within limited flight areas and distance. Reason to explain why age did not influence the frequency of orientation flight which differed from that in foraging flight might be due to different flight motivations. Foraging flight is for collecting food from a relatively distant place requiring bees to fly more and gain more experience to maximize foraging profitability. Conversely, orientation flight is to facilitate a bee's homing success from foraging flight. Within an orientation flight, bees flew around the hive

surroundings to establish feature memories of the hive exit and familiarity with the sun compass (Degen et al. 2015). Bees could perform re-orientation flight when they were disrupted during foraging flight or moved to a new place (Degen et al. 2018). Physical limits of age could be a similar factor to influence bees' performance in both orientation and foraging flights in distance, area and duration.

The sequence of behavioral events made no difference on whether bees leave the colony performing 'LR', 'OW' or 'FL'. However, it fostered bees to switch from orientation flight to foraging flight. As expected, when. bees gained more experience with more flight trips, they would increase flight distance in 'MedianDist' and 'AccuDist' of orientation and foraging flights. Bees speeded up and increased maximum distance during orientation flight, which were not indicated in foraging flight. This result was reasonable, since bees' motivations for orientation and foraging flights were different. To get more familiar with the nearby environment and improve homing success, bees explored flight areas as large as possible with the least energy consumption during orientation flights. Due to rare natural food sources, the artificial food in the experiment was the major advertised food by dancers.

'Out' was the only behavior in the study that was influenced by the daytime. This result indicated that bees' leaving the colony to the hive exit was more predictable while other behaviors might be regulated by other unpredictable factors.

Temperature and wind restricted bees to stay more inside the colony, suggesting that bees were sensitive to the external climate condition. However, they did not influence bees' switch between 'OW' and 'FL', and between orientation and foraging flights. This result indicated that bees' initiation of a flight did not necessarily depend on climate condition. However, bees' performance during a flight was more influenced by the climate. Of which, temperature significantly increased frequency of orientation flights. This result was reasonable since the maximum temperature in the study was 35 °C. However, bees continued flying even the temperature were up to 37 °C. However, the performance of foraging flight was more dependent on the environment than orientation flight.

Frequency of flights ('NoF') was the only parameter during foraging flight that was not influenced by external environment conditions (temperature, wind speed and weather). Bees tended to extend distances and speeds of flights under a partial cloudy day rather than on a sunny day. It was noteworthy that partial cloudy equally termed as partial sunny that 3/8 ~ 5/8 of the sky is covered by clouds according to

the National Weather Service (https://forecast.weather.gov/glossary.php?letter=p).
This interesting result for one aspect showed partial cloudy did not negatively
influence a bee's performance during a foraging flight. It also might indicate that bees
may sense the relatively bad weather and work harder. This hypothesis may be
supported by the result in the study of He et al. (2015) that bees foraged longer
before rainy day. This result may also suggest that bees within a tiny colony were
more resistant to environmental changes due to survival stress for a small colony.
Apparently, my result showed that usage of space inside the colony was essential for
regulating bees' outdoor activities. Bees were able to adjust behaviors by detecting
the colony need.

4.6.2 Intra- and inter-individual variation of outdoor behaviors

Understanding the inter- and intra-individual behavioral variability in a complex social
community may provide a specific insight into how individual animals develop their
social characteristics and the individuality. Variations of the outdoor behaviors among
individual bees were analyzed.

All the tested behaviors were repeatable across the life history and the behavioral
repeatability values range between 0.19 ~ 0.78 (Table 11). These behaviors were
expressed differently dependent on different non-social factors. Of which, variation of
'Out' was considerably (86%) explained by multiple non-social factors, including age,
temperature, wind speed, daytime, number of empty cells and workers. Variations of
preference between 'LR' and 'OW', between 'OW' and 'FL', and orientation flight
parameters 'MedianDist' and 'MaxDist' were slightly interpreted by non-social factors
with coefficient determination values between 10% ~ 35%. Variation of 'NoF' and
'AccuDist' of orientation and foraging flights could be intermediately illustrated by
non-social factors with R square ranging between 31% ~ 65%. As far as I know,
these results were the first time to characterize the extent of consistent behavioral
differences among individual bees in a series of outdoor behaviors helping us to
estimate all types of phenotypes.

I also demonstrated each individual bee was peculiar to their own rhythms with
different levels of variation to respond to non-social factors. The mixed models
revealed considerable within- or between-individual variation of different outdoor
behaviors. Bees possessed their own slope and intercept in the 'Out', indicating
bees' flexibility in leaving the colony to the hive exit (Figure 12). The effect of age on
the frequency of 'Out' rely on the individual bees was observed. The standard

deviations of random intercepts in the models were different for evaluating multiple outdoor behaviors (Figure 12 ~ 16). All the standard deviations of the random factor BeeID were large, in particular in the models to evaluate the frequency of switch between 'LR' and 'OW', between 'OW' and 'FL', between orientation flight and foraging flight, and 'MedianDist'. These results suggested that except for the non-social factors, different individuals behaved greatly differently in outdoor behaviors. However, what would be the differences existed in individual bees that make them behave differently among and within a group in outdoor behaviors?

Honeybees are capable of detecting and evaluating atmospheric conditions. They can not only maintain a consistent collective behavior within a group but also develop their own behavioral flexibility in the individual level. To figure out what and how these within- and between-individual and investigate how each bee develops their social roles within a colony, further studies are required for looking into the social community.

4.7 Reference

Al Toufailia, H., Couvillon, M. J., Ratnieks, F. L., & Grüter, C. (2013). Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour. *Behavioral Ecology and Sociobiology, 67*(4), 549-556.

Araya-Ajoy, Y. G., & Dingemanse, N. J. (2014). Characterizing behavioural 'characters': an evolutionary framework. *Proceedings of the Royal Society B: Biological Sciences, 281*(1776), 20132645.

Beekman, M., Sumpter, D. J. T., Seraphides, N., & Ratnieks, F. L. W. (2004). Comparing foraging behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers. *Functional Ecology, 18*(6), 829-835.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *rends in ecology & evolution, 24*(3), 127-135.

Bonoan, R. E., Feliciano, P. M. I., Chang, J., & Starks, P. T. (2020). Social benefits require a community: The influence of colony size on behavioral immunity in honey bees. *Apidologie, 51*(5), 701-709.

Chaffiol, A., Laloi, D., & Pham-Delegue, M. H. (2005). Prior classical olfactory conditioning improves odour-cued flight orientation of honey bees in a wind tunnel. *J Exp Biol, 208*(Pt 19), 3731-3737. doi:10.1242/jeb.01796.

Clarke, D., & Robert, D. (2018). Predictive modelling of honey bee foraging activity using local weather conditions. *Apidologie, 49*(3), 386-396.

Couvillon, M. J., Riddell Pearce, F. C., Harris-Jones, E. L., Kuepfer, A. M., Mackenzie-Smith, S. J., Rozario, L. A., ...Ratnieks, F. L. (2012). Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding. *Biology open, 1*(5), 467-472.

Dainat, B., Dietemann, V., Imdorf, A., & Charrière, J. D. (2020). A scientific note on the 'Liebefeld Method' to estimate honey bee colony strength: its history, use, and translation. *Apidologie*, 1-6.

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*, 45-47.

Degen, J., Hovestadt, T., Storms, M., & Menzel, R. (2018). Exploratory behavior of re-orienting foragers differs from other flight patterns of honeybees. *PLoS One, 13*(8), e0202171. doi:10.1371/journal.pone.0202171.

Dyer, F. C. (2002). The biology of the dance language. *Annual review of entomology, 47*(1), 917-949.

Ikeno, H., Akamatsu, T., Hasegawa, Y., & Ai, H. (2014). Effect of olfactory stimulus on the flight course of a honeybee, *Apis mellifera*, in a wind tunnel. *Insects*, 5(1), 92-104.

Evans, J. D., & Wheeler, D. E. (2001). Gene expression and the evolution of insect polyphenisms. *Bioessays*, 23(1), 62-68.

Farina, W. M., Gruter, C., & Diaz, P. C. (2005). Social learning of floral odours inside the honeybee hive. *Proc Biol Sci, 272*(1575), 1923-1928. doi:10.1098/rspb.2005.3172.

Farina, W. M., Grüter, C., Acosta, L., & Mc Cabe, S. (2007). Honeybees learn floral odors while receiving nectar from foragers within the hive. *Naturwissenschaften, 94*(1), 55-60.

von Frisch, K. (1967). Dance language and orientation of bees. Cambridge, MA.: Harvard University Press.

Grüter, C., & Farina, W. M. (2007). Nectar distribution and its relation to food quality in honeybee (*Apis mellifera*) colonies. *Insectes sociaux, 54*(1), 87-94.

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Grüter, C., & Farina, W. M. (2009). Past Experiences Affect Interaction Patterns Among Foragers and Hive-Mates in Honeybees. *Ethology, 115*(8), 790-797.

Guscelli, E., Spicer, J. I., & Calosi, P. (2019). The importance of inter-individual variation in predicting species' responses to global change drivers. *Ecol Evol, 9*(8), 4327-4339. doi:10.1002/ece3.4810.

Hamilton, A. R., Traniello, I. M., Ray, A. M., Caldwell, A. S., Wickline, S. A., & Robinson, G. E. (2019). Division of labor in honey bees is associated with transcriptional regulatory plasticity in the brain. *Journal of Experimental Biology, 222*(14), jeb200196.

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., . . .Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ, 6*, e4794. doi:10.7717/peerj.4794.

Hertel, A. G., Niemela, P. T., Dingemanse, N. J., & Mueller, T. (2020). A guide for studying among-individual behavioral variation from movement data in the wild. *Mov Ecol, 8*, 30. doi:10.1186/s40462-020-00216-8.

Hut, R. A., & Beersma, D. G. (2011). Evolution of time-keeping mechanisms: early emergence and adaptation to photoperiod. *Philos Trans R Soc Lond B Biol Sci, 366*(1574), 2141-2154. doi:10.1098/rstb.2010.0409.

Ikeno, H., Akamatsu, T., Hasegawa, Y., & Ai, H. (2014). Effect of olfactory stimulus on the flight course of a honeybee, *Apis mellifera*, in a wind tunnel. *Insects, 5*(1), 92-104.

Jones, B. M., Rao, V. D., Gernat, T., Jagla, T., Cash-Ahmed, A. C., Rubin, B. E., . . .Robinson, G. E. (2020). Individual differences in honey bee behavior enabled by plasticity in brain gene regulatory networks. *Elife, 9*. doi:10.7554/eLife.62850.

Khamis, A. M., Hamilton, A. R., Medvedeva, Y. A., Alam, T., Alam, I., Essack, M., ...& Bajic, V. B. (2015). Insights into the transcriptional architecture of behavioral plasticity in the honey bee *Apis mellifera*. *Scientific reports, 5*(1), 1-19.

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*, 14.

Kirchner, W. H. (1993). Vibrational signals in the tremble dance of the honeybee, *Apis mellifera. Behavioral Ecology and Sociobiology, 33*(3), 169-172.

Klein, S., Pasquaretta, C., Barron, A. B., Devaud, J. M., & Lihoreau, M. (2017). Inter-individual variability in the foraging behaviour of traplining bumblebees. *Sci Rep, 7*(1), 4561. doi:10.1038/s41598-017-04919-8.

Klein, S., Pasquaretta, C., He, X. J., Perry, C., Søvik, E., Devaud, J. M., ...Lihoreau, M. (2019). Honey bees increase their foraging performance and frequency of pollen trips through experience. *Scientific reports, 9*(1), 1-10.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*. USA, United States: Oxford University Press.

Menzel, R., Kirbach, A., Haass, W. D., Fischer, B., Fuchs, J., Koblofsky, M., . . .Greggers, U. (2011). A common frame of reference for learned and communicated vectors in honeybee navigation. *Curr Biol, 21*(8), 645-650. doi:10.1016/j.cub.2011.02.039.

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.

Moore, D. (2001). Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms. *Journal of Insect Physiology, 47*(8), 843-857.

Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R(2) and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *J R Soc Interface, 14*(134). doi:10.1098/rsif.2017.0213.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev Camb Philos Soc, 85*(4), 935-956. doi:10.1111/j.1469-185X.2010.00141.x

Nakayama, S., Laskowski, K. L., Klefoth, T., & Arlinghaus, R. (2016). Between-and within-individual variation in activity increases with water temperature in wild perch. *Behavioral Ecology*, arw090.

Pasquaretta, C., & Jeanson, R. (2018). Division of labor as a bipartite network. *Behavioral Ecology, 29*(2), 342-352.

Petelle, M. B., McCoy, D. E., Alejandro, V., Martin, J. G., & Blumstein, D. T. (2013). Development of boldness and docility in yellow-bellied marmots. *Animal Behaviour, 86*(6), 1147-1154.

Polatto, L. P., Chaud-Netto, J., & Alves-Junior, V. V. (2014). nfluence of abiotic factors and floral resource availability on daily foraging activity of bees. *Journal of Insect Behavior, 27*(5), 593-612.

Roberts, S. P., & Elekonich, M. M. (2005). Muscle biochemistry and the ontogeny of flight capacity during behavioral development in the honey bee, *Apis mellifera*. *J Exp Biol, 208*(Pt22), 4193-4198. doi:10.1242/jeb.01862.

Robinson, G. E., Page Jr, R. E., Strambi, C., & Strambi, A. (1992). Colony integration in honey bees: mechanisms of behavioral reversion. *Ethology, 90*(4), 336-348.

Rohlf, F. J., & Sokal, R. R. (1995). *Statistical tables*.

Scheiner, R., Page, R. E., & Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie, 35*(2), 133-142.

Seeley, T. D. (1992). The tremble dance of the honey bee: message and meanings. *Behavioral Ecology and Sociobiology, 31*(6), 375-383.

Seeley, T. D. (2009). The Wisdom of the Hive : the social physiology of honey bee colonies. Cambridge Massachusetts: Harvard University Press.

Seeley, T. D., Visscher, P. K., Schlegel, T., Hogan, P. M., Franks, N. R., & Marshall, J. A. (2012). Stop signals provide cross inhibition in collective decision-making by honeybee swarms. *Science, 335*(6064), 108-111. doi:10.1126/science.1210361.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: an intergrative overiew. *Q Rev Biol, 79*(3), 241-277. doi:10.1086/422893.

Smith, M. L., Koenig, P. A., & Peters, J. M. (2017). he cues of colony size: how honey bees sense that their colony is large enough to begin to invest in reproduction. *Journal of Experimental Biology, 220*(9), 1597-1605.

Stabentheiner, A., & Kovac, H. (2014). Energetic optimisation of foraging honeybees: flexible change of strategies in response to environmental challenges. *PLoS One, 9*(8), e105432. doi:10.1371/journal.pone.0105432.

Stabentheiner, A., & Kovac, H. (2016). Honeybee economics: optimisation of foraging in a variable world. *Sci Rep, 6*, 28339. doi:10.1038/srep28339.

Toma, D. P., Bloch, G., Moore, D., & Robinson, G. E. (2000). Changes in period mRNA levels in the brain and division of labor in honey bee colonies. *Proc Natl Acad Sci U S A, 97*(12), 6914-6919. doi:10.1073/pnas.97.12.6914.

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.

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.

Wang, Y., Kaftanoglu, O., Siegel, A. J., Page Jr, R. E., & Amdam, G. V. (2010). Surgically increased ovarian mass in the honey bee confirms link between reproductive physiology and worker behavio. *Journal of Insect Physiology, 56*(12), 1816-1824.

Whitman, D. W., & Agrawal, A. A. (2009). What is phenotypic plasticity and why is it important. . *Phenotypic plasticity of insects: Mechanisms and consequences*, 1-63.

5 Chapter 3: Ontogeny of social behaviors and flight in honeybee, *Apis mellifera L.*

5.1 Abstract

In honeybees, a trade-off between staying inside the colony and flying outdoor is regulated by age-dependent and experience-dependent learning procedures. It is therefore expected that individual bees behave differently depending on experiences and ages. In particular, social contacts (e.g., dance communication) are expected to greatly determine bees' outdoor behaviors. However, how indoor behaviors develop over bees' life history and regulate their outdoor behaviors is rare understood. Here, this chapter aims at mapping developmental ontogeny of behaviors inside and outside of the colony. It is hypothesized that each individual bee behaves differently within its own 'life history', and bees that are more active outdoors may have more experience in social contacts. Indoor behaviors were recorded using Raspberry pi camera and flight behaviors were tracked with harmonic radar. Out of the 87 focal bees, seventeen representative bees were monitored over their full life history. Eight indoor and five outdoor behavioral states were recorded over 15 days, respectively. Total numbers of indoor behaviors captured were 994 'dance' (dance following), 21779 'sna' (bees exchange food and social information with each other with antennae and trophallaxis) and 781 'Qinterest' (bees attended the queen). Instead, the outdoor events included 188 'Out' (bees left the colony) and 121 flights consisting of 65 orientation flights and 56 foraging flights. These data demonstrate considerable variation on the individual level. Bees at the same or similar ages behaved differently, and their life history also differed with respect to occurrence of indoor and outdoor behaviors. These results not only show that bees may start to follow dances immediately after their emergence and initiate orientation flights as early as age of 4th day, but also reveal that bees mostly begin their orientation flight after having the experience of following dances. Although bees differed in the life history, they maintained consistent frequencies of indoor and outdoor behaviors on the group level. Bees that were more active outdoors exhibited a higher level of social interaction with other workers ('dance' and 'sna') while they attended less the queen ('Qinterest'). The importance of spatial fidelity in regulating social interacts and

outdoor behaviors was studied. The results for this question indicate that spatial movements indoors at young age correlate positively with the frequency of social interactions and outdoor behaviors. Taken together, these results provide a complete life history of the indoor and outdoor behaviors and help us to better understand the correlation between the social interactions and outdoor behaviors.

5.2 Introduction

Social insects are ideal models for understanding the regulatory architecture of phenotypic behavioral specificity and flexibility. Social interactions among individuals are fundamental components of group stability. It accounts for information spreading and exchange, coordination of task allocation and collective cooperation. Individuals' roles are reflected in specific social behaviors. Thus, understanding how social behaviors develop is critical in ethology. Variation of social interactions on an individual level has received increasing interest over decades (Jones et al. 2020). Behavioral differences among individuals and within individual can either be consistent or less stable over lifetime (Réale et al. 2007, Sih et al. 2004, Wuerz and Kruger 2015). Honeybees show highly efficient labor division and cooperation (Johnson 2010a, Seeley and Visscher 1988). Their behaviors are characterized by within-caste plasticity in response to age and colony needs. Typically, they switch tasks from inside duties to outside foraging flight as they age (Jones et al. 2020, Kapheim et al. 2020, Lindauer 1953). However, bees of the same age also show behavioral variation (Huang and Robinson 1996, Johnson 2010a, Robinson et al. 1992, Seeley 1982). One explanation for this phenomenon may be intensities of information exchange via social interactions varied between individuals (Gernat et al. 2018). Antennation plays multiple roles in social contacts (Chole et al. 2019, Gernat et al. 2018). Antennae are equipped with multiple types of sensory organs such as mechanoreceptors (Esslen and Kaissling 1976), gustatory receptors and olfactory receptors (Lachér and Schneider 1963, Vareschi 1971), helping bees to differentiate conspecifics from members of other colonies through olfactory cues (Boulay et al. 2000, Breed et al. 1992, Mc Cabe et al. 2007) and to detect the queen by its pheromone (Boulay et al. 2000, Breed et al.1992, Hoover et al. 2003, Mc Cabe et al. 2007). Antennae are also involved in reinforcing appetitive learning and memory formation (Galizia et al.1997, Hammer and Menzel 1998, Joerges et al.1997, Menzel et al. 2001, Waters and Fewell 2012). Several studies have shown that information

exchange via antennation between experienced workers may be sufficient to initiate foraging flights (Balbuena et al. 2012, Cholé et al. 2019, Farina et al. 2012). Antennation is frequently accompanied with trophallaxis, at which one bee delivers liquid food to another bee via mouth-to-mouth (Wilson 1971). Trophallaxis activates mates to search for food inside and outside of the hive (Crailsheim 1998, Gil and De Marco 2005), gives arriving foragers feedbacks of food quality (Wainselboim and Farina 2000), allows receivers to establish associative learning about the chemosensory information of food (Farina et al. 2012), and reactivates bees' memory of former foraging experience (Balbuena et al. 2012). Thus, antennation and trophallaxis jointly establish an excellent system for exchanging and spreading social information inside the colony.

Dance communication, the most important communication system involves round dances (performed for food sources at distances shorter than 100 m) and waggle dances (for food sources at distances > 100 m). Dances appear mainly at the 'dancer floor', a region on the comb close to the hive exit (von Frisch 1967). Dancing foragers transmit information about the quality, distance, direction of food sources (von Frisch 1967, Riley et al. 2005) to other workers after successful foraging flights. Distance information of food sources is transferred in the duration, length and number of waggles in a waggle run while direction of the outbound flight relative to the sun azimuth at the particular time of a day is related to the direction of the waggle run relative to the gravity on the vertical comb surface (von Frisch 1967). The profitability of food resources is encoded in the thoracic vibration and the number of dance rounds (Hrncir et al. 2011, Seeley et al. 2000).

Except for the above indoor behaviors, queen retinue, feeding larvae and fanning contribute roles in structuring labor division. However, limited knowledge is known about them. Old egg-laying queens are more attractive to workers than the newly mated or virgin queens and workers that attended the queen are usually young bees (Pankiw et al.1995, Seeley et al.1979). Feeding is a behavior of nursing bees to feed larvae while fanning the wings is a behavior that middle-aged workers to regulate temperature and help evaporate nectar (Kaspar et al. 2018, Seeley 2014).

Although sleep is not a strictly social behavior, accumulated evidences suggest that it is conservatively important in consolidating recently acquired memory (Beyaert et al. 2012, Zwaka et al. 2015) and accuracy in dance communication (Klein et al. 2010).

Spatial fidelity is important for structuring information (Crall et al. 2018) and helps regulate information flow through social contacts (Mersch et al. 2013). Individuals vary in the use of space and thus may differ in social cues experienced in particular spatial zones. There has been increasing attention to spatial fidelity in social insects, particularly in the honeybees (Crall et al. 2018, Gernat et al. 2018, Wild et al. 2020) and ant (Mersch et al. 2013, Modlmeier et al. 2019, Pamminger et al. 2014). These studies shed a new light on understanding how spatial movements structure information flow that regulates social behaviors.

As a remarkable central-place navigator, the honeybees not only explore new food resources and perform back-and-forth flights to their familiar food destinations, but also execute conspicuous shortcuts on their way back home and between food sources (Menzel et al. 2005, Wehner and Srinivasan 2002). For successful foraging flights, honeybees should be familiar with the celestial compass and the surrounding layouts around the hive that are learnt during orientation flight (Capaldi et al. 2000, Degen et al. 2018). The orientation flights involve two forms: exploratory orientation and re-orientation flights (Degen et al. 2018). Exploratory orientation flights are performed by young bees when they leave hive for the first time (Capaldi and Dyer, 1999, Degen et al. 2015). Re-orientation flights are conducted by experienced bees transported to a new location or by a swarm setting down at a new nest site. During the foraging flights, bees establish a memory about multiple cues of the respective food sources (e.g., olfactory, color, shape), and the close or distant landmarks (Collett and Collett 2002, Dyer 1998, von Frisch 1965, Menzel et al. 2005, Wehner and Srinivasan 2002). Experienced bees gathered their private experience both inside and outside the colony and use this information to make decisions on when and where to forage (Grüter et al. 2011, Price et al. 2019).

Bees proceed through behaviors as they age. Socially acquired and individually gathered information do not share the same time scale. It is therefore important to ask how they interact over their life history, and which drive the other and when. For instance, how do trophallaxis, antennation and dance following influence outdoor behaviors during orientation and foraging flights? Biesmeijer and Seeley (2005) addressed these questions in an impressive endeavor. However, their study was limited to bees of unknown ages and did not include their former flight experience on an individual level. Ai et al. (2017) monitored the ontogeny of dance-related behaviors but did not consider other formats of social behaviors and flights. Degen et

al. (2015, 2016, 2018) documented age-dependent effect on flight duration leading to increasing range of exploration in sequential orientation flights but they did not collect information about social interactions before and during the execution of orientation flights. Klein et al. (2019) presented data about the frequencies of foraging flights on an individual level along bees' life history and suggested that bees improved foraging performance with experience. However, their tracking device using RFID at the hive entrance limited them to only record the time when individuals arrived and departed. They could not separate between orientation and foraging flights. In addition, they did not collect data of social behaviors inside the colony. All these studies did not provide a complete map about the developmental processes of the indoor and outdoor behaviors and their relations.

Cognitive experience during the early stage of life can shape future behavior (Arenas et al. 2013, Rittschof et al. 2015). Knowledge about the ontogeny of social behaviors and flights will help to address several questions: how do social behaviors and flight patterns of individual bees develop over the lifetime? How does pervious social experience (e.g., trophallaxis, antennation) affect future social communication (e.g., dance and dance following) and bees' flight performance? Does foraging flight only occur after orientation flights? Do all worker bees become foragers? Are bees more ready to follow dances that indicated the areas where they had explored? Are bees that are more active in social behaviors are more active in outdoor behaviors? Does spatial fidelity play an important role in information flow and regulating bees' indoor and outdoor behaviors?

Addressing these questions requires a full documentation of the behaviors inside and outside the colony along the life history of individual bees. Therefore, 17 bees with indoor behaviors (antennation, dance following, spatial fidelity, sleeping, queen-attending, feeding and fanning) were monitored with video recordings and with outdoor behaviors recorded by harmonic radar. This study establishes a comprehensive developmental document of individual bees' behaviors within the colony and outdoor behaviors (orientation and foraging flights).

These data provide a fundamental basis for revealing bees' behavioral development and uncovers the relation between the indoor and outdoor behaviors.

5.3 Materials and Methods

5.3.1 Experimental design

The experimental site was at an open pasture, with a highly structured agricultural landscape stretching from south to north and east to west with pathways, trees, bushes and creeks close to the village of Großseelheim (Hessen, Germany). A tiny colony with one comb including a queen and 1000-1500 workers (*Apis mellifera L.*) was housed in a wooden cabin (coordinate: 50°48'50.7' N, 8°52'33.9' E) (Fig 18). Small colony size allowed to clearly recognize the identity of bees marked with number tags and to monitor their behaviors on the individual basis in the dark with infrared light. The hive exit pointed towards south and was marked with a blue sticky tape. A transparent tube of 20 cm protruded from the inside hive to the outside exit making it possible to observe bees walking from the inside part of the hive exit to the outside part. The identity of each bee traveling inside the tube was detected and decisions were made in a fast sequence whether the particular bee would be caught for a catch-and-release experiment. Several experimenters worked together for this demanding exercise. A transparency plastic sheet was inserted into the tube in order to control the outbound and inbound activities (Fig 19a).

5.3.2 Introducing and marking bees

A comb with emerging bees was placed in an incubator at 33 ~ 34 °C and 65 ~ 70% RH overnight. Ninety-six newly emerged bees were collected (age 1), individually marked with number tags on the thorax (the focal bees: 201 ~ 297) and introduced into the colony. In addition, bees from the same colony were marked with numbers 100 ~ 197, 300 ~ 397 and 400 ~ 497. A few of these bees were trained to a feeding site and acted as dancers.

5.3.3 Training bees to a feeder

An artificial feeder (**FN**, feeder north) at 530 m north of the hive was established (50°49'07.8' N, 8°52'30.2' E, direction 352° relative to N). Standard training methods (von Frisch 1967) were applied to train a few bees (5 ~ 10) from the colony to **FN**. Different sucrose concentrations were used to regulate dance behavior. Bees visiting **FN** were marked with white dots on the abdomen, allowing fast identification of the dancers in the plastic tube of the hive exit (Figure 17). No odor was applied at any time of training. A complete feeder training protocol was established including the number of the foragers, the time of foragers arrival at **FN** and the concentration of sucrose solution at that time. None of the focal bees visited **FN** although many of them followed the dances from the trained foragers and performed flights toward **FN**.

Figure 17. Training bees collected food at the artificial feeder

5.3.4 Catch-and-release of focal bees

Experimenters sitting at one side of the exit observed all bees travelling in the plastic tube at the hive exit. When a focal bee appeared at the hive exit from inside, it was guided into a marking device (a plastic tube with a mesh on one side and a soft stopper on the other side), a transponder was quickly attached to the number tag on its thorax and subsequently released (Figure 19d). The focal bees carrying transponders were released at the hive exit. Flight trajectories were collected by harmonic radar (see below). A full protocol was established for these procedures at both the radar station via voice transmission through walky-talkies and at the hive. The protocol included descriptions of behaviors of the focal bees, their flight performance within visual range, time when it landed and returned into the hive after removal of the transponder. Weather conditions were recorded as described in Chapter 2.

5.3.5 Radar tracking system

Tracking the flights of the focal bees was achieved by harmonic radar system (Raytheon Marine GmbH, Kiel, NSC 2525/7 XU, located at the west of the bee colony, geographic coordinate: 50°48'52.2' N, 8°52'20.5' E), equipped with a sending unit consisting of a 9.4 GHz radar transceiver combined with a parabolic antenna providing approximately 44 dBi. The second harmonic component was the signal receiving unit, combined with the receiving unit consisted of an 18.8 GHz parabolic antenna, with a low-noise pre-amplifier directly coupled to a mixer (18.8 GHz oscillator) and a downstream amplifier with a 90 MHz ZF-filter (Figure 19b). A 60 MHz ZF-signal was used for recognizing signal from the transponder bees were

carrying. The transponder fixed to the thorax of bee was made of silver wire with a diameter of 0.33 mm and a loop inductance of 1.3 nH. The dipole of the antenna was a low barrier Schottky diode HSCH-5340 of centered inductivity. The weight and length of transponder were 10.5 mg and 11 mm, respectively. The range of harmonic radar was set to 0.5 nautical miles. Radar fixes were recorded every 3s. The raw outputs captured from radar were stored as bitmap files. An offline run custom made R-based program converted the bitmap data into x/y/t. These cartesian coordinates were aligned with the geography of the landscape using multiple stationary fix points. The geographic map was created with a commercial drone (DYI Inspire). The corrected cartesian coordinates together with the time stamps of each radar fix were used to generate flight trajectories.

5.3.6 Social behavior monitoring system

One infrared sensitive Raspberry Pi camera module (Raspberry Pi3 Model B, v2 Pi NoIR, 1.2 GHz QuadCore, 64Bit CPU) was fixed on each side of the observation hive, enabling us to record social behaviors of the whole colony under dark conditions (Figure 18f). The frame rate and the pixels of video recording were 15 fps and 1920 × 1080, respectively, allowing me to cover the full area on the comb with high resolution. Video recordings were conducted from morning to the end of daily experiment until sunset. The videos were recorded from the first day the focal bees were introduced into the hive to the day when all the focal bees had died. Since an unexpected heavy storm severely damaged the radar system, the radar-dependent experiments were terminated earlier than planned. The raw video streams were packaged with the format as H264 codec, requiring us to convert all the videos into mp4 format. The produced videos were wrapped by using MP4Box in the raspberry pi system for maximizing video quality. Since the methods of automatic tracking and classifying behaviors could not be applied in my data (see Chapter 1), the videos had to be analyzed manually using QuikTime player going frame by frame.

Figure 18. Experimental setup. 'a', cabin for the bee colony; 'b', harmonic radar; 'c', bee colony with camera and raspberry pi; 'd', experimental observer capturing bees at one side of exit; e, bees fanning at the hive exit; d, raspberry pi with camera.

5.3.7 Quantifying behavioral data inside and outside the colony

5.3.7.1 Behaviors inside the colony

5.3.7.2 Follow & No follow

Dance followings were identified by the criteria adopted from Judd (1995) and Wray et al., (2008). A focal bee was identified as dance follower when it was close to the dancer and was actively walking after the dancer within one bee's body length. A focal bee was not a follower when it did not walk after the dancer or did not direct its antennae toward the dancer although it was close to the dancer. The following parameters of dances were quantified: the number and duration of waggle run in a dance and direction of a waggle run to gravity. The relative position of the focal bees following a particular dance were noted. The numbers of other bees than the focal bees around the dancer were also counted.

5.3.7.3 Trophallaxis & Antennation

Trophallaxis and antennation (occurrence and duration) were quantified. When the duration was shorter than 1s, the information exchange was considered to be not effective. When the duration was longer than 1 s, the state was classified as 'Trophallaxis' or 'Antennation'. Workers offering food were defined as 'donor', and the food receivers as 'receiver'.

5.3.7.4 Spatial fidelity

The space of comb of each side were divided into 9 parts (L1, L2, L3, M1, M2, M3, U1, U2, U3) (Figure 19) and recorded the location where focal bees were every 5 min.
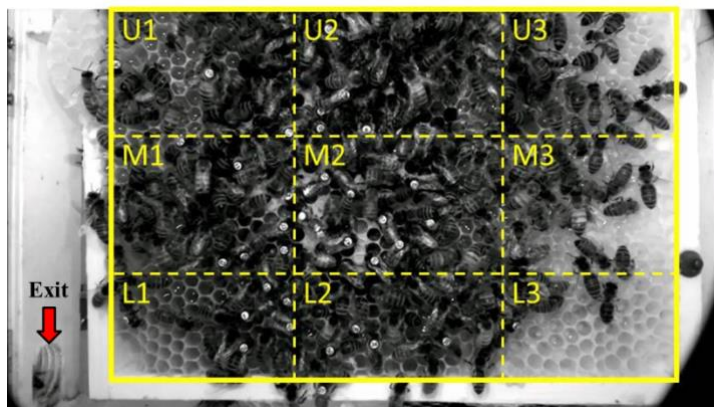
Figure 19. Division of comb space into 9 parts (L1 ~ U3). Hive exit is indicated in red arrow.

5.3.7.5 Sleeping

When a focal bee was immobile with antennae and abdomen being motionless, she was identified as in deep sleep (ds), while bees showing swaying motions or minute twitches of the antennae and abdomen were identified as resting (Klein and Seeley, 2011).

5.3.7.6 Queen retinue

A focal bee may show several responses when encountering the queen: she may keep away from queen, get close to the queen, take care of queen or show no response to the queen. Therefore, I would classify these states into two categories: 'Qinterest' and 'Qnointerest'.

5.3.7.7 Feeding

The events that when the focal bees inserted their heads into the larvae cells over 1s were counted as feeding behavior and their durations were recorded.

5.3.7.8 Fanning

The duration (> 1s) and frequency of bees fanning with wings were recoded.

5.3.7.9 Behaviors accompanied with steering towards the hive exit and flight behaviors

The following five categories were distinguished: 1) Look-Return (LR): a focal bee left the frame, steered towards the hive exit, appeared at the inside part of the tube and returned to the colony immediately. This behavior was interpreted as an attempt to leave. 2) Out-Walk (OW): a focal bee left the comb, walked fast to the outside of the hive exit. The focal bee was captured with a marking device and a transponder was attached on her thorax. She walked around the exit or on the wall of cabin but did not fly away from the hive exit. 3) Fly-Return (FR): a focal bee with a transponder flew away but soon returned to the hive exit. 4) Flight (FL): a focal bee released with transponders performed flights. These flights were differentiated into orientation and foraging flights. What to mention is, most of the foraging flights discussed in the Chapter were toward the direction of the artificial feeder (**FN**) but did not landed at the feeder while a few flights were toward natural food.

5.4 Data analyses

This study aims at exploring the variation of specific behaviors within and between individual bees. The analyses were as follows:

Seventeen focal bees were included in the analyses. They are bees 201, 203, 214, 215, 216, 218, 243, 244, 246, 251, 257, 262, 267, 271, 272 and 282. Nine indoor behaviors (i.e., 'dance following', 'Antennation', 'Trophallaxis', 'Qnointerest', 'Qinterest', 'ds', 'resting', 'fanning' and 'feeding') were recorded. Outdoor behavior 'Out' (bees left the colony to the hive exit) was categorized into four states: 'LR', 'OW', 'FR' and 'FL'. 'FL' was differentiated into 'O' (orientation flight) and 'F' (foraging flight). The potential relationship between the indoor and outdoor behaviors was firstly quantified by examining the correlations based on the average frequencies of behaviors.

To understand age-dependent variability of indoor and outdoor behaviors among the 17 focal bees, the daily variation of these behaviors between individuals and within each individual was calculated over 15 days. The onsets of these behaviors were

traced and the age when the first occurrence of these behaviors was compared. Intensities of behaviors inside the colony at the between-individual and within-individual levels: '**between-individual**' denotes the frequencies and durations of a bee's behaviors of dance following, social interaction ('sna') and attending the queen ('Qinterest') relative to the sum of the respective measure within the same day of all other focal bees. '**within-individual**' denotes the respective measure of the same behavior of a bee within a day relative to the sum of the respective measure on the rest of lifetime of the bee.

To demonstrate the relation between 'sna' and 'dance following', 1) frequency and duration of 'sna' on the day when bees followed or did not follow dances were compared at the group and individual levels. 2) numbers of dances followed on a day were categorized into 4 groups: n <= 5, 5 < n < 10, 10 < n < 15, n > 20. The intensity of 'sna' in the frequency and duration at the **between-** and **within-individual** levels were compared. Meanwhile, the age when bee have their first dance following was compared with the frequency and duration of 'sna' at the between-individual and within-individual levels, respectively.

Based on the frequency of 7 outdoor behavioral states, bees were classified into different functional groups manually and by PCA. Following these categorizations, 'sna' and 'dance following' among different functional groups were compared. To further explore potential correlation between the indoor and outdoor behaviors, bees at different functional groups were scored based on the frequency of different outdoor behaviors. In the analysis of comparing Out', each event of 'Out' was scored 1. In the case of comparing 'LR', 'OW', 'FR' and 'FL', each event was scored as -1, 0, 1 and 2, respectively. To compare the occurrence of 'Orientation' and 'Foraging' flight, each event was correspondingly scored as 1 and 2. Pearson correlation analysis was applied to correlate the average scores of outdoor behaviors and the indoor behaviors in frequency and duration.

Additionally, in addressing the question of whether the number of bees around the dancer would influence the bees' motivation to follow dances, the number of dance followers was counted.

The spatial patterns on the combs for each bee were investigated by daily recording their spatial location on the combs (Figure 19) every 5 minutes. The index of daily spatial dispersion indicating how active each bee was in spatial use ('D') was calculated. High values of D indicated bees were in high tendency to move around

different areas of the colony and active in social behaviors while low spatial dispersion showed bees maintained in a relatively stationary state in a single area with less chance to access social contacts. The index of dispersion was calculated as the ratio of the variance to the mean: $D = \sigma^2 / u = k*(N^2 - \Sigma f^2) / N^2 * (k-1)$ (Walker 1999) (https://www.statisticshowto.com/index-of-dispersion/), where k was the number of area categories, N was the total observation number of areas where bees occupied, and f was the observation number of each area category.

To assess whether spatially specialized bees was a predictor for its role in the colony, the connection between patterns of spatial fidelity and social contacts of dance following and 'sna' was explored at **between-** and **within-individual** levels.

5.5 Statistics

Normality and homogeneity of variance for each behavioral category were checked in order to determine the statistical methods. When the dataset was parametric, one-way ANOVA test was applied or otherwise Kruskal-Wallis one-way analysis of variance test ('K-W') was conducted. After one-way ANOVA test or K-W test showed statistically significant differences ($p < 0.05$), the corresponding post hoc analysis TukeyHSD or dunn test was used to determine the pair-wise differences between two bees. Wilcoxon test was applied to compare the differences between two groups of non-parametric data of independent and paired samples. The following packages were applied in data analysis: 'GGally' and 'Hmisc' with 'pearson' method were used for plotting correlation matrixes and testing correlation of significance, respectively. 'dplyr' was used to check normality. 'stats' and 'FSA' were used to conduct parametric and non-parametric statistical analyses, respectively. 'ineq' was used to plot cumulative plots. 'FactoMineR' and 'factoextra' were applied to perform and visualize PCA. All the data analyses were performed in R version 4.0.4. Software 'Tableau' was applied to produce behavioral heat maps.

5.6 Results

5.6.1 General characters of indoor behaviors

The tendency of frequencies of 'Waggle dance following' and 'Waggle run following' were similar, and this was also similar between 'Antennation' and 'Trophallaxis' (Figure S3). Thus, I would focus on analyzing 'Waggle dance following' and combined data of 'Antennation' and 'Trophallaxis' as social contact 'sna' for further

comparative analyses. The frequencies of the indoor behaviors ('Waggle dance following', 'sna', 'Qinterest') were positively correlated with the frequency of bees leaving the colony ('Waggle run following', $p < 0.01$, 'sna', $p < 0.01$, 'Qinterest', $p < 0.05$). 'ds', 'resting', 'fanning' and 'feeding' were neither correlated with the outdoor behavior 'Out', nor with other indoor behaviors. 'QNointerest' was positively correlated with 'sna' ($p < 0.05$), but not with 'Out'. Therefore, 'Qnointerest', 'resting', 'ds', 'fanning' and 'feeding' were excluded from further analyses. The total numbers of behavioral events analyzed for the 17 focal bees within 15 days' observations were: 994 'Waggle dance following', 21779 social contacts 'sna' and 781 'Qinterest', 188 'Out' and 121 flight trips with 65 orientation flights of 14 bees and 56 foraging flights of 5 bees. Another three behavioral states of 'Out' included 17 'LR' of 9 bees, 17 'OW' of 7 bees and 5 'FR' of 3 bees.

Figure 20: Correlation matrix of the indoor and outdoor behaviors. '*' and '**' indicated p-value < 0.05 and p-value < 0.01, respectively. The correlation coefficient in each cell shows the correlation between two behavioral states.
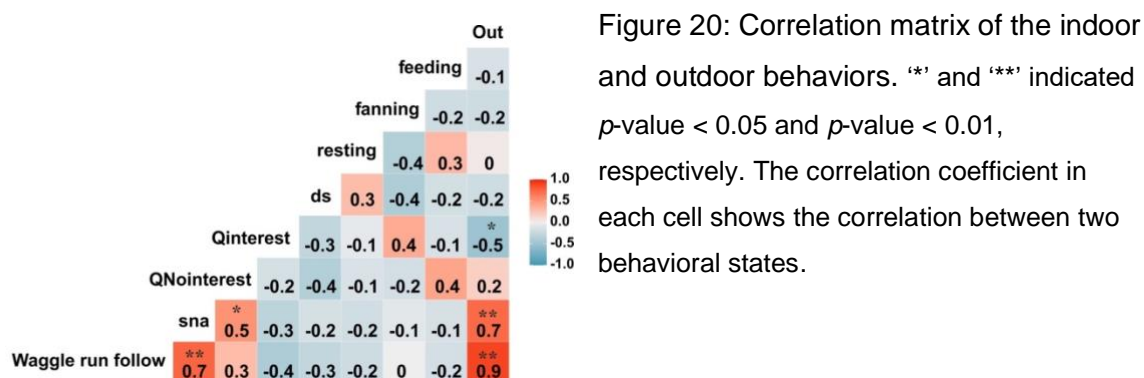
5.6.2 Most indoor and outdoor behaviors were performed by a minority of elite bees

The 17 focal bees exhibited considerable variation in the frequency and duration of indoor and outdoor behaviors (Figure 21 ~ 23, Table S5a). Bees 216, 244, 262, 267 and 271 displayed the highest activity of dance following, bees 218, 243, 257 and 276 followed less, and bees 201, 203, 214, 215, 246, 251, 272 and 282 followed the least dances (Figure 21A, Table S5b). Similarly, bees 216, 244, 267 and 271 remained highest levels in 'sna'. Although bee 262 was significantly lower in the level of 'sna' than bee 216 ($p = 0.04$), she did not show significant differences in 'sna' from bees 244, 267 and 271 (p-values > 0.05, Table S5b). The remaining bees had a lower frequency of 'sna'. Of which, bee 218 was more active than bees 214, 272 and 282 (Figure 21B, Table S5b). The overall frequency of bees acting as 'receiver' of social signals was not significantly different (K-W, $p = 0.45$). Thus, 'receiver' and

'sender' were not further separately studied for the 'sna'. When bees 215, 216, 251, 262, 267 and 282 came close to the queen, they showed little response to the queen. Bees 201, 244 and 271 showed moderate level of response, while bees 203, 214, 218, 243, 246, 257, 272 and 276 displayed the highest level of caring the queen (Figure 21C). These results indicated an opposite tendency between 'Qinterest' and dance following and 'sna'. Across all the focal bees, the distributions of duration of 'sna' and 'Qinterest' showed the same effect as the frequencies (Figure 22). Taken together, bees 216, 244, 262, 267 and 271 outperformed in social interaction ('dance following' and 'sna') than what other bees did.

Bees also presented extreme differences in outdoor performances (Figure 23). Bees 216, 244, 267 and 271 had the highest activity in 'Out', orientation and foraging flights. Although bee 262 did not perform as much flights as these four bees, she was also highly active in performing orientation and foraging flights. Bees 257, 272 and 282 frequently left the colony, however, they did not perform flights. Bees 201, 243, 276, 214 and 218 showed intermediate level in the frequencyof orientation flights, but they did not perform foraging flights

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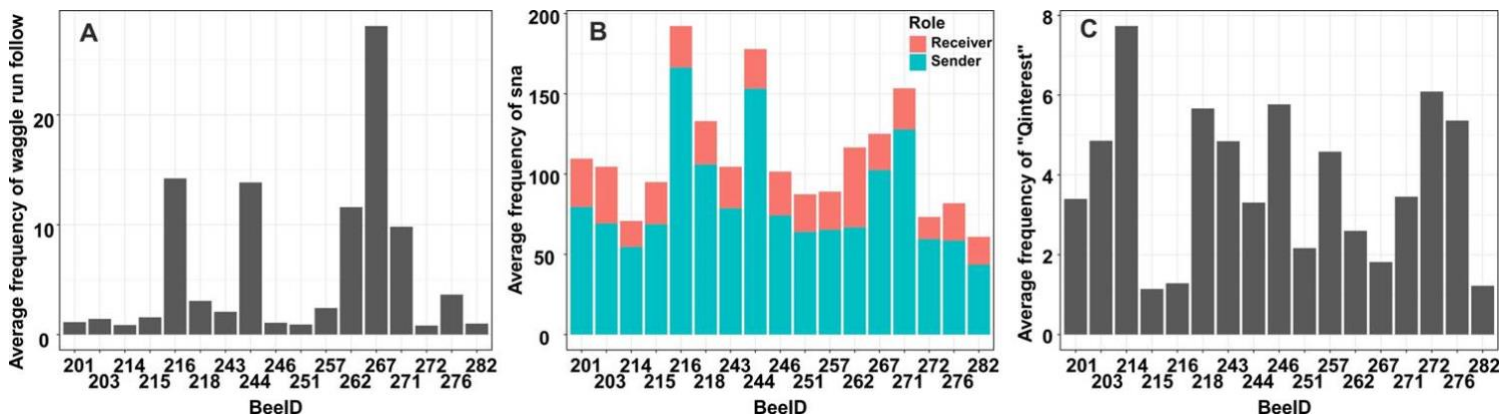
Figure 21. Frequency of indoor behaviors 'Waggle dance following', 'Waggle run follow' and 'Qinterest' among 17 bees over their life spans.
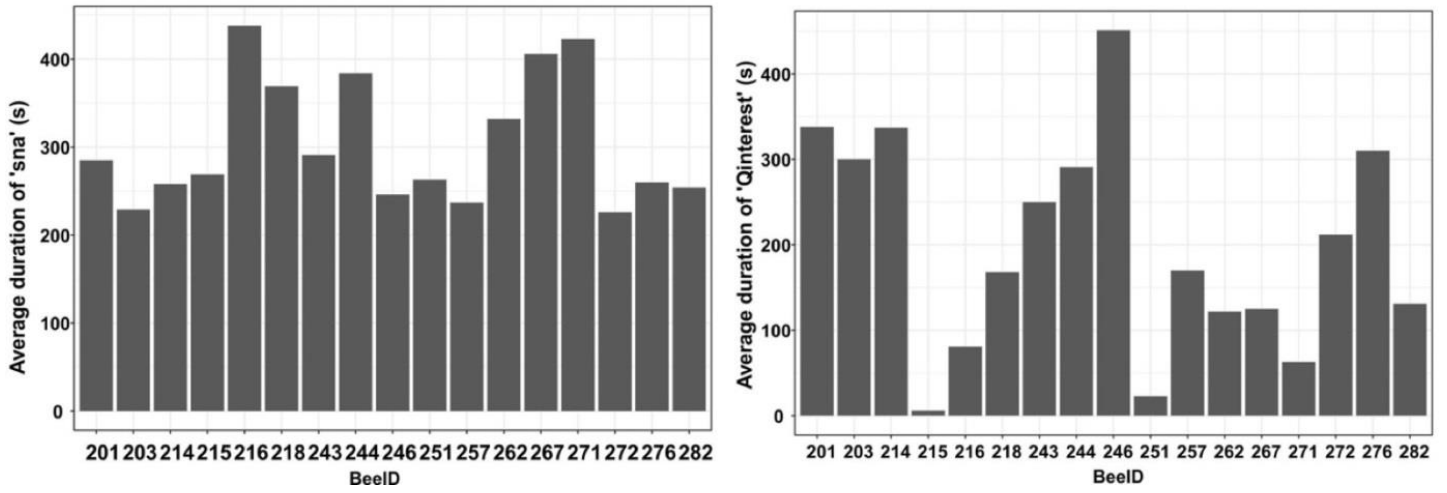
Figure 22. Duration of indoor behaviors 'sna' and 'Qinterest' among 17 focal bees.
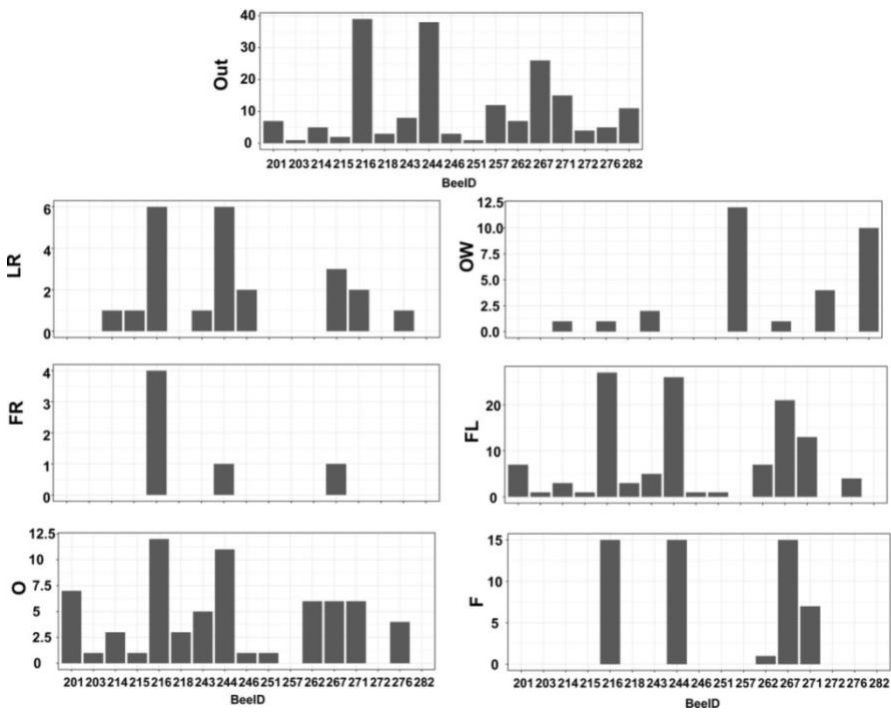
Figure 23. Frequency of different outdoor behaviors over the 17 bees' life spans.

5.6.3 Variation in indoor and outdoor behaviors between and within individuals

The quantification of daily variations across indoor and outdoor behaviors among the 17 focal bees showed high variability at within- and between-individual levels over 15 days (Figure 24). 'sna' occurred every day while dance following, 'Qinterest' and outdoor behaviors only existed occasionally.

Generally, the highest daily frequency and duration of 'sna' of most of bees were less than 200 and around 250s, respectively (Figure 25). However, the daily frequency and duration of 'sna' remained to be highly varied within and among individuals. Among individuals, except for bees 203, 251, 257, 272 and 282 remained in relatively stable frequency levels of 'sna' less than 200 events, other bees were changing in social contacts. Bees 201, 214, 216, 218, 244, 246, 267 and 271 showed peaks at young ages (1st ~ 4th days), middle (7th ~ 8th days) and older age (10th ~ 13th days), whereas bees 215 and 262 were most active in 'sna' on the 3rd and 4th day. Bees 243 and 276 reached a peak in the frequency of 'sna' on between 5th ~ 7th and 4th days, respectively. The daily changes in the duration of 'sna' showed a similar dynamic as the frequency for most bees, except for bees, e.g., bee 257 had a particularly high frequency of 'sna' in the older age but contacted with other bees in shorter durations, while bee 282 had a stable frequency of contacts but suddenly showed extremely longer durations of 'sna'.
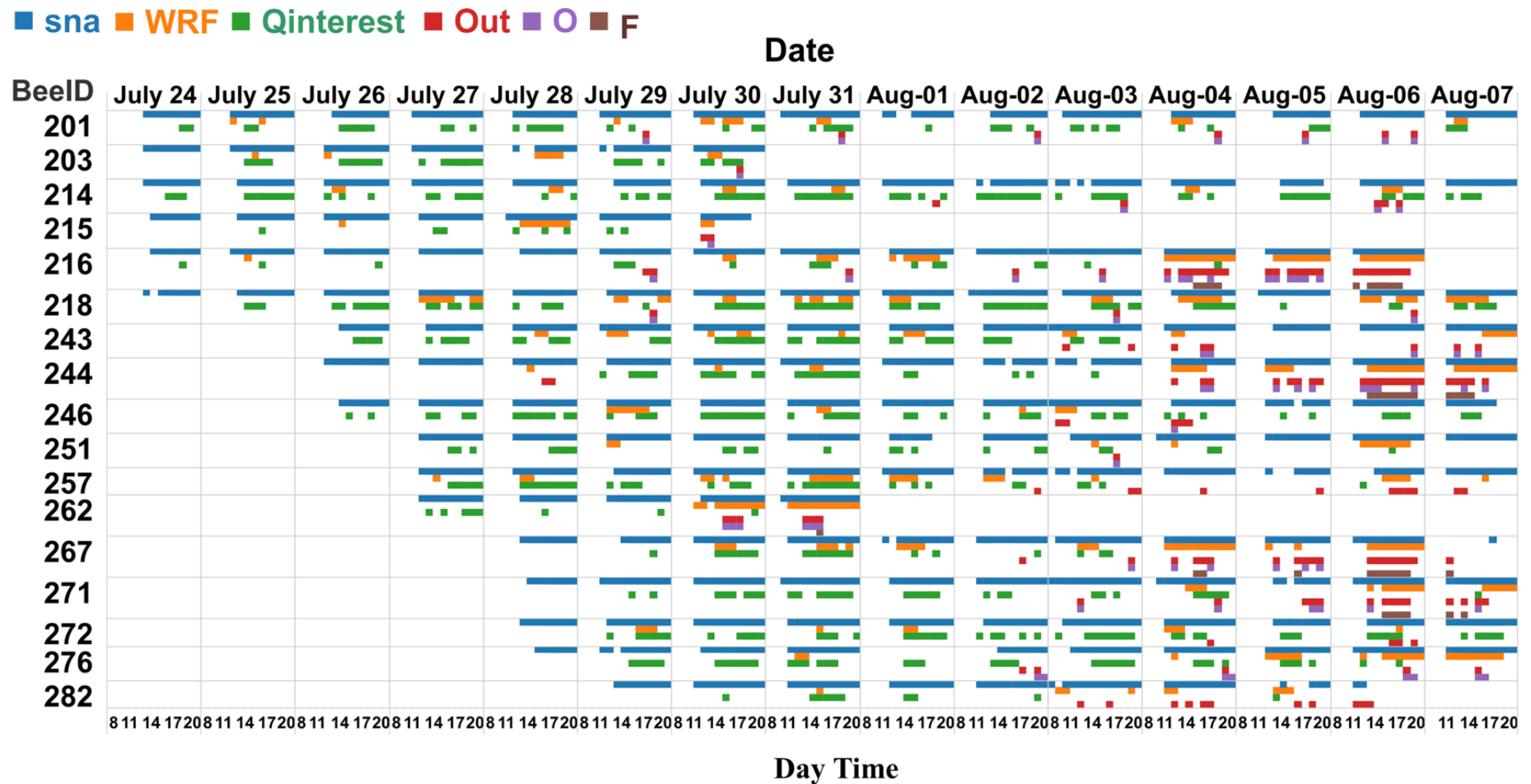
Figure 24. Traces of the daily indoor and outdoor behaviors of the 17 focal bees over their lifespans. X axis at the bottom and on the top indicates the daytime and date during which bees follow dances ('WRF', orange), make social contact with other bees ('sna', blue), attend the queen ('Qinterest', green), leave the colony ('Out', dark red) and perform orientation flights ('O', purple) and foraging flights ('F', brown), respectively. The lengths of bars with different colors indicates the frequency of different behaviors. The vertical number 201 ~ 282 refer to bee identity. Bees 201 ~ 218 emerged on 24th, July; bees 243 ~ 246 on 26th, July; bees 251 ~ 262 on 27th, July; bees 267 ~ 276 on 28th, July; bee 282 on 29th, July.
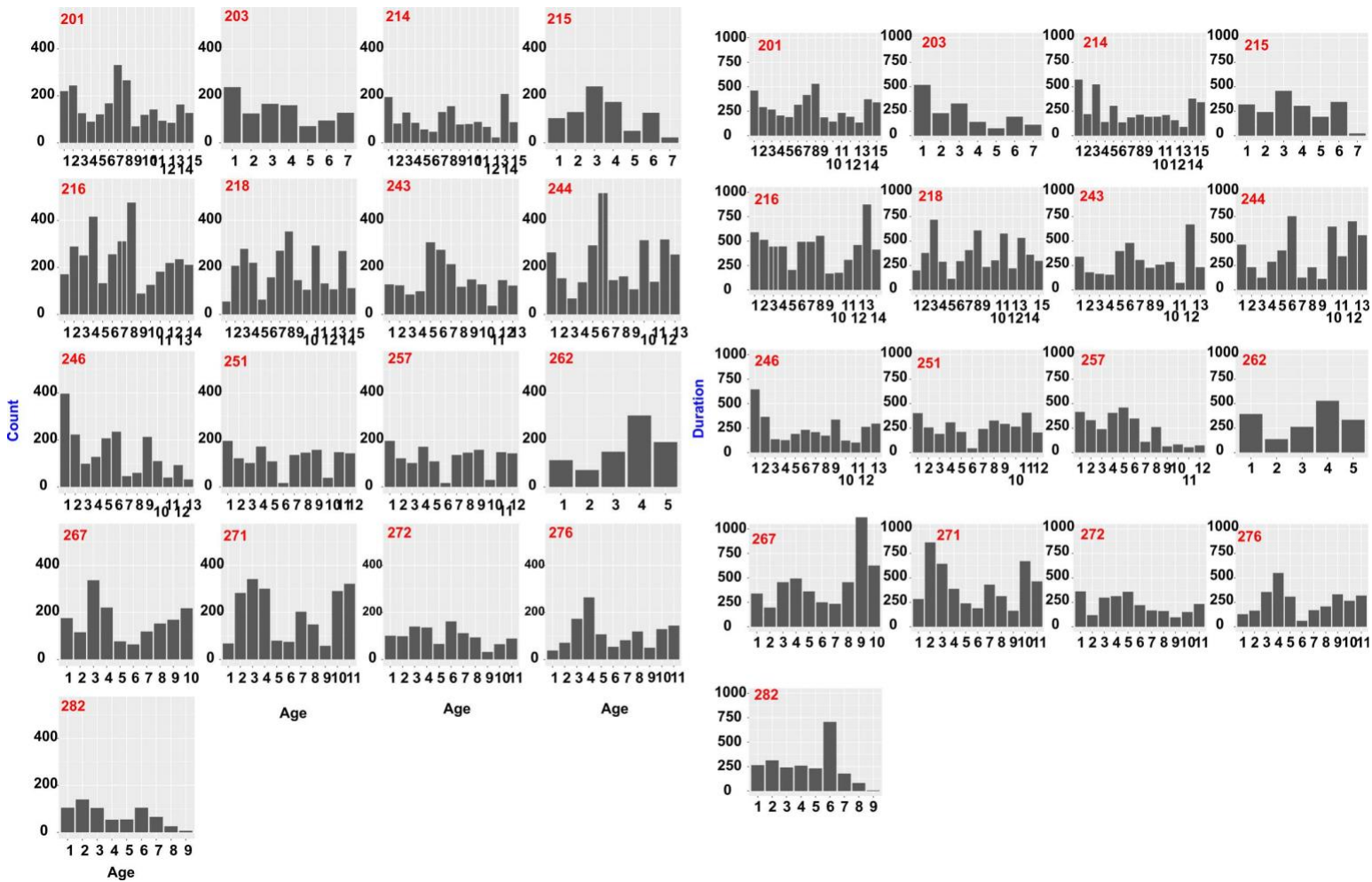
Figure 25. Daily levels of social contacts 'sna' in the frequency and duration (in second) among the 17 focal bees.

Bees varied greatly in following dances within and between individuals over age. The days of age when bees started to follow dances differed among the 17 focal bees (Figure 24). Bees could follow dances as early as the first day of age (e.g., bee 257) and as late as the 8th day of age (e.g., bee 271). However, most bees started dance following at the age of 3rd day. The rate of dance following among the 17 bees differed. For example, comparing to the other bees, bees 218, 243 and 257 followed dances consecutively over some few days of age while bees 201, 216 and 276 occasionally followed dances with a gap of 2 days or 4 days. Daily numbers of dance followings varied greatly within and between individual. For instance, bees 216, 243, 244, 251, 267, 271 and 276 followed only 1 or 3 dances during the first few days and reached high levels of dance following after 10 days (e.g., over 70 dance followings within a day). Bees 218 and 246 followed more dances at the beginning of age and

decreased over the later days of age. Other bees followed more dances in the middle age, e.g., bees 201, 203, 215, 262 and 282 (Figure 27, left). These results showed the day when bees reached high level of dance following differed greatly indicating that these effects were not cause by more or less the performance of dances of dancers. What to mention, although bee 262 did not follow dances for many days, she followed over 20 dances in the first two days (Figure 25, left).

The daily frequency and duration to attend the queen varied greatly within and between bees (Figure 27). Bees 215, 251 and 262 were least attracted by the queen over days. Although the frequencies and duration bees attended the queen fluctuated, e.g., bee 201, 214, 243 and 272, etc., most bees were least attracted by the queen in the older ages, except for bee 214. The tendency of durations bees spent on attending the queen was less consistent than with the changes of frequency. For example, bees 201, 214, 218, 243, 267, 272, 276 and 282 occasionally spent extremely longer durations of queen attendance, even though they showed low frequency in caring the queen.

As for the outdoor behaviors, bees normally left the colony ('Out') at an age of 6 days, but they could also go out as early as 3 days of age (e.g., bee 244, Figure 24). At early age, bees intermittently left the colony with a gap of 1 day and 6 days (e.g., bee 244, Figure 25). At older age, they went out more frequently the day after another. Orientation flights occurred before foraging flights. Here, what to remind was that the term 'foraging flight' did not mean that bees forage at a feeding site but rather that she steered toward the feeding site. Commonly, bees started foraging flight after 5 ~ 8 of orientation trips, but they could initiate foraging flight after 2 trips of orientation flights (e.g., bee 267). They flexibly switched between orientation and foraging flights (Figure 26, right). My observations showed that bees started their first orientation flight only after having experience of dance following. Although bees 257, 272 and 282 went to the hive exit multiple times, they did not perform a flight. Thus, no flight data are shown for these bees in Figure 26 (right). The numbers of orientation and foraging flights were different among the 17 bees over days. Most of bees only performed 1 ~ 2 orientation flights each day, e.g., bee 201 (Figure 26, right). Bees 216, 244, 262, 267 and 271 had both orientation and foraging flights and both the numbers of flights increased as they aged.
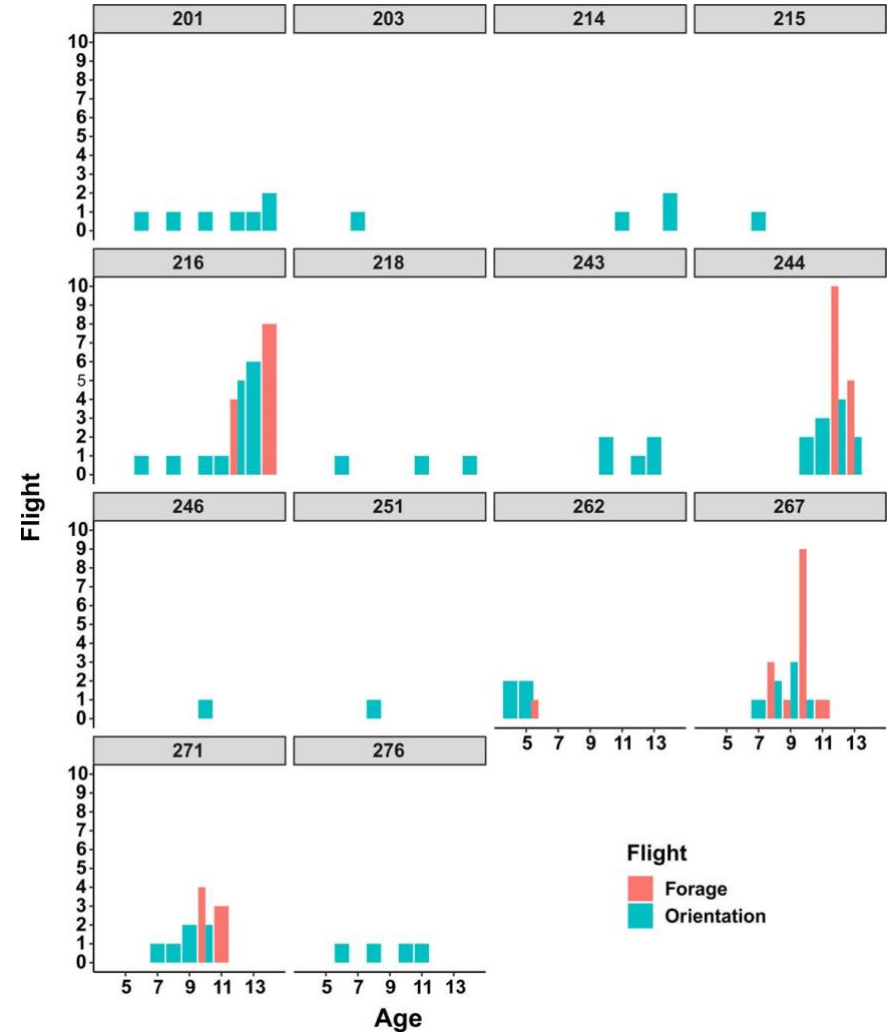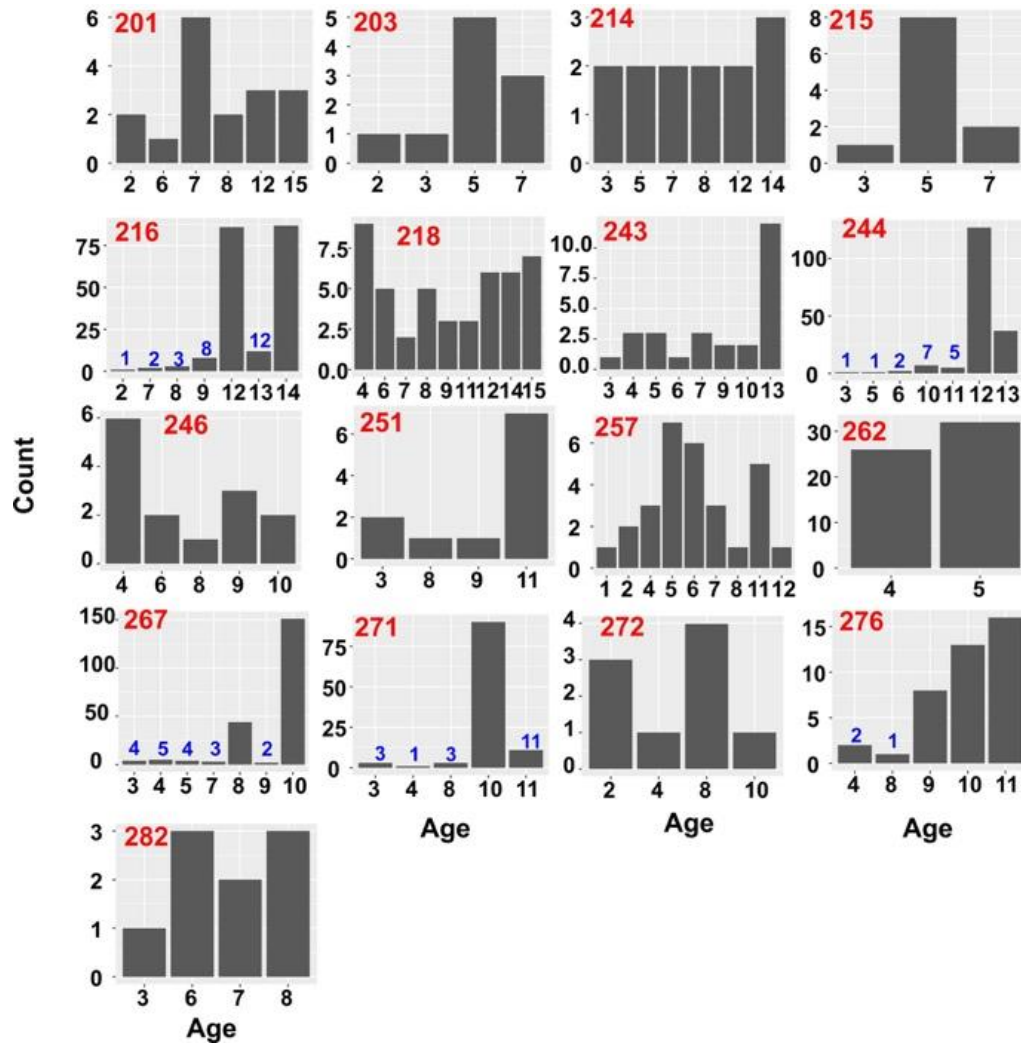
Figure 26. Daily levels of dance following (left) and flights (orientation and foraging flights) (right) among the 17 focal bees. Due to the different scales within a single plot, some small values that are not shown visually are marked in blue on the left figure.
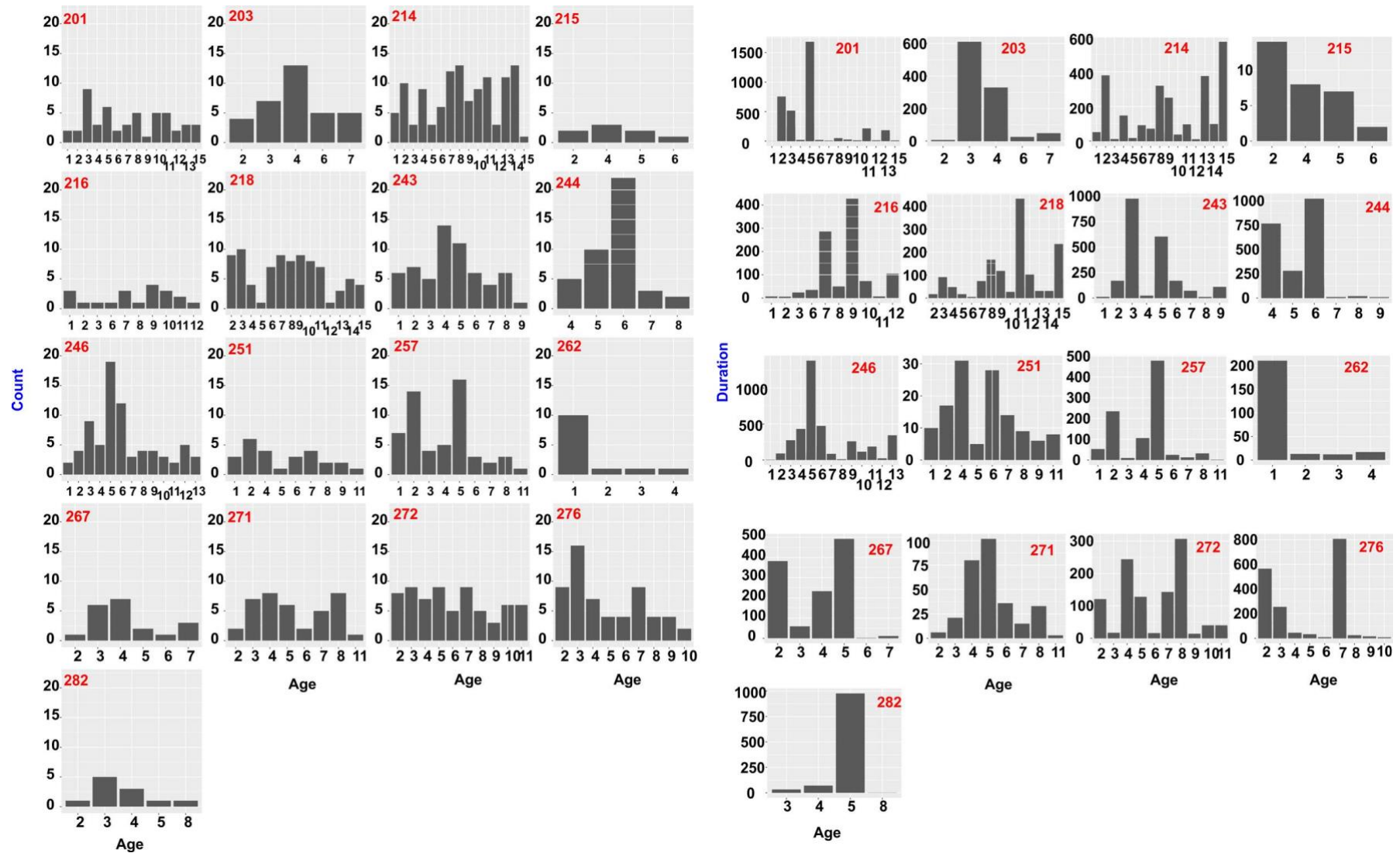
Figure 27. Daily levels of 'Qinterest' in the frequency and duration (in seconds) among the 17 focal bees.

In general, variations of intensities of indoor behaviors in frequency and duration differed at **between- and within-individual** levels (Figure 28). In particular, the daily intensity of dance following in frequency **between** bees was more stable and similar in the early days and varied more greatly as bees aged (Figure 28A, e.g., bee 271 at between the 8th ~ 10th days of age, bees 216, 218, 244 and 271 after the 10th day of age). Compared to this, the intensity of dance following in frequency was more variable **within individual** over days (Figure 28B), e.g., bees 203, 215, 246 and 251. These results were separately similar to the intensities of 'sna' both in frequency (Figure 28C, D) and duration (Figure 28G, H) at **between-** and **within-individual** levels. The overall development intensities of 'Qinterest' at **between-individual** level in frequency and duration stayed similar for most bees, except for bees 201, 214, 218 and 246. These bees increased the care for the queen at older ages (Figure 29E, I). At **within-individual level,** most bees had low but stable levels of interest in attending the queen over their lifetime, except for bees 203, 215, 244 262, 267 and 282 showed fluctuating intensity of care for the queen at their younger ages (Figure 29F, J).
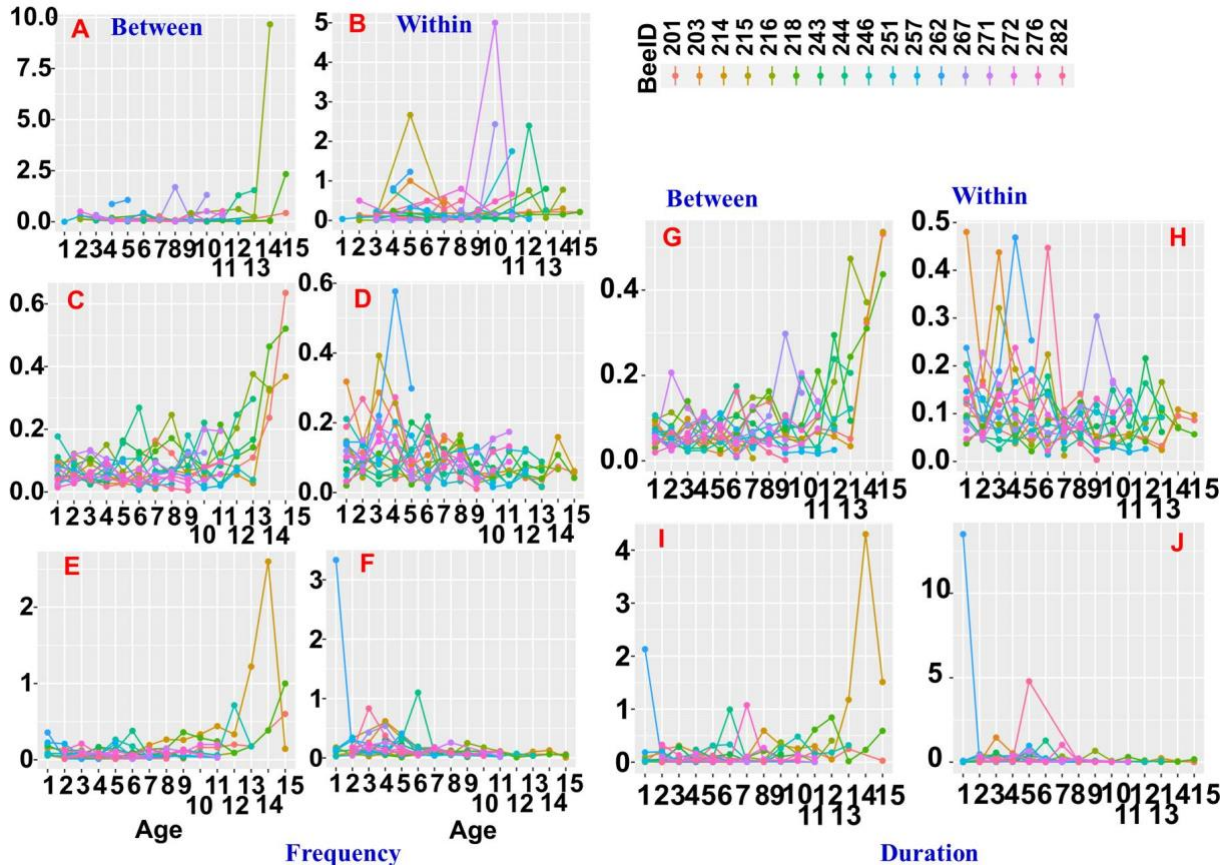
Figure 28. Intensities of indoor and outdoor behaviors in the frequency (left) and duration (right) of the 17 focal bees at within- and between-individual levels. The intensities of behaviors in the frequency and duration at **Within-** and **Between-individual** levels were calculated separately as: the frequency and duration of a behavior of a day relative to the respective measures of the rest days within an individual bee ('**Within-individual'**), and of a bee relative to the respective measures of the other bees on the same day ('**Between-individual'**). A, C, E, G, I show the intensities 'dance following', 'sna' and 'Qinterest' at **between-individual** level, and B, D, F, H, J figures show these corresponding parameters at **within-individual** level.

5.6.4 Bees active in social interaction were more active in outdoor behaviors

With the description of development of indoor and outdoor behaviors above, it was found that each bee behaved differently over their lifetime. Bees were differentiated into 4 groups to show similar characteristics in their life history. The method to make the groups were based on a comprehensive comparison of frequency of 'Out', 'Orientation', 'Foraging' and 'OW': bees 216, 244, 267, 262 and 271 in group 'A' were characterized with highest frequencies in orientation and foraging flights. Bees 201, 203, 214, 215, 218, 243, 246, 251 and 276 in 'B' group showed intermediate level of numbers of orientation and foraging flights. Bees 257, 272 and 282 in group 'C' were with intermediate level of numbers of 'Out', but highest frequencies of 'OW' without a flight (Figure 23). Cumulative distributions in the frequency and durations of indoor and outdoor behaviors were shown in Figure 29. Compared to the bees in the groups of 'C' and 'B', the bees in the group 'A' outperformed more in 'sna' and dance following (Figure 29, right) and less in 'Qinterest'. No significant differences in the frequencies of 'dance following', 'Qinterest' and 'Out' between bees observed in the groups of 'B' and 'C'. Furthermore, bees in the group 'B' were more active in 'sna' than bees in the group 'C'.
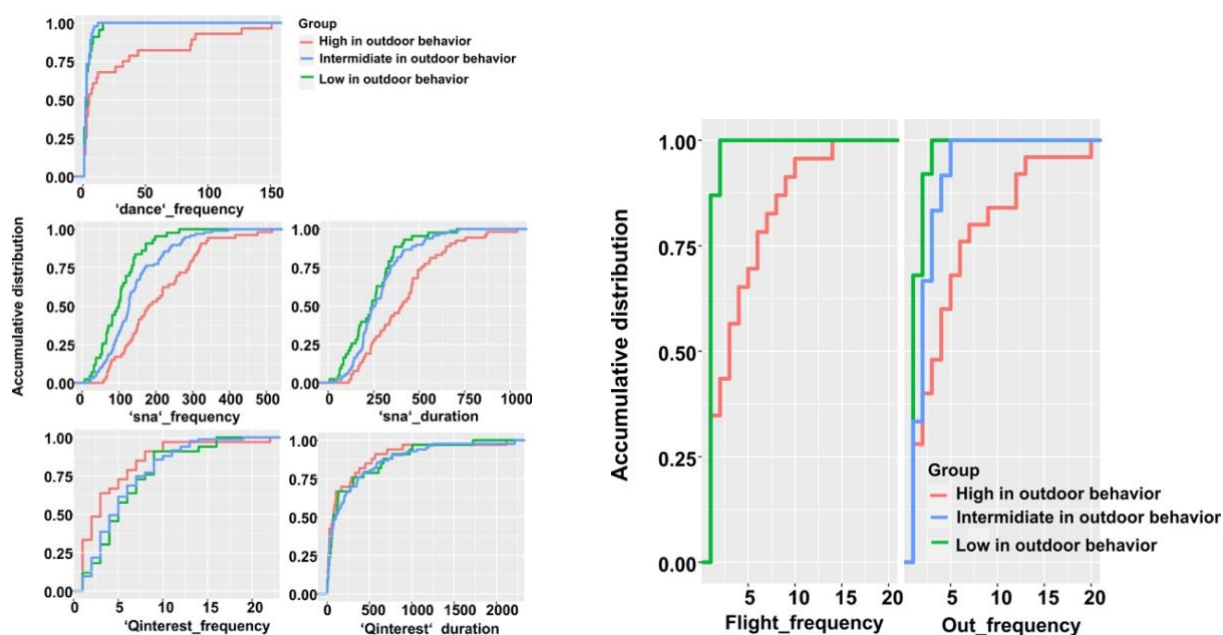
Figure 29. Cumulative plot of the indoor and outdoor behaviors for grouping bees with different outdoor performance. 'dance'_frequency, 'sna'_frequency and 'Qinterest'_frequency are the frequencies of 'dance following', 'sna' and 'Qinterest', respectively. 'sna_duration', 'Qinterest_duration' represent the duration of 'sna' and 'Qinterest', respectively. 'High in outdoor behavior', 'Intermediate in outdoor behavior' and 'Low in outdoor behavior' were group 'A', 'B' and 'C' as defined in the result part of **5.6.4.**

To be more accurate in classifying the focal bees, PCA was applied to mean frequency of outdoor behaviors (Figure 30, Table S6). Two PCs with loading values were retained and clusters for behaviors and individuals were given in Figure 30. PC1 explained 66.2% of the total variance and PC2 13.2%. The results indicated that PC1 was positively associated with the frequency of outdoor behaviors 'Out', 'Flight', 'Foraging', 'LR', 'Orientation' and 'FR'. PC2 was largely positively influenced by 'OW'. Bees in group 1 (bee 216, 244 and 267) were differentiated from bees 262 and 271 in group 2. Bees 201, 203, 214, 215, 218, 243, 246, 251, 272 and 276 were in group 3 and bees 257 and 282 were in group 4. This clustering corroborated the manual grouping.
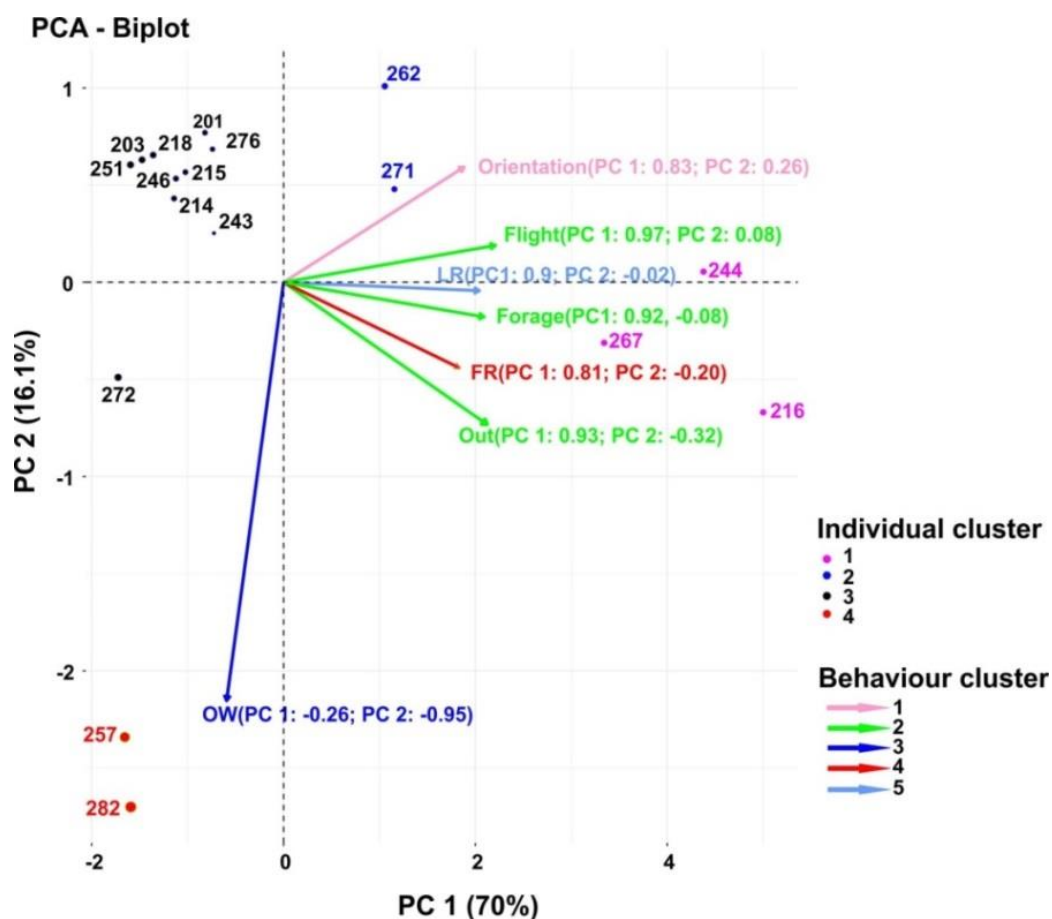
Figure 30. Principal Component Analysis (PCA) of outdoor behaviors. The figure shows tow plots in one for the individual bees and the behaviors. Loading values of PCA of the two main components (PC1-PC2) for outdoor behaviors of the 17 focal bees. Vectors pointing in similar directions indicate positively correlated variables, vectors pointing in opposite directions indicate negatively correlated variables, and vectors at proximately right angles indicate low or no correlation. Individuals with different colors were grouped by k-means (0.95-probability level).

In the next step, the average frequencies of outdoor and indoor behaviors were separately compared. (Figure 31). Bees in the group 2 were not significantly different from bees in the group 1 in most indoor and outdoor behaviors, except for 'FR' (K-W test, $\chi2 = 8.8$, df = 3, $p < 0.01$). Although the difference of 'Out' was not significant between groups of 1 and 2 ($\chi2 = 12.2$, df = 3, $p > 0.05$), the higher occurrence of 'FR' in the group 1 might be due to the higher frequency of 'Out'. The frequencies of 'Flight' ('Orientation' and 'Foraging' flights) were different among the four groups, which were highest in the groups of 1 and 2, followed by in the group 3 and lowest in group 4. Significant differences were observed between group 1 and 4 ('Flight': $\chi2 = 11.9$, df = 3, $p < 0.01$, 'Foraging': $\chi2 = 14.8$, df = 3, $p < 0.05$, 'Orientation': $\chi2 = 11.6$,

df = 3, *p* < 0.01), group 1 and 3 ('Flight': χ2 = 11.9, df = 3, *p* < 0.05, 'Foraging': χ2= 14.8, df = 3, *p* < 0.05, 'Orientation': χ2 = 11.6, df = 3, *p* < 0.05), group 2 and 3 ('Flight': χ2= 11.9, df = 3, *p* < 0.05, 'Foraging': χ2 = 14.8, df = 3, *p* < 0.05, 'Orientation': χ2 = 11.6, df = 3, *p* < 0.05), and group 2 and 4 ('Flight': χ2 = 11.9, df = 3, *p* < 0.05, 'Foraging': χ2 = 14.8, df = 3, *p* < 0.05, 'Orientation': χ2 = 11.6, df = 3, *p* < 0.05). These results were consistent with indoor behavior 'Dance following' and 'sna' (Figure 31). Although bees in the group 3 had more flights and 'sna' than in the group 4, the differences were not significant. Bees in the group 4 had significantly higher frequencies of outdoor behaviors ('Out') than in the group 3 (χ2 = 7.2, df = 3, *p* < 0.05), but only 'OW'. Although there was no significant difference in response to the queen between four all groups, bees in the group 3 and 4 showed the highest frequency and longest duration in attending the queen.
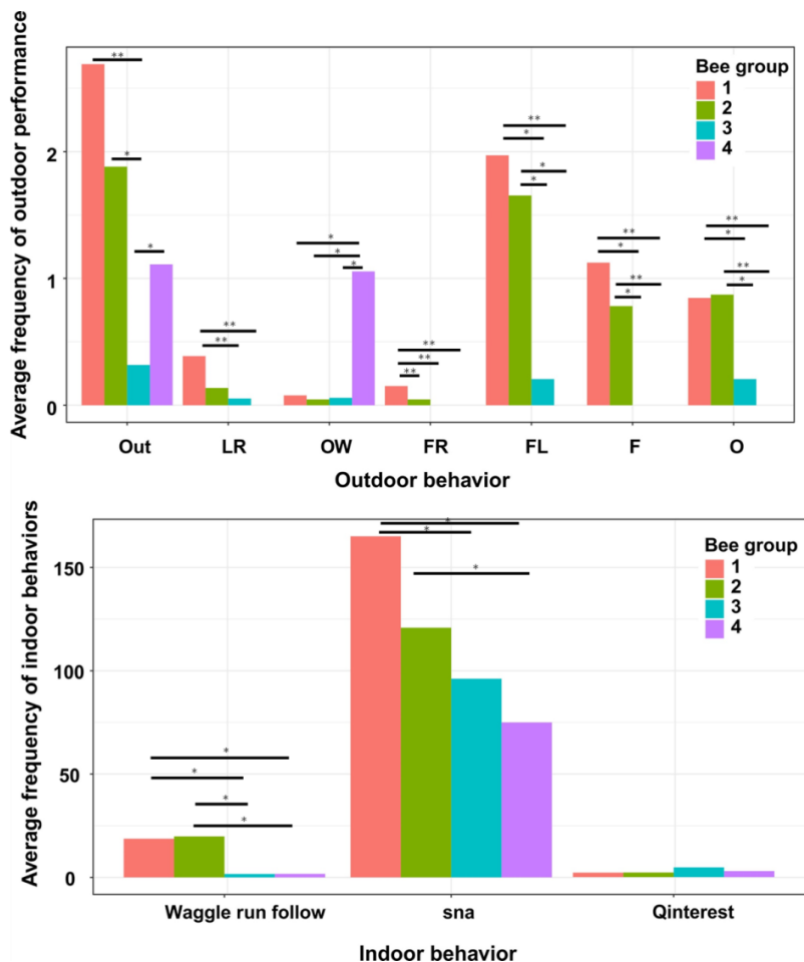
Figure 31. Average frequency of outdoor and indoor behaviors after grouping the 17 focal bees into four groups according to the PCA in Figure 30. Group 1 consisted of bees 216, 244 and 267, group 2 consisted of bees 262 and 271, group 3 consisted of bees 201, 203, 214,

215, 243, 246, 251, 272 and 276, and group 4 consisted of bees 257 and 282. '*' and '**' indicate the *p*-values < 0.05 and < 0.01, respectively.

The relation between multiple social behaviors was further explored. Bees on the days with dance following had more and longer 'sna' (Figure 32A ~ D, Wilcoxon test, Frequency: $p = 0.64$, Duration: $p < 0.01$). Although the difference for these frequencies was not significant, more 'sna' for each bee was on the days when they followed dances, except for bees 272 and 282. This result is interesting. Unlike other bees, bees 272 and 282 left the colony multiple times without any flight (Figure 23). The days with different numbers dance followings were also further differentiated into 4 groups (n < = 5, 5 < n < 10, 10 < n < 15, n > 20). The analyses were conducted at within- and between-individual levels (Figure 32E ~ H). Bees followed more dances on the days with higher frequencies of 'sna'. This effect was supported by the intensities of 'sna' in frequency and duration at the between-individual level (K-W test, RIsnainF_between: $p < 0.01$, RIsnainD_between: $p < 0.01$) (Figure 32F, H). Although the respective measures were not significantly different within-individual level, increasing intensities of 'sna' in frequency and duration were still observed when bees followed more dances (Figure 32E, G).
It was assumed that the higher frequencies of 'sna' bees had before, the earlier bees initiated the first dance following. However, statistical analysis did not support this hypothesis. Correlations between the age bees started the first dance following and intensity of 'sna' in both frequency and duration were not significant (Pearson test, RIsnainF_between: $R = -0.072$, $p = 0.78$, RIsnainD_between: $R = 0.11$, $p = 0.68$, RIsnainF_within: $R = 0.031$, $p = 0.91$, RIsnainD_within: $R = -0.0029$, $p = 0.99$). These results suggested that bees were more random to initiate the first dance following.
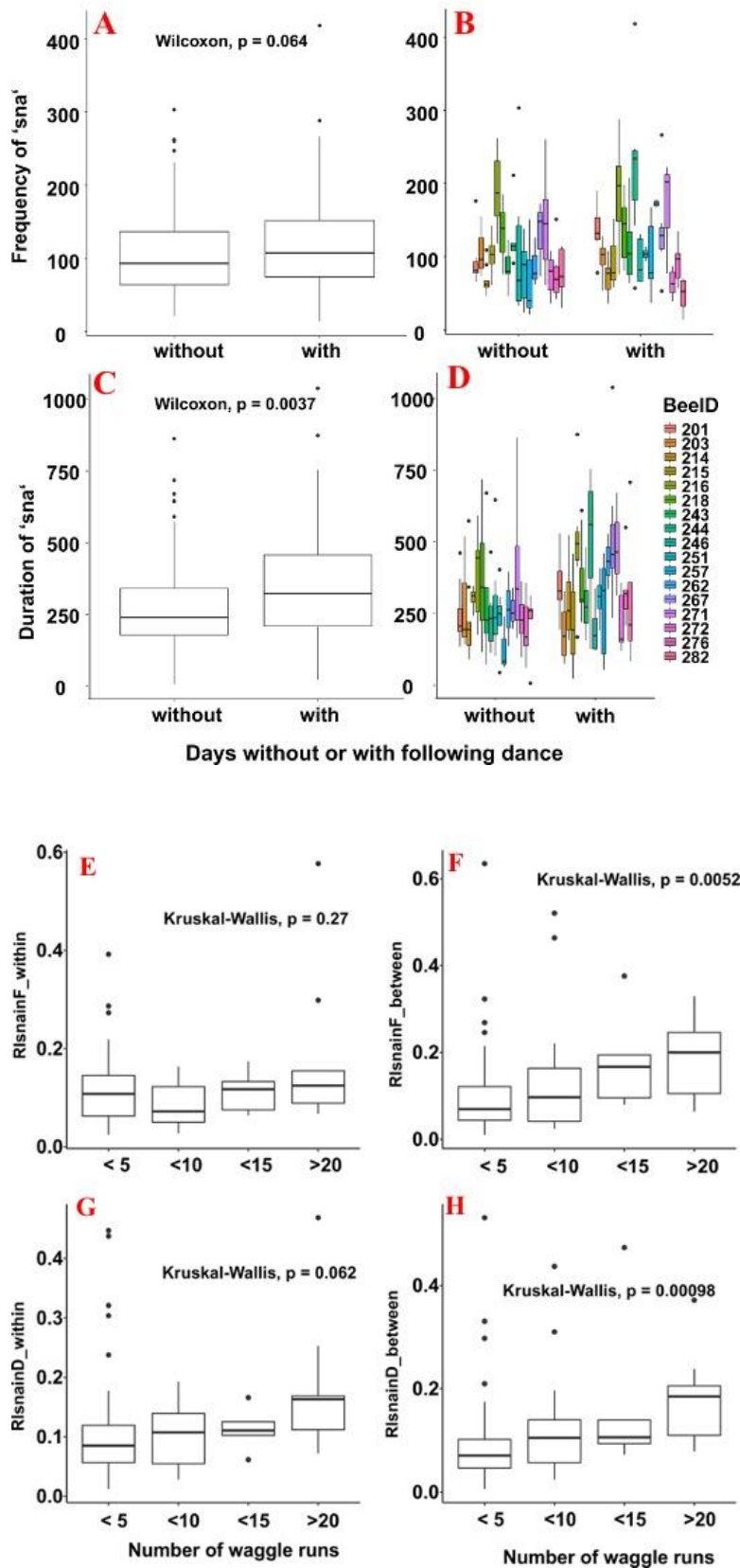
Figure 32. Correlation between intensity of 'sna' and dance following. 'with' and 'without', bees follow and do not follow dances. RIsnainF_within: intensity of sna calculated by the frequency relative to that of other days' within an individual. RIsnainF_between: intensity of sna

calculated by the frequency relative to that of other bees' within the same day. RIsnainD_within: intensity of sna calculated by the duration relative to that of other days' within individual. RIsnainD_between: intensity of sna calculated by the duration relative to that of other bees' within same day.

On the basis of these analyses, there were two hypotheses: 1) bees that were more active in 'sna' before, they initiated outdoor behaviors earlier. To solve this question, correlation between the age when bees initiate the first outdoor behaviors ('Out', 'Orientation flight', 'Forage flight') and intensity of 'sna' in the frequency and duration at within-individual level was analyzed. However, the results were not significant (Figure S4, Pearson test, RIsnainF_within versus: 'Out': R = -0.3, $p = 0.24$, 'Orientation': R = -0.47, $p = 0.09$, 'Foraging': R = -0.82, $p = 0.08$, RIsnainD_within versus: 'Out': R = -0.36, $p = 0.15$, 'Orientation': R = -0.43, $p = 0.12$, 'Foraging': R = -0.68, $p = 0.21$). 2) bees were more active outdoors if they started dance following at a younger age before. To solve this question, correlation between the age when bees started to follow dance and requency of outdoor behaviors were analyzed. Correlation analyses did not support this hypothesis (Pearson test, age versus: 'Out', R = 0.15, $p = 0.56$, 'Orientation', R = 0.24, $p = 0.36$, 'Foraging', R = 0.028, $p = 0.91$). Based on the result of PCA grouping bees with different outdoor performances with different scores calculated by the frequency, analyses on correlations between outdoor performance and frequency and duration of indoor behaviors were studied. The overall frequency of 'Qinterest' had a significantly negative effect on the scores of 'Out' but not on other outdoor behaviors (Figure 33a, b, c). 'dance following' and 'sna' were significantly positively correlated with flight frequencies (Figure 33a ~ c). Furthermore, duration of 'sna' was only significantly positively correlated with flight frequencies (Figure 34b).

In the final step, whether number of recruits following dances would influence the 17 focal bees' motivation to follow dances was asked. Significant positive correlations were found between the number of recruits around the dancers and the frequency of dance followings of the 17 focal bees (Figure S5, left, Pearson test, R = 0.17, $p < 0.01$), and between the number of recruits and duration of dance following (Figure S5, right, R = 0.12, $p = 0.011$) were found.

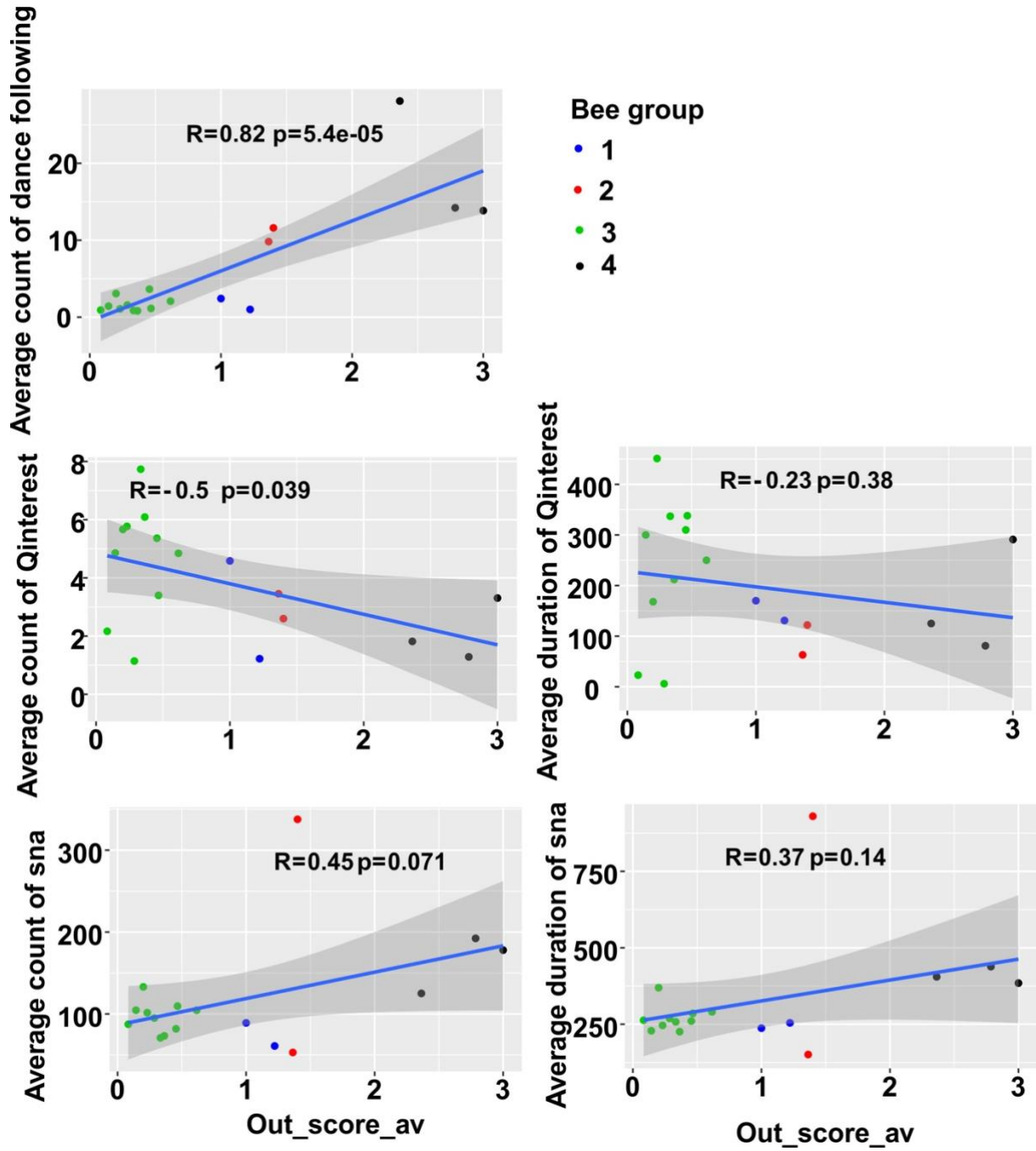Figure 33a. Correlation analyses for the indoor and outdoor behaviors of bees in four groups differentiated by PCA show in Figure 31. 'Out_score_av', average score of 'Out' over each individual's life span. Pearson test was performed for the correlation analysis.
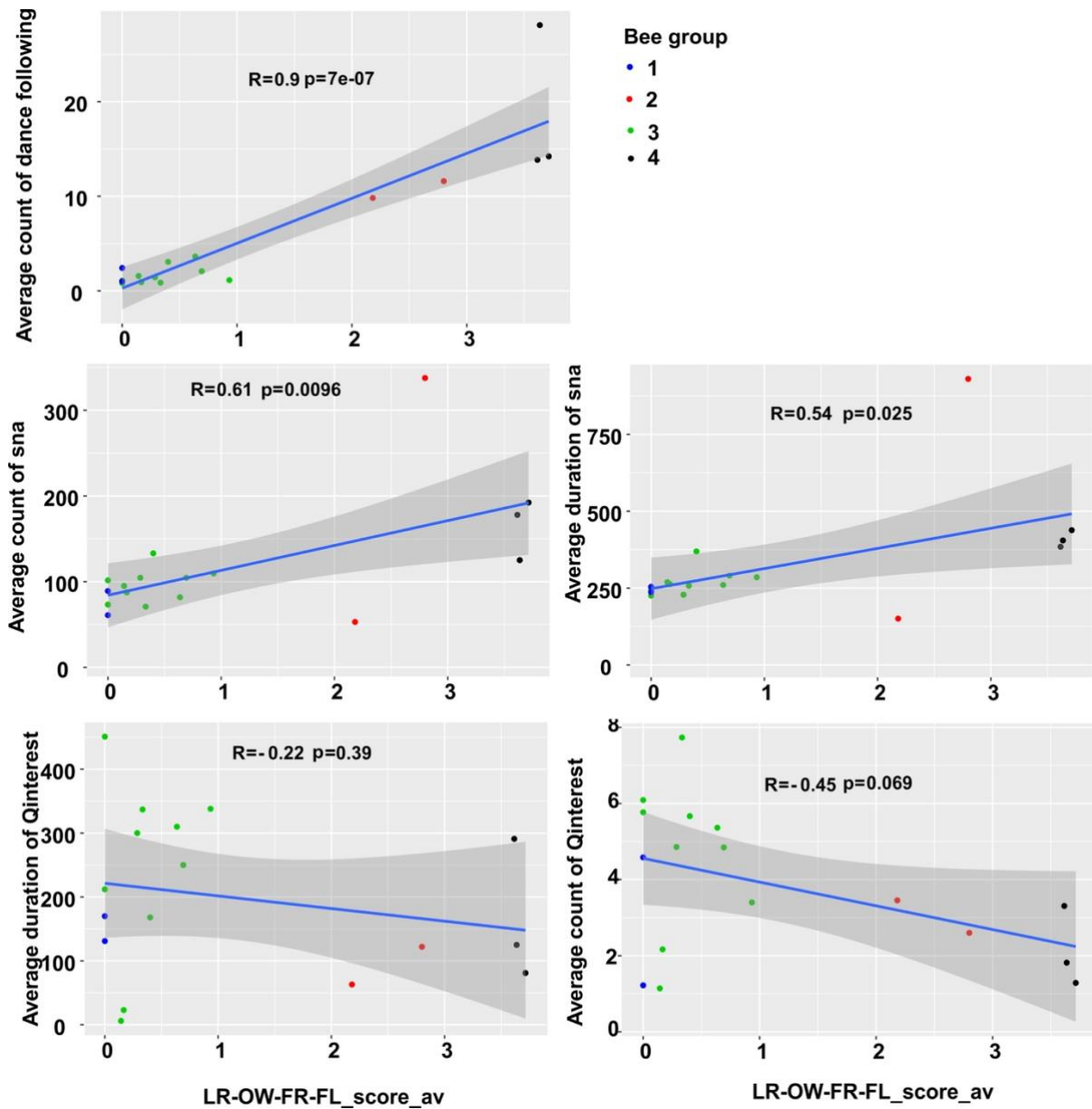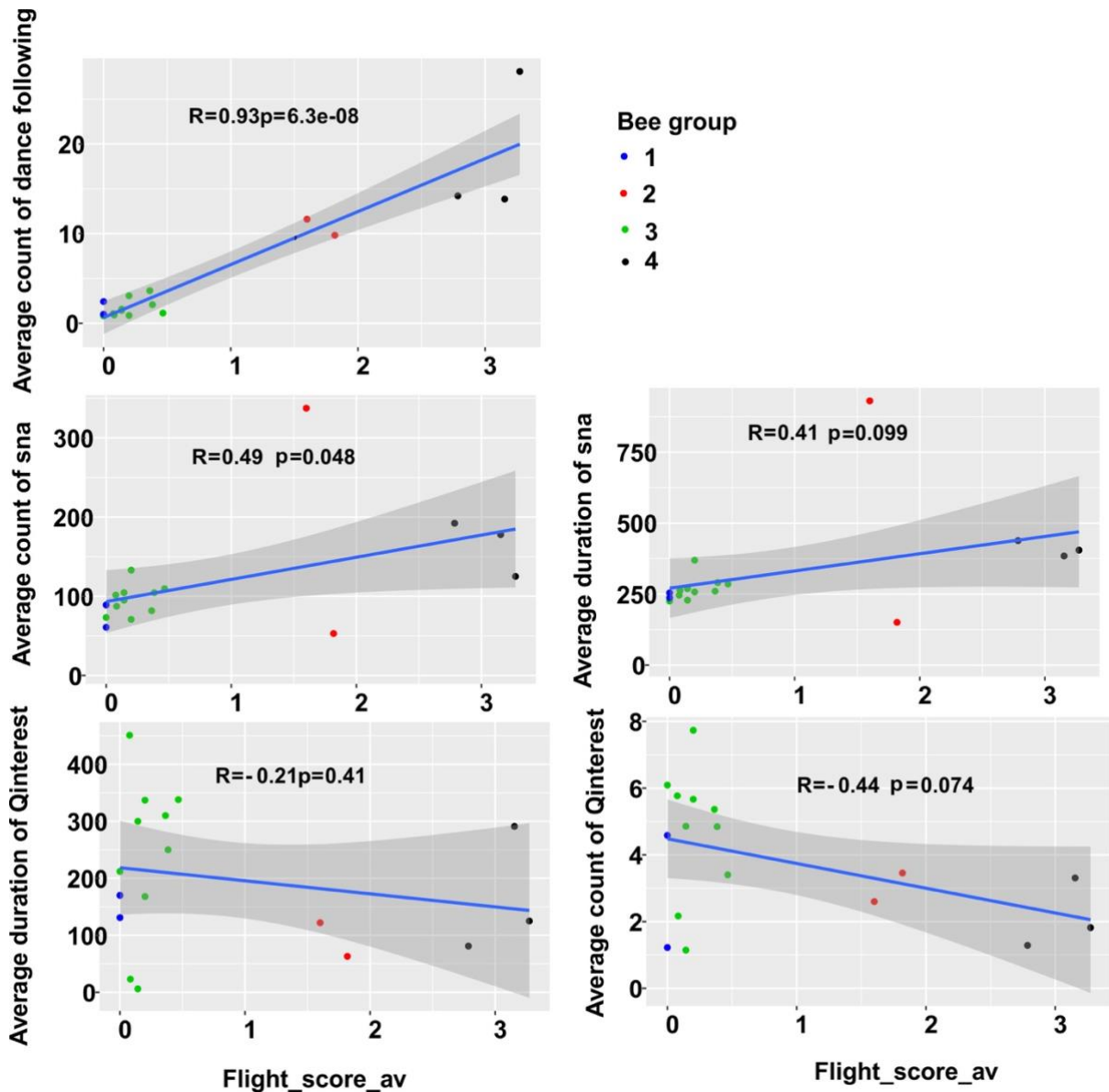
Figure 33b. Correlation analyses for the indoor and outdoor behaviors of bees in four groups differentiated by PCA show in Figure 31. 'LR/OW-FR-FL_score_av', average score calculated by frequency of 'LR', 'OW', 'FR' and 'FL' over each individual's life span. 'LR', bees appear at the hive exit but soon return into colony, 'OW', bees leaving the colony are caught and released but don't fly up, 'FR', bees return to the hive exit soon after flying up close to hive entrance, 'FL', bees performed flights.

Figure 33c. Correlation analyses for the indoor and outdoor behaviors of bees in four groups differentiated by PCA show in Figure 31. 'Flight_score_av', average scores of 'Orientation' and 'Foraging' flight calculated by frequency over each individual's life span.

5.6.5 Spatial fidelity led to individual difference in social behaviors

Next, I addressed the question whether spatial distribution on the comb influenced frequencies of indoor behaviors. The two sides of the comb were divided in 6 rectangles (Figure 34, right). First, bees danced mainly in the L1 area close to the hive exit was confirmed. A relatively small proportion of dancers were also seen in the neighboring areas M1 and L2, and occasionally at M2 that was close to L2 and M2, and between M1 and M2. They rarely danced at L3, M3 and U1 ~ U3 areas.

Unlike dancers, the queen spent her activities mostly at M1 ~ M2, U1 ~ U2 and L2, and less at L1, L3, M3 and U3. Since dancers were more focused on particular areas of 'dancing floor' while the queen moved evenly on the different areas of the comb. Therefore, the spatial occupation at dancing areas as an indicator for the social behaviors related with outdoor behaviors.
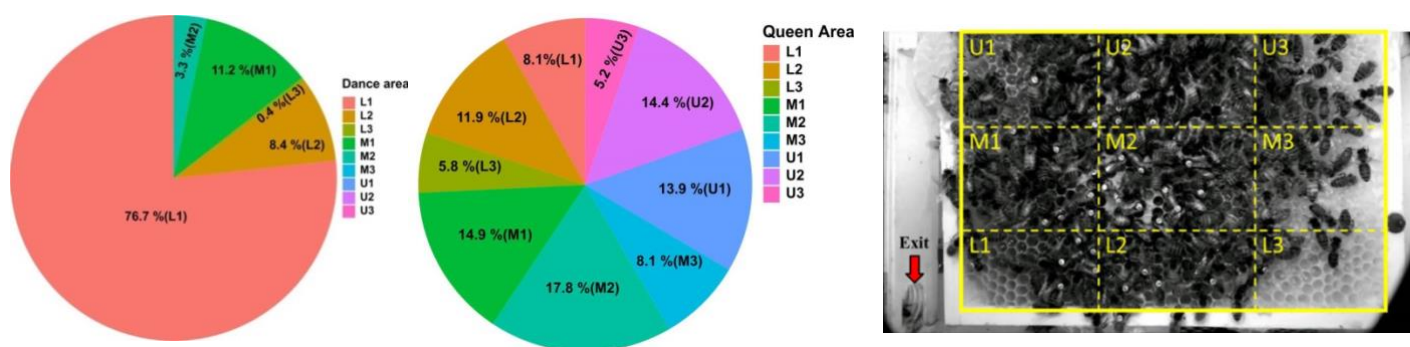

Figure 34. Percentages of areas on the combs occupied by dances and the queen. The figure on the left shows the proportion of areas where dances occur. The figure on the middle presents the percentage of areas where bees attend the queen. The right figure shows areas on the comb are divided into 9 parts. L1 is the closest area near the hive exit (red arrow). L2 and M1 are also close to the hive exit. M2 occupies the largest brood area and a small proportion of broods is located at M1, U2 and L2. Areas L3, M3 and U1 ~ U3 are mainly the food storage areas.

Data of tracking the spatial occupancy over bees' lifetime allowed to explore the age dependence of space use (spatial fidelity) in the colony. Overall, bees showed highly various patterns of spatial fidelity over ages (Figure 35). During the first three days of age, bees were more at U areas where less social interactions related to the outdoor behaviors occurred, except for bees 215, 216, 218, 243, 244, 251, 257 and 272. As bees were aged, most of them occupied more at the areas of L and M, except for bees 203, 214, 246 and 276. This result was consistent with age-dependent indoor and outdoor behaviors (Figure 24). The frequencies of switching within the areas of L1 ~ U3 over days of age of the 17 focal bees were distinctly different between individuals. For example, bee 282 occupied more frequently at U areas during the first 6 days, later she appeared more at areas of L1 and M1. Bee 216 walked more around the whole comb during the first 11 days and tended to stay at the areas of L1 and M1 afterwards. A similar pattern was seen for bee 244. Bee 243 switched frequently among different areas during the first 8 days and was then found in the U3

area on the 9th day of age. Then, she was active again in the areas of L1 ~ U3, and later she stayed more in the areas of L1 and L2 on the 12nd and 13rd days of age. Bees 267 and 271 appeared frequently in the areas of L1, L2 and M1 throughout her lifetime.
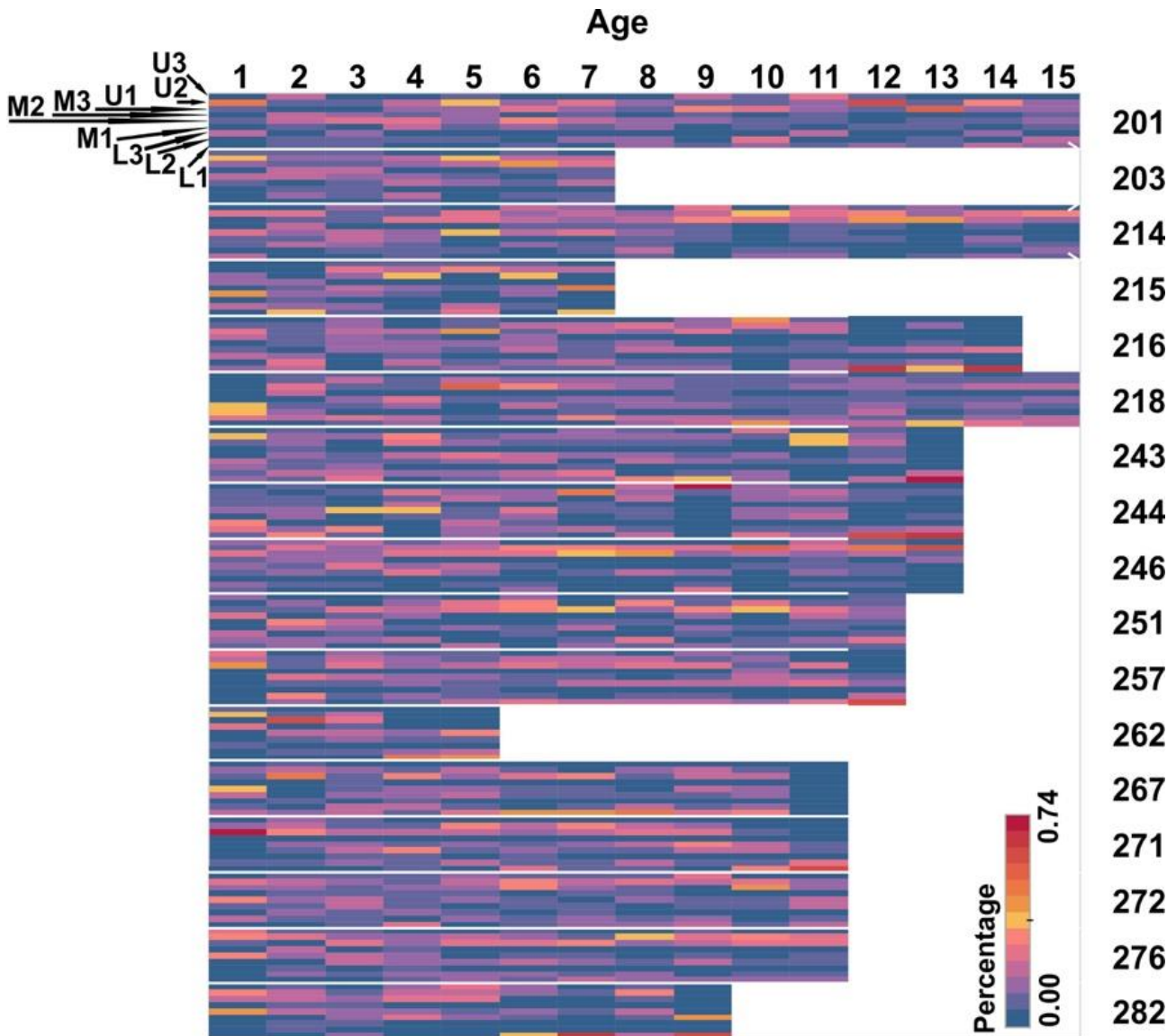

Figure 35. Spatial appearance within the colony of the 17 focal bees over their respective lifetime. The daily probability of the bees' appearance at L1 ~ U3 relative to the total observations within a day of each bee.

The index of spatial dispersion showed bees' locomotion varied over age. Overall, bees increased the spatial dispersion during the first three days of age (Figure 36, left) and then behaved slightly different among three manually clustered groups (Figure 36, right). Bees in the group 1 (216 ~ 271) and group 3 (257 ~ 282) gradually

decreased the spatial dispersion to a lower level compared to the bees in the group 2 (201 ~ 276). Bees in the group 2 showed a stable and high level of spatial movement over the days of age, this tendency increased even when bees were older (Figure 36, right).
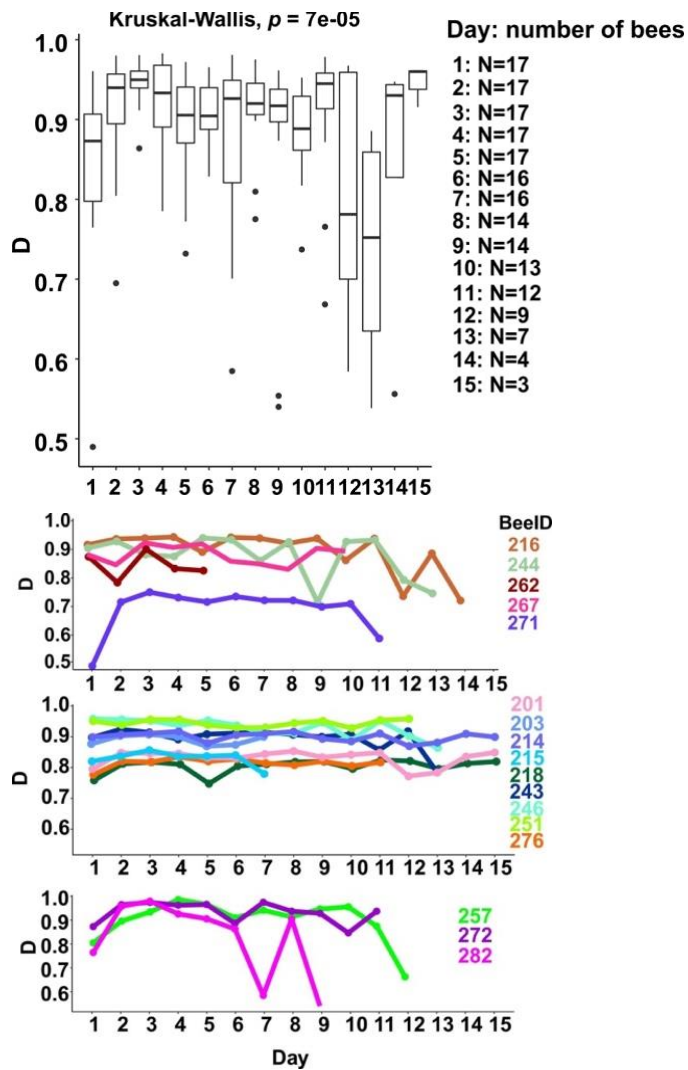

Figure 36. Spatial dispersion among the 17 focal bees over the lifetime. 'D' in the y axis is the index of variation of spatial dispersion within a day of each individual. Figures left and right show the pooled and individual data, respectively.

Next, the connection between spatial fidelity and behaviors indoors and outdoors was studied. The spatial fidelity in the areas of L1 ~ U3 were significantly different between '**on**' and '**before**' days, which were denoted as the days when bees had followed dances and outdoor behaviors ('**on**'), and the days before these behavioral events

occurred ('**before**') (Figure 37). Bees moved on the '**on**' days more preferentially around the main 'dancing floor' L1 (K-W test, $p < 0.01$). Higher but not significant different frequencies in areas L2 ($p > 0.05$) and M1 ($p = 0.055$) were also found on '**on**' days. In contrast, bees on '**before**' days were more frequent in the areas M3 ($p = 0.04$), U1 ($p = 0.013$), U2 ($p = 0.005$) and U3 ($p = 0.008$), where more nest related tasks were performed. The occupancies at M2 (the brood area) did not differ between '**on**' and '**before**' days. These results provided a cue about the potential relation between spatial fidelity and 'dance following' and 'Out'. Following this result, I further asked whether spatial fidelity regulated the information flow via the 'sna'. The spatial dispersion and 'sna' in frequency and duration were compared on the '**on**' and '**before**' days, respectively (Figure 38a, b). Although the spatial dispersion was not significantly different, it remained to be higher on the day '**on**' (Figure 38a, left). The intensity of spatial dispersion at between-individual level was significantly higher on the day '**on**' than '**before**' (Wilcoxon test, $p < 0.01$) (Figure 38a, right). Correspondingly, the frequency and duration of 'sna' on the day '**on**' were significantly higher than those on days '**before**'. The intensities of 'sna' in frequency and duration at **between-individual** level were also significantly higher on the day '**on**' than '**before**' (Figure 38b). Thus, the correlations between 'sna' and spatial fidelity at **between-** and **within-individual** levels were then documented. Frequency and duration of 'sna' were significantly positively correlated with spatial dispersion, though the coefficients were not high, with higher spatial dispersion following with higher frequencies and longer durations of 'sna' (frequency: R = 0.19, $p < 0.01$, duration: R = 0.14, $p < 0.05$) (Figure 39a). Strong positive correlations between intensity of 'sna' and intensity of spatial dispersion were identified at **between-** and **within-individual** levels (Figure 39b, **between-individual**: R = 0.6, $p < 0.01$, **within individual**: R = 0.76, $p < 0.01$).

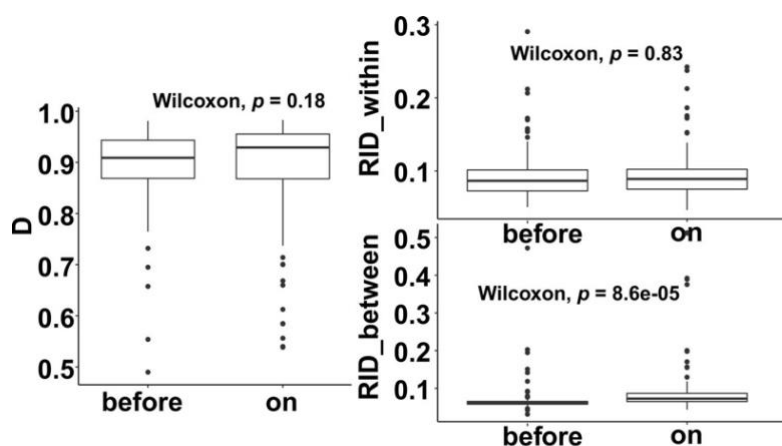Figure 37. Spatial dispersion on the combs between **'on'** and **'before'** days. **'on'**: the days when bees follow dance or leave the colony (n=111). **'before'**: the days when these behavioral events occurred (n=82). K-W test is applied for separately comparing the differences of space locations at L1 ~ U3 areas between **'on'** and **'before'** bee groups. 'D' (y axis) indicates the spatial dispersion at each day of individual bees. RID_within: the intensity of spatial dispersion relative to the respective measure in other days within individual. RID_between: the intensity of spatial dispersion relative to the respective measure of other bees within the same day.
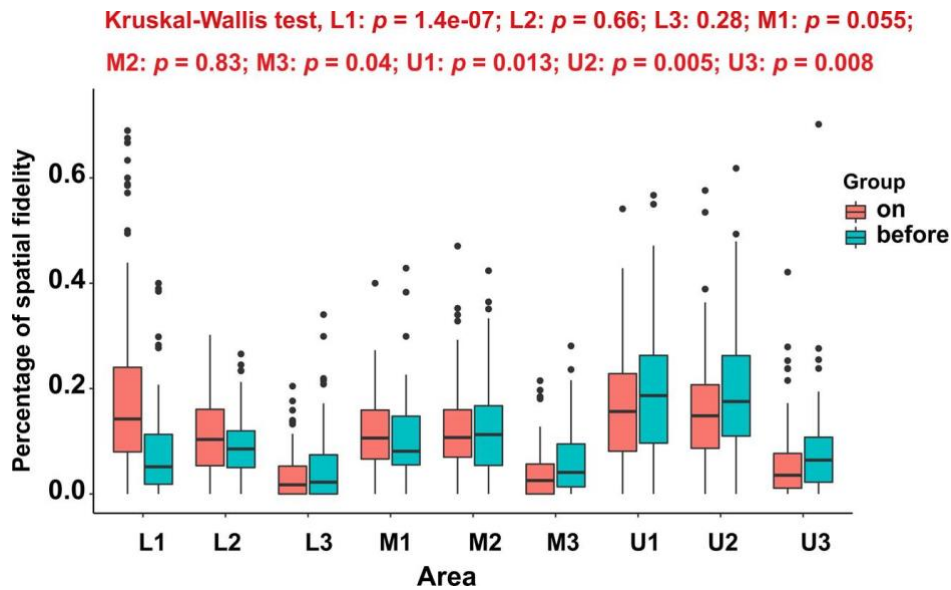

Figure 38a. Difference of spatial dispersion and relative intensity of spatial dispersion between **'on'** and **'before'** days. *p*-values of Wilcoxon test are presented. 'Percentage of spatial fidelity' is the daily probability of bees' appearance at L1 ~ U3 relative to the total observations on the days of 'on' and '**before**'.
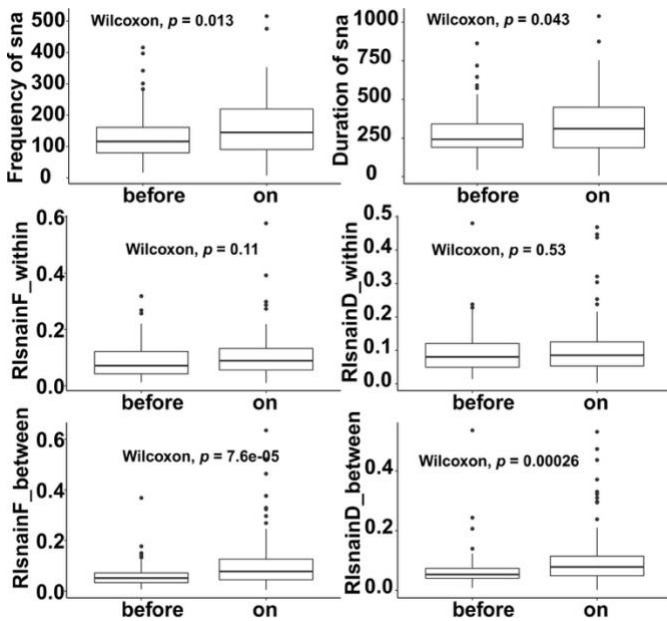
Figure 38b. Differences of 'sna' between **'on'** and **'before'** days. 'Frequency of sna' and 'Duration of sna' represent the total amount of frequency and duration of 'sna' in each day of an individual correspondingly. RIsnainF_within: intensity of sna in frequency relative to the respective measure in other days within individual. RIsnainF_between: intensity of sna in frequency relative to the respective measure of other bees within same day. RIsnainD_within: intensity of sna in duration relative to te respective measure in other days within individual. RIsnainD_between: intensity of sna in duration relative to the respective measure of other bees within same day.
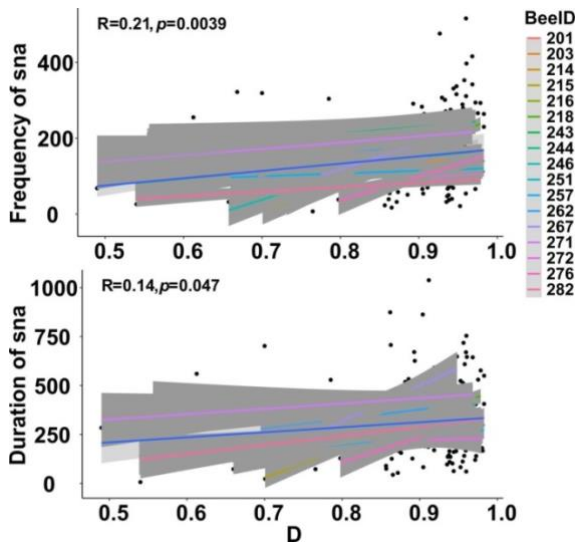

Figure 39a. Correlation between the frequency and duration of 'sna' and spatial dispersion. 'D' indicates the spatial dispersion at each day of individual bees. The dark blue lines represent the regression line of sum of the17 bees, while the other lines show the regression lines for each individual bee.
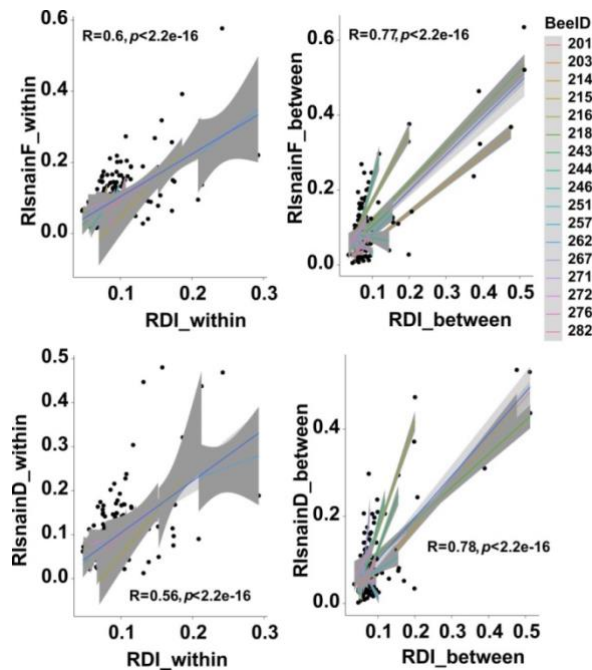
Figure 39b. Correlation between relative intensity in the frequency and duration of 'sna' and the intensity of spatial dispersion within and between individuals.

5.7 Summary and discussion

In a community of social insects like honeybees, animals behave independently and cooperatively. Individuals specialize on duties that are age and experience-dependent but also adapts flexibly to colony need (Johnson, 2010b, Jones et al. 2020, Lindauer, 1952). Uncovering the regulation mechanisms involved in behavioral variation and plasticity is fundamental for understanding how individual and collective decisions are made. Within a colony, behaviors are characterized by social contacts between community members, conditions that differ drastically from outdoor behavior in which each individual acts separately and alone.

Although there were amounts of work studying on the indoor behaviors (e.g., dance and dance following) and outdoor behaviors (e.g., foraging flight, nest scouting, exploration), systematic studies on relationship between these two lifestyles are rare. This unfortunate condition results from experimental limitations in following individuals both indoor and outdoor throughout their life history. Here infrared movie recording of indoor behaviors and harmonic radar tracking of flights were taken advantage of. As far as I know, this is the first study to relate the honeybees' indoor and outdoor behaviors on a quantitative basis along their full life history on individual and group levels.

These results uncover considerable skews in the occurrence of outdoor and indoor behaviors among the 17 focal bees. Bees at the same or at similar ages may differ in the occurrence of both the outdoor and indoor behaviors. The age dependence also showed high individual differences among individuals. For example, bee 257 started to follow dances immediately after her emergence while bee 271 began her first dance following at an age of 8th day. Bee 216 left the colony over 36 times while bee 203 with the same age went out only once. Bee 257 left the colony 12 times without performing flights while bee 262 of the same age left the colony 7 times and performed full flight each time. Bee 244 started the first orientation flight at an age of 4th day while bee 214 had her first flight on an age of 11th day. This wide range of age-dependent task allocation differs from the picture established on the observations in large colonies. Bees usually start to leave the hive at 2 ~ 3 weeks old in a normal colony with more bees. However, bees in my study commonly left the colony at an age of 4 ~ 11 days. This might be explained by reasons that a small colony may require bees to forage earlier and it may be less flexible for a small colony to make use of comb space. The higher sensitivity to allocating the limited physical space for food storage and brood may lead to the stricter control of number of workers indoor for indoor (Huang and Robinson, 1996, Smith et al. 2017). Although this might be a special situation because of the very small size of colony, it demonstrates a high plasticity of bees' life history on an individual basis. Indoor and outdoor behaviors also vary considerably within individual over days. For example, bee 282 kept a stable and low level of social interaction 'sna' with other workers while bee 271 fluctuated strongly in the levels of 'sna' over days. Bee 216 followed 1 ~ 3 dance in the early days and over 70 in a day as she were older. These observations question the traditional picture of rather strict age-dependent and external factors-dependent labor division.

What causes these behavioral variations? The individuals in question may differ much more strongly in their experience both inside and outside the colony, and this is also the topic I explored in my study. Despite bees showed high levels of behavioral variations, they can be grouped according to consistent behavior patterns. Overall, only a minority of 'elite' bees were more active in outdoor behaviors and social interactions. e.g., bees 216, 244, 262, 267, 271 that performed orientation and foraging flights more frequently than the other bees and were more active in 'sna' and dance following. This result suggests potential correlations between social

behaviors, and between outdoor behaviors and social interactions. Our further analyses confirm positive relations between the frequency of 'sna' and dance following (Figure 32), and between the frequencies of these social interactions and outdoor behaviors (Figure 29, 33a, b, c).

Symbolic communication via waggle dance and learning of olfactory information from other members via trophallaxis intimately connected in the colony. Cholé et al. (2019) documented appetitive learning via antennation and trophallaxis, the trained feeder in my experiment did not provide any odor cues, however, social contact via antennation and trophallaxis in this study still motivated bees to follow dances more actively and encouraged them to leave the colony for exploratory flights more frequently. What processes are involved in such a communication process excluding olfactory information from the food sources? And, when do bees rely more on 'sna' or dance information?

My observation shows that bees initiate orientation flights only after having the experience of dance followings. It is most likely that dances experienced by naïve bees unfamiliar with the sun compass and environment provide a motivational component because these bees will not be able to decode the vector information of direction and distance in the dances. However, innate basic information about the relation between the sun azimuth and direction of waggle run relative to gravity as well as basic information between distance of food sources and numbers of waggle runs could potentially be used by the naïve bees as guide in the exploratory orientation flights (Menzel, 2019). It will therefore be important to examine whether the direction of orientation flight is reflected in the orientation of dances a bee follows before she leaves the hive. A topic addressed in the next chapter.

Another interesting finding is that bees 257, 272 and 282 were released multiple times after exiting the hive, but they did not perform flights. This result raises a question whether dance following is particularly important in determining bees' decision to perform flights. This question will be studied in the next chapter.

Multiple empirical studies have been performed to investigate spatial aspects of social organization in insect colonies (Crall et al. 2018, Jandt and Dornhaus 2009, Mersch et al. 2013, Pinter-Wollman, 2015). These studies documented importance of spatial fidelity for regulating information flow and initiating collective behaviors. Here I reveal the temporal-spatial movement patterns over the life history of the 17 focal bees, which differed in at the within- and between-individual levels. The bees

maintained individually specific patterns of spatial occupancy across days (Figure 35). This result is different from a previous study which shows that the spatial distributions between individual bumble bees were repeatable over their lifetime. The discrepancy may be due to different social structures. This difference may also be an indication of a higher task flexibility in the honeybees. My observation also showed that bees had common patterns in the spatial mobility within multiple functional groups with different levels of outdoor activities. Overall, bees in particular those would be more active outdoors were characterized by a higher spatial mobility during younger age. As bees were older and more active in joining more foraging tasks, they were less dispersed in the spatial movement and occupied consistently the areas close to the hive exit. This result indicates that spatial fidelity at the early age critically influenced bees' future outdoor behaviors and while the spatial patterns at bees' older age may suggest the main tasks bee have in the colony. To sum up, all these results provide evidences for an important role of spatial fidelity in regulating information flow. Investigating spatial fidelity over bees' lifetime provide a predictor about bees' outdoor behaviors. My results also support an idea that social interactions via 'sna' and 'dance following' connected with spatial fidelity appears to regulate outdoor behaviors (Figure 38 ~ 39).

5.8 References

Ai, H., Kobayashi, Y., Matake, T., Takahashi, S., Hashimoto, K., Maeda, S., …Tsuruta, N. (2017). Development of honeybee waggle dance and its differences between recruits and scouts. bioRxiv. doi:https://doi.org/10.1101/179408.

Arenas, A., Ramírez, G., Balbuena, M. S., & Farina, W. M. (2013). Behavioral and neural plasticity caused by early social experiences: the case of the honeybee. *Frontiers in physiolog, 4*, 41.

Balbuena, M. S., Molinas, J., & Farina, W. M. (2012). Honeybee recruitment to scented food sources: correlations between in-hive social interactions and foraging decisions. *Behavioral Ecology and Sociobiology, 66*(3), 445-452.

Beyaert, L., Greggers, U., & Menzel, R. (2012). Honeybees consolidate navigation memory during sleep. *Journal of Experimental Biology, 215*(22), 3981-3988.

Biesmeijer, J. C., & Seeley, T. D. (2005). The use of waggle dance information by honey bees throughout their foraging careers. *Behavioral Ecology and Sociobiology, 59*(1), 133-142.

Boulay, R., Soroker, V., Godzinska, E. J., Hefetz, A., & Lenoir, A. (2000). Octopamine reverses the isolation-induced increase in trophallaxis in the carpenter ant Camponotus fellah. *Journal of Experimental Biology, 203*(3), 513-520.

Breed, M. D., Stiller, T. M., Blum, M. S., & Page, R. E. (1992). Honeybee nestmate recognition: effects of queen fecal pheromones. *Journal of chemical ecology,, 18*(9), 1633-1640.

Capaldi, E. A., & Dyer, F. C. (1999). The role of orientation flights on homing performance in honeybees. *Journal of Experimental Biology, 202*(12), 1655-1666.

Capaldi, E. A., Smith, A. D., Osborne, J. L., Fahrbach, S. E., Farris, S. M., Reynolds, D. R., . . . Riley, J. R. (2000). Ontogeny of orientation flight in the honeybee revealed by harmonic radar. *Nature, 403*(6769), 537-540. doi:10.1038/35000564.

Chole, H., Carcaud, J., Mazeau, H., Famie, S., Arnold, G., & Sandoz, J. C. (2019). Social Contact Acts as Appetitive Reinforcement and Supports Associative Learning in Honeybees. *Current Biology, 29*(8), 1407-+. doi:10.1016/j.cub.2019.03.025.

Cholé, H., Carcaud, J., Mazeau, H., Famié, S., Arnold, G., & Sandoz, J. C. (2019). Social contact acts as appetitive reinforcement and supports associative learning in honeybees. *Current Biology, 29*(8), 1407-1413.

Collett, T. S., & Collett, M. (2002). Memory use in insect visual navigation. *Nature Reviews Neuroscience, 3*(7), 542-552.

Crailsheim, K. (1998). Trophallactic interactions in the adult honeybee (Apis mellifera L.). *Apidologie, 19*(1-2), 97-112.

Crall, J. D., Gravish, N., Mountcastle, A. M., Kocher, S. D., Oppenheimer, R. L., Pierce, N. E., & Combes, S. A. (2018). Author Correction: Spatial fidelity of workers predicts collective response to disturbance in a social insect. *Nat Commun, 9*(1), 2180. doi:10.1038/s41467-018-04598-7.

Degen, J., Hovestadt, T., Storms, M., & Menzel, R. (2018). Exploratory behavior of re-orienting foragers differs from other flight patterns of honeybees. *PLoS One, 13*(8), e0202171. doi:10.1371/journal.pone.0202171.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., . . .Menzel, R. (2016). Honeybees Learn Landscape Features during Exploratory Orientation Flights. *Current Biology, 26*(20), 2800-2804. doi:10.1016/j.cub.2016.08.013.

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*, 45-47.

Dyer, F. C. (1998). Spatial cognition: lessons from central-place foraging insects. *Animal cognition in nature* (pp. 119-154): Academic Press.

Esslen, J., & Kaissling, K. E. (1976). Zahl und Verteilung antennaler Sensillen bei der Honigbiene (Apis mellifera L.). *Zoomorphologie, 83*(3), 227-251.

Farina, W. M., Grüter, C., & Arenas, A. (2012). Olfactory information transfer during recruitment in honey bees. In *Honeybee neurobiology and behavior* (pp. 89-101): Springer.

von Frisch K. (1965). *Tanzsprache und Orientierung der Biene*: Springer.

von Frisch K. (1967). *Dance language and orientation of bees*. Cambridge, MA.: Harvard University Press.

Galizia, C. G., Joerges, J., Küttner, A., Faber, T., & Menzel, R. (1997). A semi-in-vivo preparation for optical recording of the insect brain. *Journal of neuroscience methods, 76*(1), 61-69.

Gernat, T., Rao, V. D., Middendorf, M., Dankowicz, H., Goldenfeld, N., & Robinson, G. E. (2018). Automated monitoring of behavior reveals bursty interaction patterns and rapid spreading dynamics in honeybee social networks. *Proc Natl Acad Sci U S A, 115*(7), 1433-1438. doi:10.1073/pnas.1713568115.

Gil, M., & De Marco, R. J. (2005). Olfactory learning by means of trophallaxis in Apis mellifera. *J Exp Biol, 208*(Pt 4), 671-680. doi:10.1242/jeb.01474.

Grüter, C., Czaczkes, T. J., & Ratnieks, F. L. (2011). Decision making in ant foragers (Lasius niger) facing conflicting private and social information. *Behavioral Ecology and Sociobiology, 65*(2), 141-148.

Hammer, M., & Menzel, R. (1998). Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learning & memory, 5*(1), 146-156.

Hoover, S. E., Keeling, C. I., Winston, M. L., & Slessor, K. N. (2003). The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften, 90*(10), 477-480.

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.

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.

Jandt, J. M., & Dornhaus, A. (2009). Spatial organization and division of labour in the bumblebee Bombus impatiens. *77*(3), 641-651.

Joerges, J., Küttner, A., Galizia, C. G., & Menzel, R. (1997). Representations of odours and odour mixtures visualized in the honeybee brain. *Nature protocols, 387*(6630), 285-288.

Johnson, B. R. (2010a). Division of labor in honeybees: form, function, and proximate mechanisms. *Behavioral Ecology and Sociobiology, 64*(3), 305-316.

Johnson, B. R. (2010b). Spatial effects, sampling errors, and task specialization in the honey bee. *Insectes sociaux, 57*(2), 239-248.

Jones, B. M., Rao, V. D., Gernat, T., Jagla, T., Cash-Ahmed, A. C., Rubin, B. E., . . .Robinson, G. E. (2020). Individual differences in honey bee behavior enabled by plasticity in brain gene regulatory networks. *Elife, 9*. doi:10.7554/eLife.62850.

Kapheim, K. M., Jones, B. M., Pan, H., Li, C., Harpur, B. A., Kent, C. F., . . .Wcislo, W. T. (2020). Developmental plasticity shapes social traits and selection in a facultatively eusocial bee. *Proc Natl Acad Sci U S A, 117*(24), 13615-13625. doi:10.1073/pnas.2000344117.

Kaspar, R. E., Cook, C. N., & Breed, M. D. (2018). Experienced individuals influence the thermoregulatory fanning behaviour in honey bee colonies. *Animal Behaviour, 142*, 69-76.

Klein, B. A., & Seeley, T. D. (2011). Work or sleep? Honeybee foragers opportunistically nap during the day when forage is not available. *Animal Behaviour, 82*(1), 77-83.

Klein, B. A., Klein, A., Wray, M. K., Mueller, U. G., & Seeley, T. D. (2010). Sleep deprivation impairs precision of waggle dance signaling in honey bees. *Proceedings of the National Academy of Sciences of the United States of America, 107*(52), 22705-22709. doi:10.1073/pnas.1009439108.

Klein, S., Pasquaretta, C., He, X. J., Perry, C., Søvik, E., Devaud, J. M., ...Lihoreau, M. (2019). Honey bees increase their foraging performance and frequency of pollen trips through experience. *Scientific reports, 9*(1), 1-10.

Lachér, V., & Schneider, D. (1963). Elektrophysiologischer Nachweis der Riechfunktion von Porenplatten (Sensilla placodea) auf den Antennen der Drohne und der Arbeitsbiene (*Apis mellifica* L.). *Zeitschrift für vergleichende Physiologie, 47*(3), 274-278.

Lindauer, M., & Watkin, B. (1953). Division of labour in the honeybee colony. *Bee world, 34*(4), 63-73.

M., L. (1952). Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. *Z. vergl. Physiol., 34*, 299-345.

Mc Cabe, S. I., Hartfelder, K., Santana, W. C., & Farina, W. M. (2007). Odor discrimination in classical conditioning of proboscis extension in two stingless bee species in comparison to Africanized honeybees. *Journal of Comparative Physiology A, 193*(1), 1089-1099.

Menzel, R. (2019). The Waggle Dance as an Intended Flight: A Cognitive Perspective. *Insects, 10*(12), 424.

Menzel, R., Greggers, U., Smith, A., Berger, S., Brandt, R., Brunke, S., . . .Watzl, S. (2005). Honey bees navigate according to a map-like spatial memory. *Proceedings of the*

National Academy of Sciences of the United States of America, 102(8), 3040-3045. doi:10.1073/pnas.0408550102.

Menzel, R., Manz, G., Menzel, R., & Greggers, U. (2001). Massed and spaced learning in honeybees: the role of CS, US, the intertrial interval, and the test interva. *Learning & memory, 8*(4), 198-208.

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. doi:10.1126/science.1234316.

Modlmeier, A. P., Colman, E., Hanks, E. M., Bringenberg, R., Bansal, S., & Hughes, D. P. (2019). Ant colonies maintain social homeostasis in the face of decreased density. *Elife, 8*. doi:ARTN e3847310.7554/eLife.38473.

Pamminger, T., Foitzik, S., Kaufmann, K. C., Schutzler, N., & Menzel, F. (2014). Worker Personality and Its Association with Spatially Structured Division of Labor. *PLoS One, 9*(1). doi:ARTN e7961610.1371/journal.pone.0079616.

Pankiw, T., Winston, M. L., & Slessor, K. N. (1995). Queen attendance behavior of worker honey bees (Apis mellifera L.) that are high and low responding to queen mandibular pheromone. *Insectes sociaux, 42*(4), 371-378.

Pinter-Wollman, N. (2015). Persistent variation in spatial behavior affects the structure and function of interaction networks. *Current Zoology, 61*(1), 98-106.

Price, R. A., Dulex, N., Vial, N., Vincent, C., & Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. *Science advances, 5*(2), eaat0450.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological reviews, 82*(2), 291-318.

Riley, J. R., Greggers, U., Smith, A. D., Reynolds, D. R., & Menzel, R. (2005). The flight paths of honeybees recruited by the waggle dance. *Nature, 435*(7039), 205-207.

Rittschof, C. C., Coombs, C. B., Frazier, M., Grozinger, C. M., & Robinson, G. E. (2015). Early-life experience affects honey bee aggression and resilience to immune challenge. *Scientific reports, 5*(1), 1-8.

Robinson, G. E., Page Jr, R. E., Strambi, C., & Strambi, A. (1992). Colony integration in honey bees: mechanisms of behavioral reversion. *Ethology, 90*(4), 336-348.

Seeley, T. D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behavioral Ecology and Sociobiology, 11*(4), 287-293.

Seeley, T. D. (2014). *Honeybee ecology*. Princeton: Princeton University Press.

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*(4), 229-237.

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, 186*(9), 813-819.

Seeley, T. D., Morse, R. A., & Visscher, P. K. (1979). The natural history of the flight of honey bee swarms. *Psyche, 86*(2-3), 103-113.

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: an intergrative overiew. *Q Rev Biol, 79*(3), 241-277. doi:10.1086/422893.

Smith, M. L., Koenig, P. A., & Peters, J. M. (2017). he cues of colony size: how honey bees sense that their colony is large enough to begin to invest in reproduction. *Journal of Experimental Biology, 220*(9), 1597-1605.

Judd, T. M. (1995). The waggle dance of the honey bee: which bees following a dancer successfully ac- quire the information? *J. Insect Behav., 8*, 343– 354.

Vareschi, E. (1971). Duftunterscheidung bei der Honigbiene—Einzelzell-Ableitungen und Verhaltensreaktionen.*, 75*(2), 143-173.

Wainselboim, A. J., & Farina, W. M. (2000). Trophallaxis in the honeybee Apis mellifera (L.): the interaction between flow of solution and sucrose concentration of the exploited food sources. *Animal Behaviour, 59*(6), 1177-1185.

Walker, J. T. (1999). *Statistics in criminal justice: Analysis and interpretation*. Boston, MA: Jones and Barlett Publishers.

Waters, J. S., & Fewell, J. H. (2012). Information processing in social insect networks. *PLoS One, 7*(7), e40337. doi:10.1371/journal.pone.0040337.

Wehner, R., & Srinivasan, M. V. (2002). Path integration in insects. In *The biological basis of navigation* (pp. 9-30). Oxford Oxford University Press.

Wild, B., Dormagen, D. M., Zachariae, A., Smith, M. L., Traynor, K. S., Brockmann, D., ... & Landgraf, T. (2020). Social networks predict the life and death of honey bees. *Nature communications, 12*(1), 1-12.

Wilson, E. O. (1971). *The insect societies*. Cambridge, Mass.: Harvard University Press.

Wray, M. K., Klein, B. A., Mattila, H. R., & Seeley, T. D. (2008). Honeybees do not reject dances for 'implausible' locations: reconsidering the evidence for cognitive maps in insects. *76*(2), 261-269.

Wuerz, Y., & Kruger, O. (2015). Personality over ontogeny in zebra finches: long-term repeatable traits but unstable behavioural syndromes. *Front Zool, 12 Suppl 1*, S9. doi:10.1186/1742-9994-12-S1-S9.

Zwaka, H., Bartels, R., Gora, J., Franck, V., Culo, A., Gotsch, M., & Menzel, R. (2015). Context odor presentation during sleep enhances memory in honeybees. *Curr Biol, 25*(21), 2869-2874. doi:10.1016/j.cub.2015.09.069.

6 Chapter 4: Dance communication shapes exploratory and foraging flight behaviors on the level of individual bees

6.1 Abstract

In the honeybees, dance and dance following are the most recognized mechanism of social communication, of which the direction and distance of food sources are encoded in the waggle dances. In the Chapter 3, the ontogeny development of social behaviors and flights across bees' lifetime have been mapped, which reveals a positive relation between social communication and flight performance. However, how the information conveyed in the dances maps into bees' outbound flights in the orientation and foraging flights and how the former experience of dance followings and flights determines bees' future flight performances remain open. Raspberry pi and harmonic radar are used to monitor behaviors of dance communication and flights over the life history of 17 representative bees. Nine hundred ninety-four dance followings, 65 orientation flights and 56 foraging flights were recorded. Waggle dances play important roles in motivating both orientation and foraging flights. When bees followed more dances, bees performed more orientation and foraging flights. However, the influences of vector information on motivating bees to perform orientation and foraging flights are different, with the higher variation of direction in the dances, the higher frequency of orientation flights, while with the higher variation of direction and distance, less foraging flights. Meanwhile, dances serve as a vector component to guide bees to fly in certain directions and distances. Bees were able to average the vector information of the dances only followed within 10 min to guide their flights. Bees can evaluate the values of dances and determine to follow the information in the dances or forage by their own experience. When the ranges of variation and dispersion of direction were larger than ± 12° and ± 5°, respectively, bees would prefer to forage with their own experience rather than relying on dance information. However, during this procedure, bees showed individuality in decoding and calibrating the dance information. My descriptive analysis also revealed that the influence of former experience of dance following and orientation/foraging flights on the future flight performance. However, this impact was also individually different.

These results are used to create an individual-orientated model relating the connection between social communication and flight performances over the life history, enabling us to understand how experience-dependent variation of social behaviors and flight performances over ages among individual bees.

6.2 Introduction

Dance communication is a fascinating symbolic form of social interaction (*Apis* sp.) (von Frisch 1967). Honeybees upon returning from a successfully located food place more than a few hundred meters away from the hive transmit information of location, odor, quality and quantity of food sources or new nest site for swarming to their nest mates through waggle dances (Dyer 2002, Riley et al. 2005, von Frisch 1967). A waggle dance is finished as stereotyped dance movements on the comb when a bee walks straight in one direction while vibrating her abdomen from one point to another (a waggle run), then she stops and returns to the starting point (return phase) and start another repeated waggle run. Foraging bees rely on the sun azimuth to obtain the compass information (von Frisch 1965). The direction to a food source from the hive relative to the sun azimuth is encoded in the orientation of dancer's body from the vertical in a waggle run when she vibrates her body from one side to another on the comb, and the distance is encoded in the length and duration in a waggle run, the number of waggles in a waggle run, or the number of dances (Seeley et al. 2000, von Frisch 1967).

Foragers may repeat multiple waggle runs within a dance. However, there is considerable variation between sequential waggle runs in both direction and distance encoding components (Preece & Beekman 2014, Okada et al. 2014, Schürch et al. 2016, Tanner & Visscher 2010). These social signals not only vary between individuals, but also within individual (Schürch et al. 2016). The question has been discussed controversially of whether the variation or imprecision benefits or constrains bees' foraging precision and flexibility to locate the advertised food sources (Okada et al. 2014, Preece & Beekman, 2014, Towne et al. 1988, Tanner & Visscher 2008, 2010, WeidenmuÈller & Seeley 1999). In particular, it is not known how the variance of dance information translates into the variance of bees' flight performance. This question relates strongly to recruits' knowledge about the guiding factors in a flight (e.g., the sun compass, the distance measure and the role of landscape features). Given that dance communication is a time-consuming process

(Price et al. 2019) and noisy (De Marco et al. 2008, Okada et al. 2014, Schürch et al. 2016), experienced foragers may learn the value of dance information, compare the cost and risk between applying the instruction of dances or exploring other food sources based on their flight experience (Price et al. 2019, Wray et al. 2012). Therefore, bees could flexibly adjust foraging strategies (Al Toufailia et al. 2013, Dawson et al. 2013) and decide whether to use the socially acquired information from dancers to forage or whether to explore a new food site independently (Price et al. 2019).

Young bees learn the outdoor conditions during orientation flights that usually occur before foraging flights. Orientation flights help bees to get familiar with visual and olfactory cues of the immediate surround at the hive entrance, the sun's ephemeris function, and the layout of the landmarks at further distance (Menzel et al. 2001, von Frisch, 1967). Foraging involves multiple cognitive faculties. Bees with unique learning experiences from social communication and exploratory flights may behave individually different in their future foraging flights (Bracis et al. 2015). Inexperienced workers are thought to rely on dance information to look for new food sources (Farina et al. 2012, Gilley et al. 2018, Thom et al. 2007), and experienced foragers may be more selective in the dance following and goal decisions. In the other side, bees may have different expectations about the natural environment when they leave the colony if social communication contributes in a proactive way to their experience in the orientation and foraging flights (Menzel et al. 2012).

When are the above processes activated in the life history of bees? Asking this question requires to acknowledge multiple functions of dance communication in a flight. Dance communicates two major components, the motivational and instructive components (Menzel et al. 2011). It has been speculated that young bees with limited outdoor experience may only be motivated to explore the surrounding environment while more experienced bees may be guided by the transmitted spatial and olfactory information. However, evidences for such speculations are very rare. Are young bees more prepared to leave the hive after more intensive social contacts? Do more experienced bees collect olfactory gustatory and symbolic information and guide their exploratory flights accordingly? So far, no study has directly compared the correlation between dance following and flight performance on an individual level taking into account individual bee's life history both inside and outside the colony. Most of the above studies on dance communication and flights

were independently investigated, for example, Ai et al. (2017) investigated the development of dance related behaviors by long-term individual tracking at the hive entrance using RFID technology and video recording. Their observations indicated that dance following preceded the first dance. They also differentiated foragers that use dance information as recruits and those that did not as scouts. Biesmeijer amd Seeley (2005) followed small groups of foragers and found that they used dance information both for finding new foraging sites and resuming former foraging sites. The impressive material collected by these elaborate studies highlighted multiple conditions in which dance communication regulates foraging activities. Dukas (2008), Klein et al. (2019) and Schippers et al. (2006) showed that bees increased foraging flights with experience over a long-term foraging career. Their studies were also limited to the RFID technology that only allowed them to record the departure and arrival time, lacking any hints on what kind of flights bees might have performed and how flights develop dynamically. Furthermore, they did not collect any data of dance communication and could not explain why increased experiences improved their foraging performances.

Therefore, I will focus on the following specific questions: 1) whether the dances bees followed contains both motivational and instructive components for orientation and foraging flight? 2) How does spatial information embedded in dances determine spatial preference in the orientation and foraging flights of naïve and experienced bees? 3) How does the experience of orientation flights together with dance following interactively determine bees' foraging flights? Addressing these questions requires a full protocol of tracking the life history of individual bees both inside and outside the colony. Here, with the advantage of infrared camera module of Raspberry Pi and harmonic radar tracking technique (Riley et al. 2005), data of dance followings and trajectories of orientation and foraging flights over the first 15 day of the lifetime of the 17 individual bees were collected. Taken together, these data provide an opportunity to address directly whether age and experience dependent process existed in a colony.

6.3 Materials and Methods (see Chapter 3)

6.3.1 Data analyses

Seventeen representative bees were selected for behavioral analysis. Video recording and radar tracking allowed quantifying the dance followings and flights of individually identified bees.

Orientation and foraging flights were differentiated (Figure S6). An orientation flight was characterized by excursions around the hive and durations shorter than 10 min. And bees did not carry nectar or pollen. A foraging flight lasted longer than 10 min and was directed toward the feeding sites of north feeder (NF) or some recognized natural pollen sites. What to mention is, most of the foraging flights discussed in the Chapter were toward the direction of the artificial feeder (**FN**) but did not landed at it while a few flights were toward natural food. Two important parameters characterized for a flight trajectory were '**α**', direction between each flight fix and the hive, and '**dist**', distance between each flight fix and the hive. The densest '**α**' was calculated as the densest point of the direction distribution by the r package 'flexmix', and maximum '**dist**' ('max **dist** ') was also computed. Two important parameters of characterizing waggle dance were '**β'**, direction to the indicated food source, and '**Dist',** distance of the indicated food sources from the hive. Average vector information of direction ('**β'**) and distance ('**Dist'**) were computed.

Time intervals between a flight and dance followings were differentiated into 4 categories: (0 ~ 10 min), (10 ~ 30 min), (30 min ~ 24 h) and (> 24 h), expressing the duration of time between the flight considered and the dance following after the former flight. When there was no dance following within these time intervals, the flight was regarded as performed without dance followings.

Trajectories of foraging flight were decomposed into outbound vector part, search part and inbound homing part (Figure S7). Transitions from the vector flight to the search flight and from the search flight to the homing flight were characterized by a sharp turn of ≥60°. Correspondingly, the directions **α** were differentiated into '**v_α**' (from the end of vector flight to the hive), '**h_α**' (the start of homing flight to the hive) and '**s_α**' (from each search flight fix to the hive). The **dist** was separated into '**LVL'** (regression distance from the end of vector flight to the hive), '**LHL'** (regression distance from the start of homing flight to the hive) and **'LSL'** (regression distance from each search flight fix to the hive), respectively. The direction and distance of the feeder in the north (**FN**) to the hive were '**FN_α**' (352.5°) and **FN$_{dist}$** (550 m), respectively.

Accumulated distances between sequential flight fixes were separately measured for the vector, search and homing parts (assigned by: '**AVL**', '**AHL**' and '**ASL**'). The '**duration**' and '**velocity**' (a ratio between accumulated distance and duration) were also calculated for these three parts of flights. In addition, straightness ('**Str.**', a ratio between regression distance and accumulated distance) in the vector and homing flights were also computed.

To explore whether dances were involved in motivating a bee to perform orientation and foraging flights, overall numbers of dance followings and the standard deviation ('**sd**') and errors ('**se**') of the directions and distances indicated in the dances were calculated in Table S7 and Figure S12. These parameters were compared among bees with different numbers of orientation and foraging flights. Orientation flights with long distances and those around the entrance were separated and the numbers of dances followed before these flights were compared.

To determine whether experience of dance following has a long-lasting impact on bees' performance in the orientation and foraging flights, following analyses were performed for different time intervals between dance following and flight: 1) correlation between the densest α and β, 2) correlation between the maximum **dist** and **Dist**, 3) correlation between Δ (densest α - β) and variation of directions indicated in the dances, 4) correlation between Δ (**Dist** – maximum **dist**) and variation of distances indicated in the dances were analyzed in orientation and foraging flights.

When bees performed the foraging flights toward FN, the following differences were calculated between 1) β and **FN_α**, 2) β and **v_α**, 3) **v_α** and **FN_α**, 4) **h_α** and **FN_α**, 5) β and **h_α**. The respective parameters in the distances were calculated between 1) **Dist** and **FN$_{dist}$**, 2) **Dist** and **LVL**, 3) **FN$_{dist}$** and **LVL**, 4) **Dist** and **HVL**, 5) **HVL** and **FN$_{dist}$**. These parameters accompanied with **Str.**, **duration** and **velocity** were investigated how the accuracies of dances indicating the directions and distances of food sources influence bees' performances in directing toward the target food sources and homing.

When bees performed foraging flights after dance followings for natural food sources rather than FN, the differences between the flight direction α and the direction indicated in the dances ('**β'**'), between flight distance ('**dist**') and distance indicated in the dances ('**Dist'**') were calculated.

6.3.2 Statistics

Generalized linear model (GLM) and generalized linear mixed model (GLMM) were used to investigate the co-influence of parameters, e.g., ages, flight trips, flight type on the orientation and foraging flights. Pearson's correlation was used to analyze the correlations between the direction and distance indicated in the dances and the corresponding variables in the flight. Wilcoxon test was applied to compare differences between two groups of non-parametric data of independent samples. All the data analyses were performed in R version 4.0.4.

6.4 Results

In the chapter 3, the results have descriptively revealed that bees started the first orientation flights after having the experience of dance following and flew more with more dance followings. In this chapter, I will quantitatively explore how dances influence bees' flight performances. Except for bees 257, 272 and 282, 14 bees performed 69 orientation flights, of which, bees 216, 244, 262, 267 and 271 had 13, 16, 1, 15 and 5 foraging flights, respectively (Figure 41, Table S7).
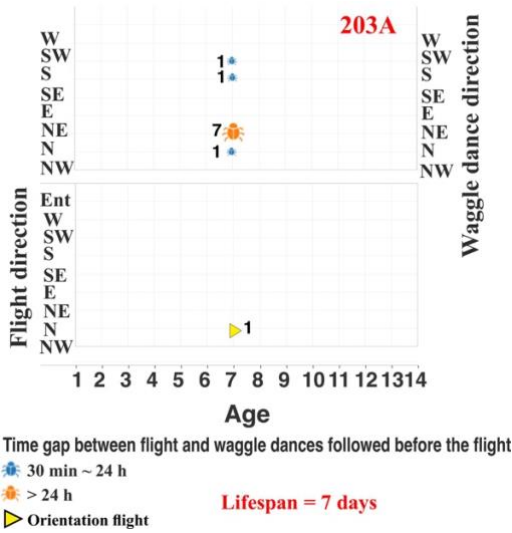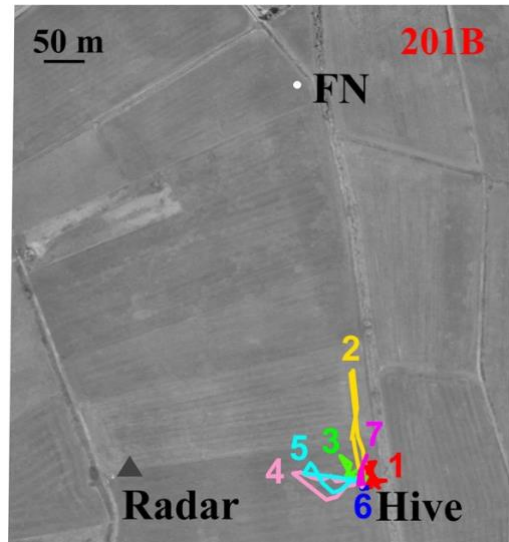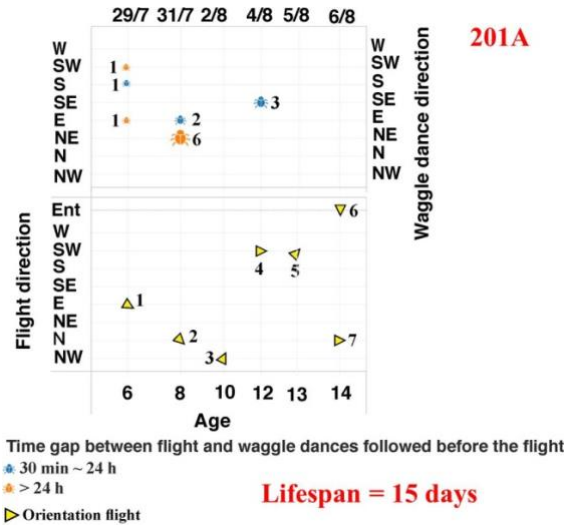
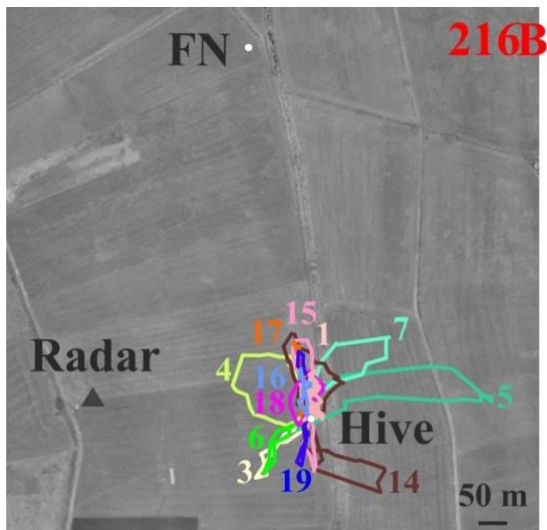### 6.4.1 Does dance following motivate bees to perform orientation and foraging flights?
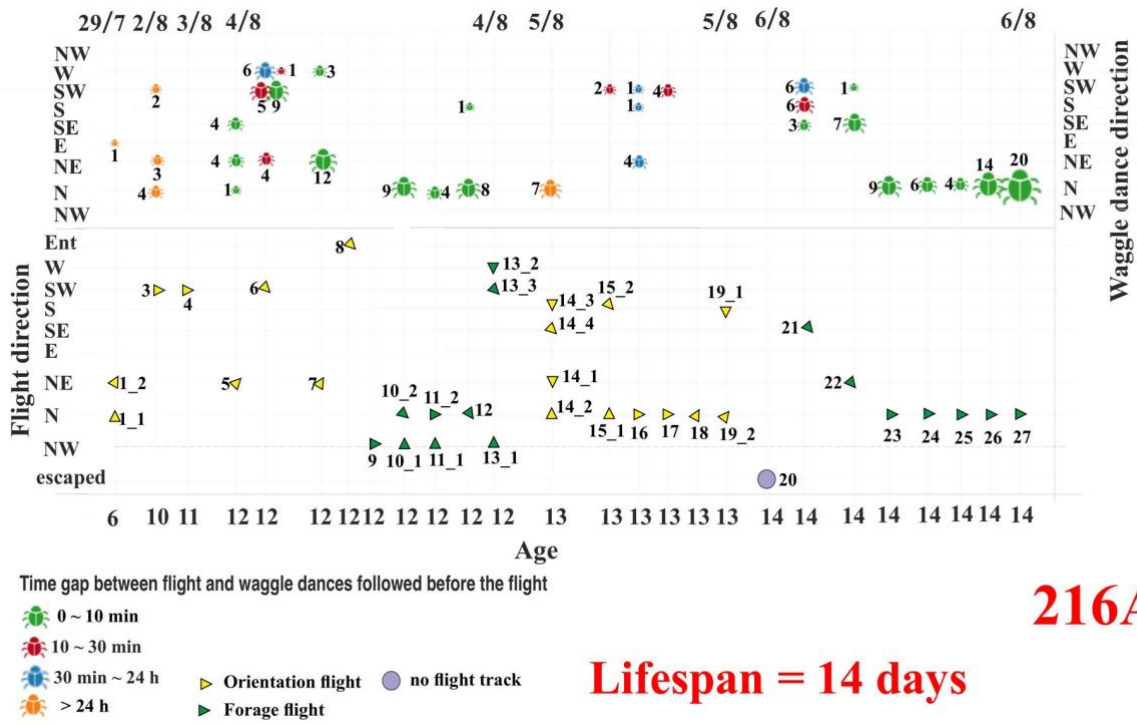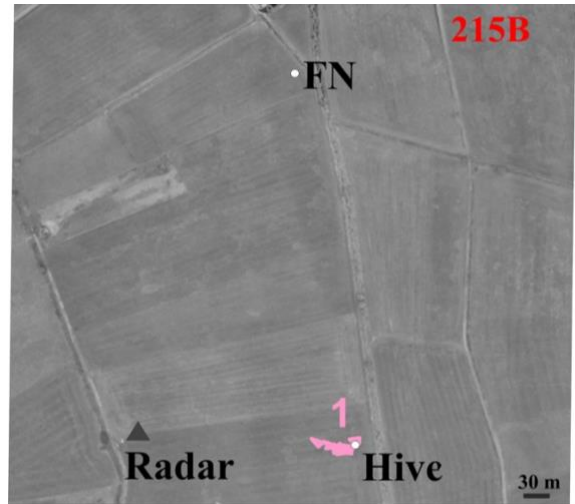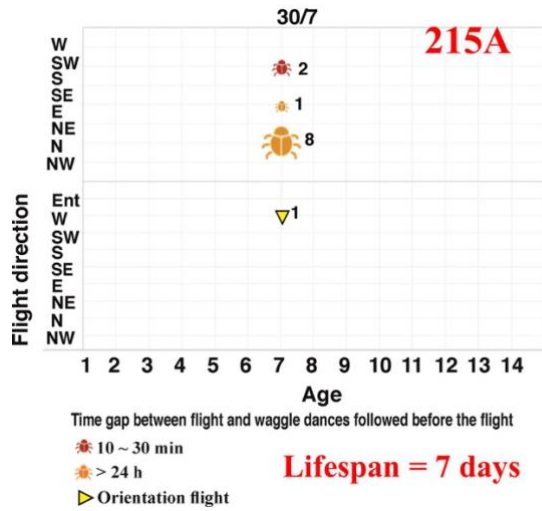
Figure 41 shows the sequences of orientation and foraging flights after dance following. Twenty-three orientation flights and eight foraging flights were performed without dance following (Figure S8). The number of dances followed before the flight, the age of bee, the flight type and the frequency of 'orientation' and 'foraging' flights were taken together to study whether bees performed flights with or without dance following relating with age, flight type and sequence of flight. GLM analysis revealed that age ($p = 0.26$, df = 1, $\chi^2 = 1.3$) and flight sequence ($p = 0.51$, df = 1, $\chi^2 = 0.42$) did not correlated significantly with whether bees performed flight with or without dance following. However, numbers of dance followings (coef. = -1.5, $p = 0.011$, df = 1, $\chi^2 = 6.5$) were significantly lower when bees performed orientation flights than foraging flights. This result further indicated that potential impacts of dance following on the orientation and foraging flight were different.
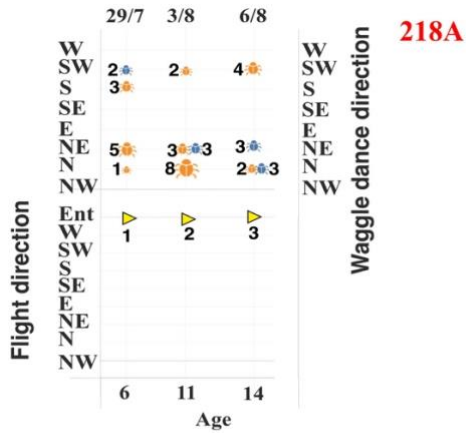
Of the 68 orientation flights evaluated, 10 flights were very short close to the entrance, 5 of them were initiated without dance following, 4 of them were performed more than 1 day after the last flight and before this flight, and 1 was done immediately after dance following. The other 59 were long distance flights, 37 of

which were performed after dance following. The numbers of dance followings, the age and flight sequence were combined to search for the relation between dance following and the distances of orientation flights. Although the interaction of flight sequence and the number of dance following did not show significantly different impacts on the bees' tendency to fly over long distances or stay close to the hive entrance (GLMM, $p = 0.18$, df $= 1$, $\chi^2 = 1.8$), this analysis gave a hint that as bees performed more flights depended less on dance following and lead them further away from the hive entrance (Figure S9). Next, I asked whether long-distance orientation flights performed after dance following depended on bees' age and flight sequence. The age of bee was not significantly different between flights without and with dance following ($p = 0.62$, df $= 1$, $\chi^2 = 0.24$) (Figure S10A). GLMM results suggested that as bees gained more experience in the orientation flight, they had significantly less followed dances ($p = 0.009$, df $= 1$, $\chi^2 = 6.8$) (Figure S10B). The former flight direction ($p = 0.62$, df $= 1$, $\chi^2 = 0.24$) and distances ($p = 0.47$ df $= 1$, $\chi^2 = 0.52$) also had no influence on whether bees followed dances before the flight. No significant differences were found on the impact of age ($p = 0.6$, df $= 1$, $\chi^2 = 0.3$), flight sequence ($p = 0.8$, df $= 1$, $\chi^2 = 0.16$) and direction and distance of the former flight ($p = 0.55$, df $= 1$, $\chi^2 = 0.35$) on foraging flights with or without dance followings (Figure S11A, B). One may expect that the variance and dispersion of the directions and distances encoded in the dances rather than the number of dances may have a stronger impact on the subsequent flights (Table S7, Figure S10). The frequencies of orientation flights were higher after bees following dances with larger overall standard deviations in the directions encoded in the dances (GLMM analysis, coef. $= 0.02$, $p = 0.004$, df $= 1$, $\chi^2 = 8.4$) while higher standard errors of directions reduced bees' orientation flight frequencies (GLMM analysis, coef. $= -0.12$, $p < 0.01$, df $= 1$, $\chi^2 = 34.8$). In the foraging flights, the overall increased standard deviations of direction code (GLM analysis, coef. $= -0.05$, $p < 0.01$, df $= 1$, $\chi^2 = 10.4$) and distance code (GLM analysis, coef. $= -0.02$, $p < 0.01$, df $= 1$, $\chi^2 = 8.4$) reduced bees to perform foraging flights. These results are also seen in Figure S12. Bees 216, 244, 262, 267 and 271 were the most active foragers after following dances with lowest standard errors of directions.
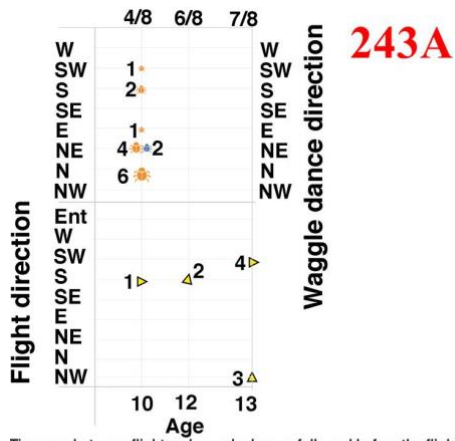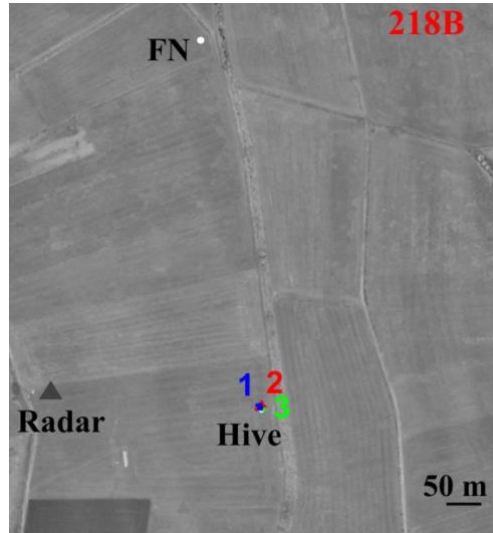
215A

30/7

2
1
8

Ent
1

Flight direction

Waggle dance direction

Age

Time gap between flight and waggle dances followed before the flight

10 ~ 30 min
> 24 h
Orientation flight

Lifespan = 7 days

215B

FN

Radar   Hive

30 m

29/7 2/8 3/8 4/8   4/8 5/8   5/8 6/8   6/8

Waggle dance direction

Flight direction

Age

Time gap between flight and waggle dances followed before the flight

0 ~ 10 min
10 ~ 30 min
30 min ~ 24 h   Orientation flight   no flight track
> 24 h   Forage flight

216A

Lifespan = 14 days

216B

FN

Radar

Hive

50 m

216C

FN

Radar   Hive

50 m

218A

218B

Lifespan = 15 days

243A

243B
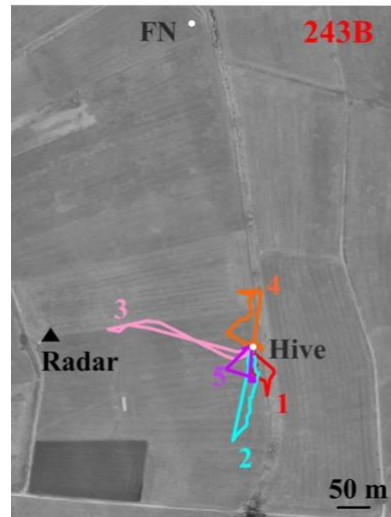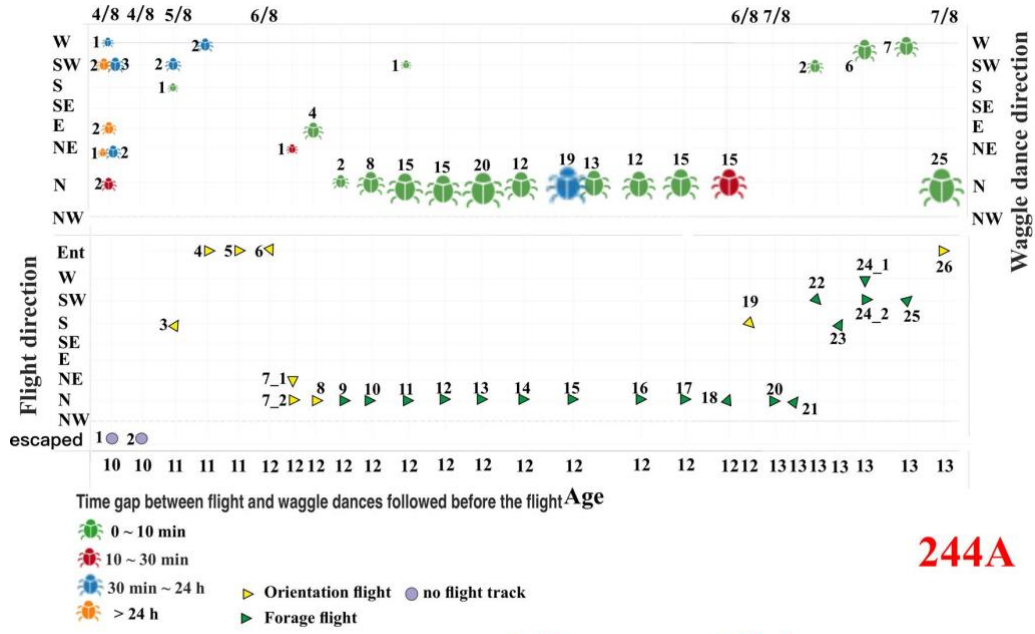
Lifespan = 13 days

244A

Lifespan = 13 days

244B

244C
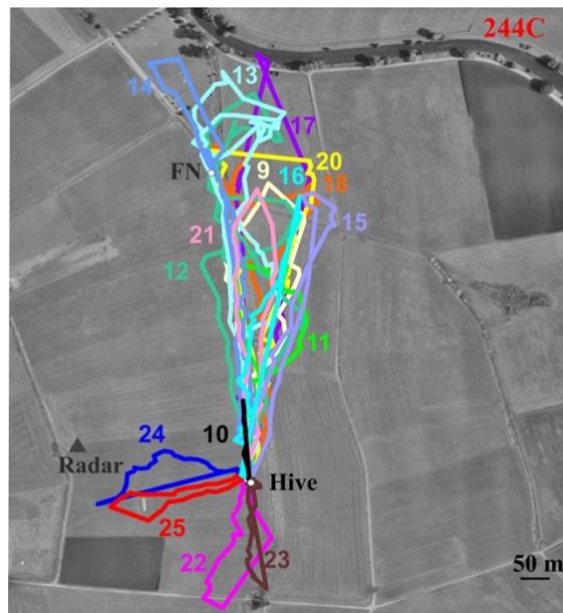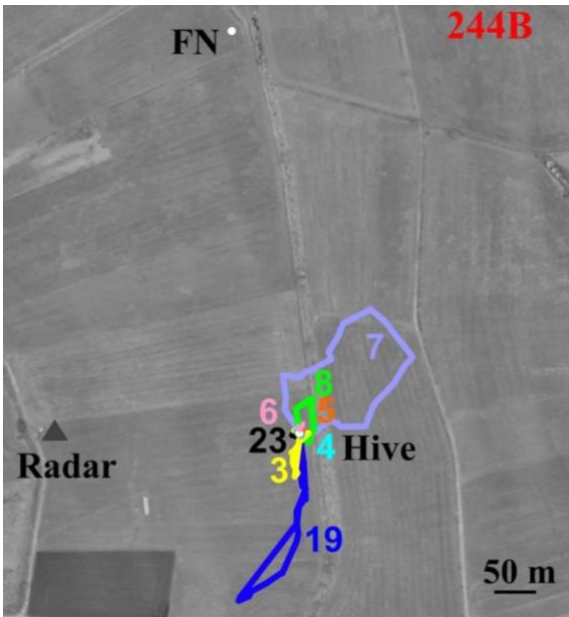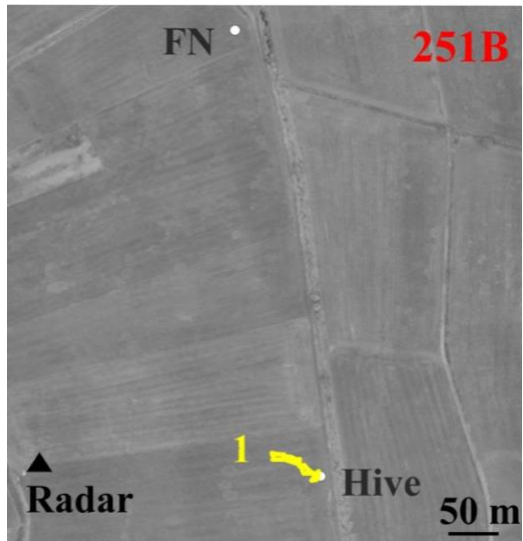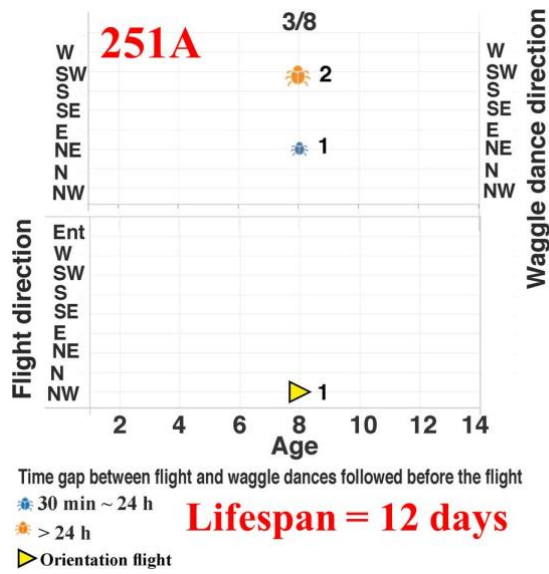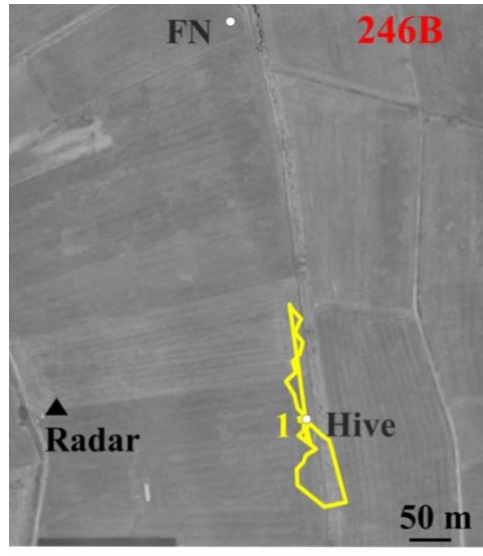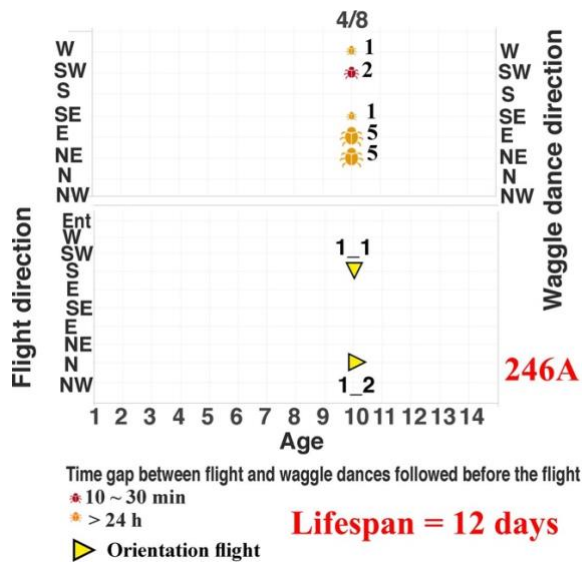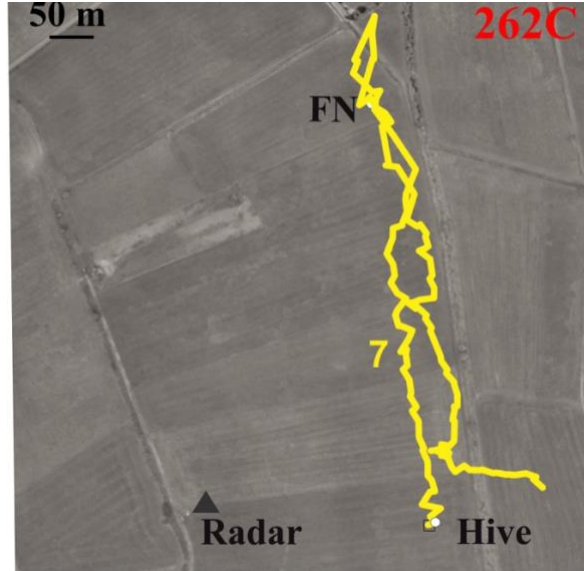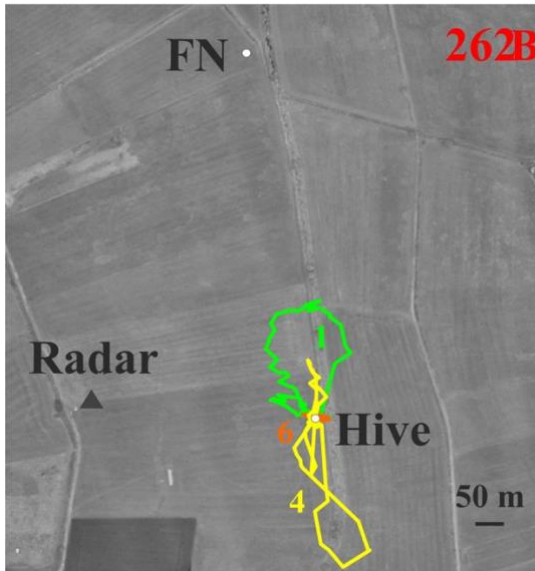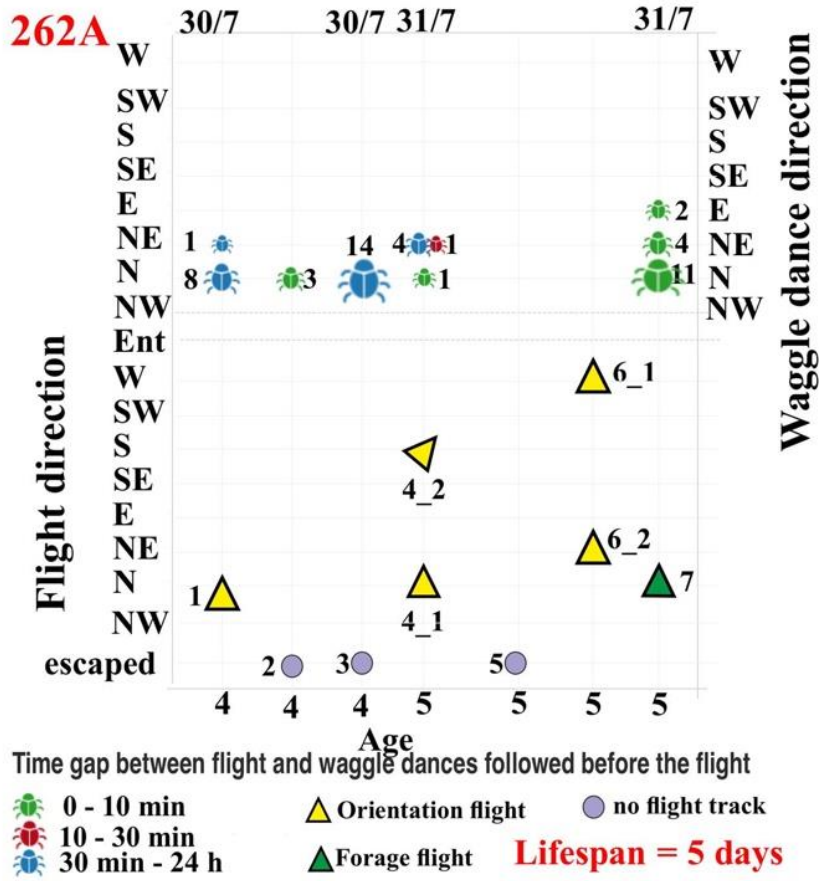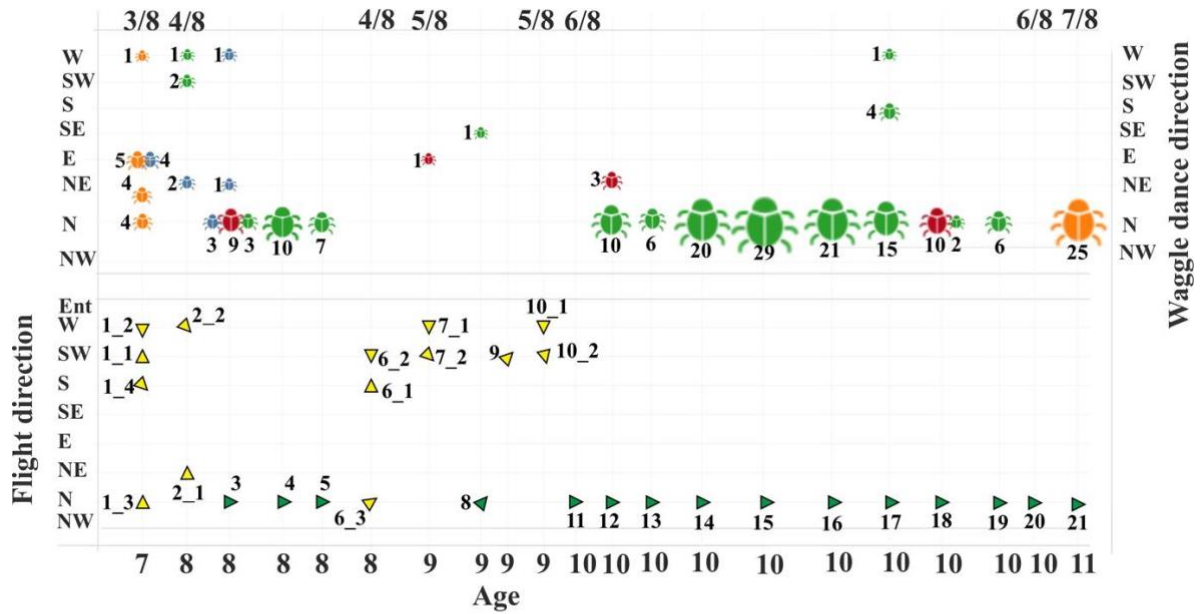
267A

Time gap between flight and waggle dances followed before the flight

- 0 ~ 10 min
- 10 ~ 30 min
- 30 min ~ 24 h
- > 24 h
- ▷ Orientation flight
- ▶ Forage flight

Lifespan = 11 days

267B

267C

Time gap between flight and waggle dances followed before the flight

🐞 0 ~ 10 min
🐞 10 ~ 30 min
🐞 30 min ~ 24 h   ▷ Orientation flight
🐞 > 24 h   ▶ Forage flight

271A

Lifespan = 11 days
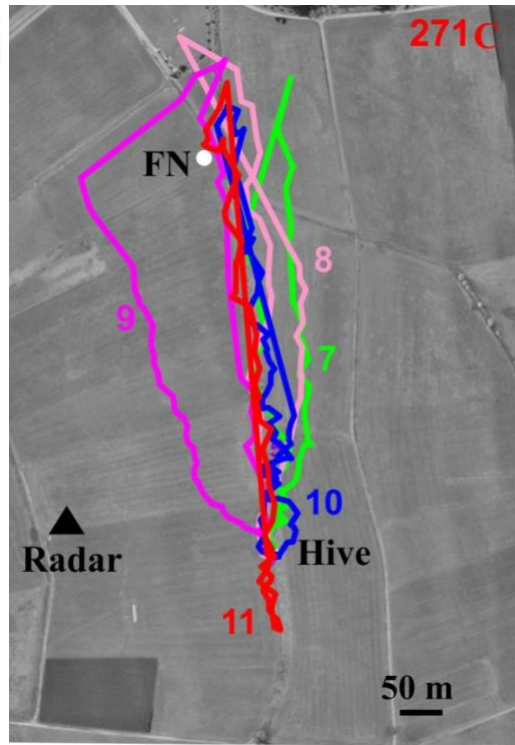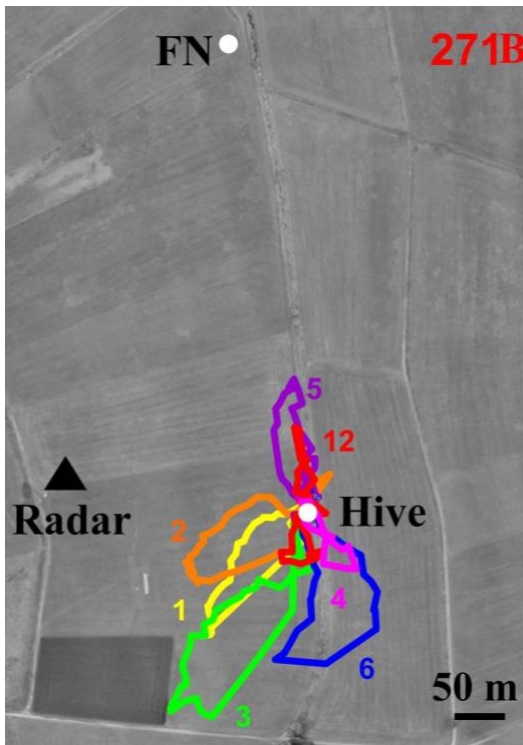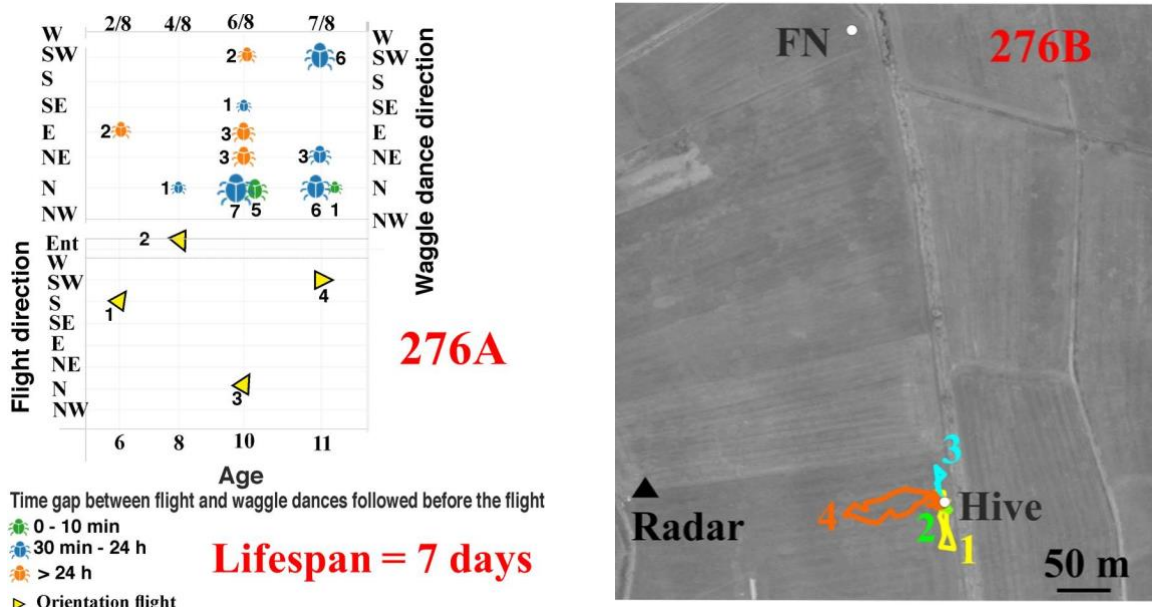
Figure 40. Number of dance followings after the former flight and before the flight across days and the sequential flights. Figures with letter 'A' show the dance followings at different time intervals (green 'bee symbol': 0 ~ 10 min; red 'bee symbol': 10 ~ 30 min; blue 'bee symbol': 30 min ~ 24 h; yellow 'bee symbol': > 24 h), numbers of dance followings are around 'bee symbol', the corresponding direction and distance codes in the dance, flight type ('orientation flight' in the yellow triangle and 'foraging flight' in the green triangle) and flight sequence (number around the triangle). 'Ent' on the left y-axis denotes bee fly around the entrance without preferred direction. 'W', 'SW', 'S', 'SE', 'E', 'NE', 'N' and 'NW' of the same axis show flight direction while the corresponding direction on the right y-axis show the direction code. The respective dates and ages when bees perform the flight are shown on the top and bottom of each sub-figure, respectively. Purple circle shows that bees fly away without radar tracking. Figures with letter 'B' (orientation flight) and 'C' (foraging flight) show the sequential flight trajectories of different bees. 'FN', 'Radar' and 'Hive' are the positions of the north feeder, radar and hive. The number with different colors shows the flight sequence.

6.4.2 Do bees care about the instructive components of dances in their orientation and foraging flights?

In addressing this question, I separated the time intervals of dance followings before the flight in 4 categories: 0 ~ 10 min, 10 ~ 30 min, 30 min ~ 24 h and (>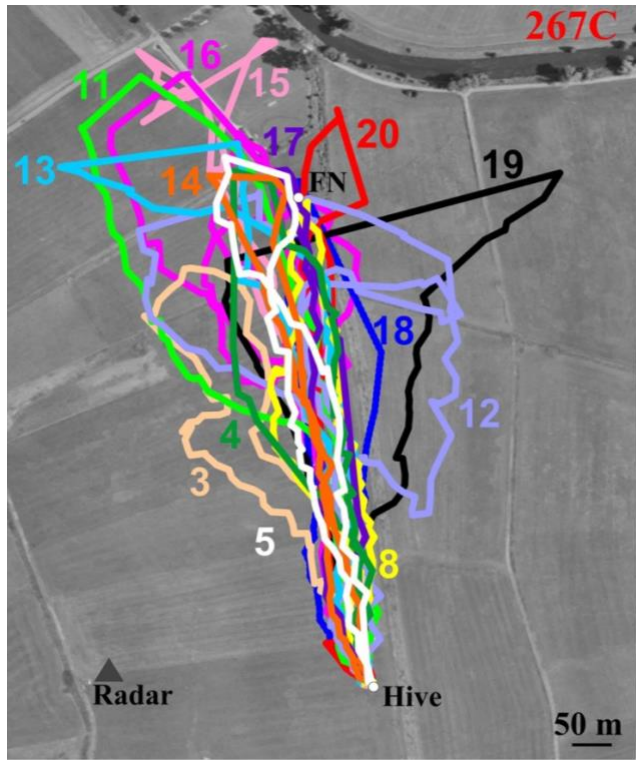 1 day). The respective numbers of orientation flights within these time intervals were: 14, 10, 25 and 21. The corresponding numbers of foraging flights were: 39, 5, 4 and 2.

The densest **α** of a flight indicating the most preferred flight direction was computed. The densest **α** of orientation flights correlates positively with the average direction ('**β**') encoded in the dances followed for the interval of 0 ~ 10 min ($R^2_{adj} = 0.49$, *p* <

0.01, Figure 41A). This is not the case for longer intervals (Figure 41C, E, G). Although the average distances ('**Dist**') encoded in the dances were far longer than the maximum flight distances ('**dist**'), they were also positively correlated (R^2_{adj} = 0.26, p = 0.03, Figure 41B). No correlations were found between the variation and dispersion of direction and distance indicated in the dances followed within different time intervals and the corresponding Δ (densest **α** - **β**) and Δ (**Dist** – maximum **dist**) (Figure S13a, b). Based on these results, further regression models were run including age, flight sequence and former flight range and direction in order to test whether bees' preferred direction and maximum distance of flight were influenced. However, these factors did not fit in the regression model analyses (all R^2_{adj} <= 0, figures were not shown).

Similar analyses were performed for foraging flights (Figure 42, S18a, b). Since 76% of foraging flights were performed after bees followed the dances within 0 ~ 10 min, the analyses were focused on the dances followed within 0 ~ 10 min. The densest flight direction of flight increased as the average directions indicated in the dances increased (R^2_{adj} = 0.75, R = 0.98, p < 0.01, Figure 42A), and the maximum flight distance was not correlated with the average distance indicated in the dances (R^2_{adj} = 0.049, p > 0.05 Figure 42B). Similar results were found for the direction (R^2_{adj} = 0.93, R = 0.96, p < 0.01, Figure 42C) and distance (R^2_{adj} = 0.058, p > 0.05, Figure 42D) of the vector flight, that is the initial straight part of the outbound component of the foraging flight. Although the average distances indicated in the dances were mostly distributed close to **FN$_{dist}$**, the difference between **FN$_{dist}$** and **Dist** ranged between -100 ~ 200 m, and **Dist** varied considerably (Figure 43A, C). The distribution of **LVL** in flights showed highly different from **FN$_{dist}$** (Figure 43B, D). These results may indicate less accurate distance encoding in the dance may thus influence the bees' accuracy in reaching the indicated goal, the north feeder (**FN**). Therefore, I next addressed the question whether more accurate encoding of distance **Dist** of the FN in the dances or **LVL** in the vector flight would lead to more precise steering toward FN during bees' search flights. I also asked whether these parameters influence the bees' decision to switch from search flight to homing flight. Regression analysis showed that the accuracy of **Dist** did not influence bees' maximum flight distance during the searching and **LHL** of the homing flights (all R^2_{adj} <= 0, figures are not shown). Instead, with increasing deviation of **LVL** in the vector flight from **FN$_{dist}$**, the maximum flight distance in the search flight (Figure 44A) and the **LHL** of the homing flight

decreased (Figure 44B). Furthermore, I analyzed whether less accurate direction or distance codes separately influence **LVL** and **v_α** of vector flight toward FN. However, no correlations were found between Δ (**v_α** – **FN_α**)) and Δ (**LVL** - **FN**$_{dist}$), Δ (**β** – **FN_α**)) and Δ (**LVL** - **FN**$_{dist}$), Δ (**Dist** – **FN**$_{dist}$)) and Δ (**v_α** – **FN_α**)) (all adjusted $R^2 < = 0$, data are not shown).

Next, I examined the impact of variation and dispersion in the codes for direction and distance on the performance of foraging flight (Figure S14a, b, Figure 45). As the overall standard deviation (**sd**) (adjusted $R^2 = 0.43$, Figure S14a) and error (**se**) ($R^2_{adj} = 0.21$, Figure S14b) of direction code increased, the densest **α** of foraging flight deviated more from the direction code. The overall standard deviation (**sd**) and error (**se**) of distances code did not influence bees' reliance on the dance and foraging flight performance (Figure 18C, D). Interestingly, as the ranges of **sd** and **se** of directions were within ± 12° and ± 5°, respectively, the densest flight directions and the directions indicated in the dances were almost similar (Figure S14b). Additional analyses excluded impacts of age, flight sequence for both direction and maximum distance of the foraging flight (all adjusted $R^2 < = 0$, data not shown). Similar results were found for the correlation between the related measures of vector flight and the dance codes (Figure 45A, B, E, F). Although the direction code for FN varied considerably, the direction of vector flight toward FN was highly precise (Figure 45C, D). Although the distance of vector flight did not correlate with **FN**$_{dist}$, the range of difference between **LVL** and the average distance code (Figure 45E, F) remained more variable than that between **LVL** and **FN**$_{dist}$ (Figure 45G, H).
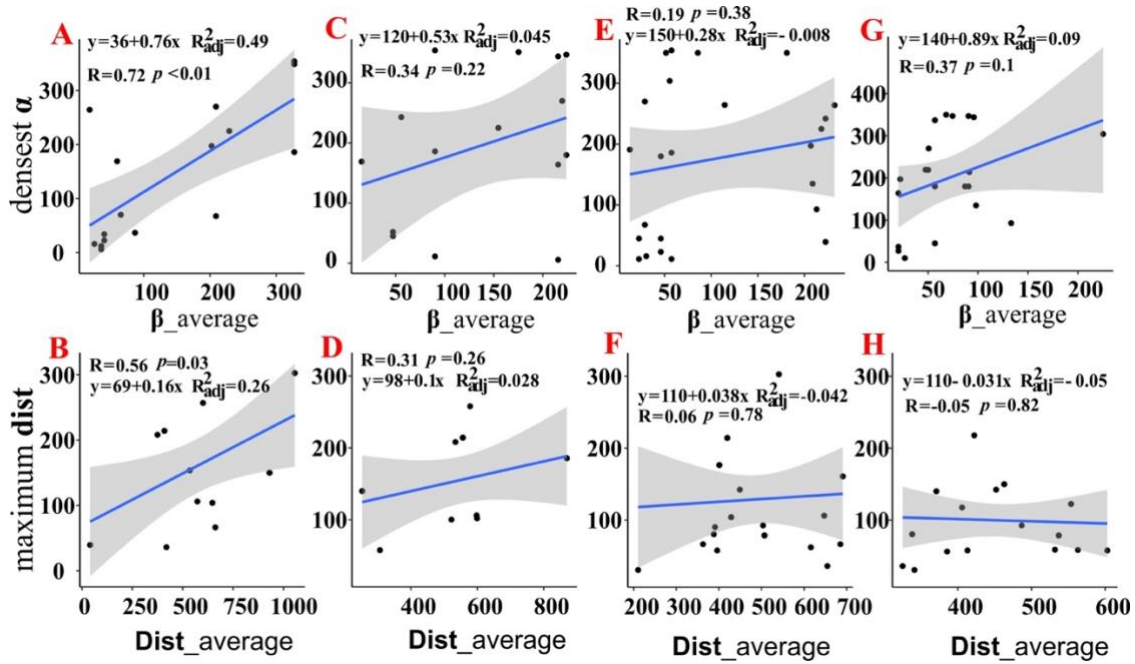
Figure 41. Correlation between the average direction and distance indicated in the dances followed within different time intervals before the orientation flight and the respective flight performance. 'Densest **α**' and 'maximum **dist**', '**β_**average' and '**Dist**_average' represent the densest direction and maximum distance of the orientation flight, and the average direction and distance encoded in the dances. A-B, C-D, E-F and G-H show the respective correlations in the time intervals of (0 ~ 10 min), (10 ~ 30 min), (30 min ~ 24 h) and (> 1 day). Values of R^2_{adj}, R and p-values explain how significant the regression model fit.
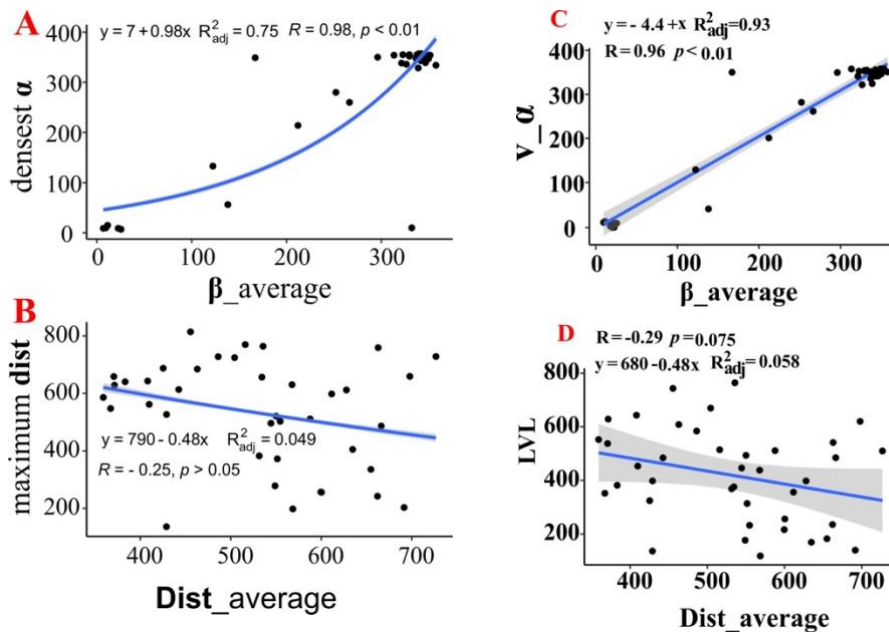
Figure 42. Correlation between the average directions and distances indicated in the dances followed before the foraging flight and the flight performance. 'Densest **α**' and 'maximum **dist**', '**v_α**' and '**LVL**', '**β_**average' and '**Dist**_average' represent the densest direction and the maximum

distance of the whole foraging flight, the direction and the distance from the end of vector flight to the hive, the average direction and distance conveyed in the dances, respectively.
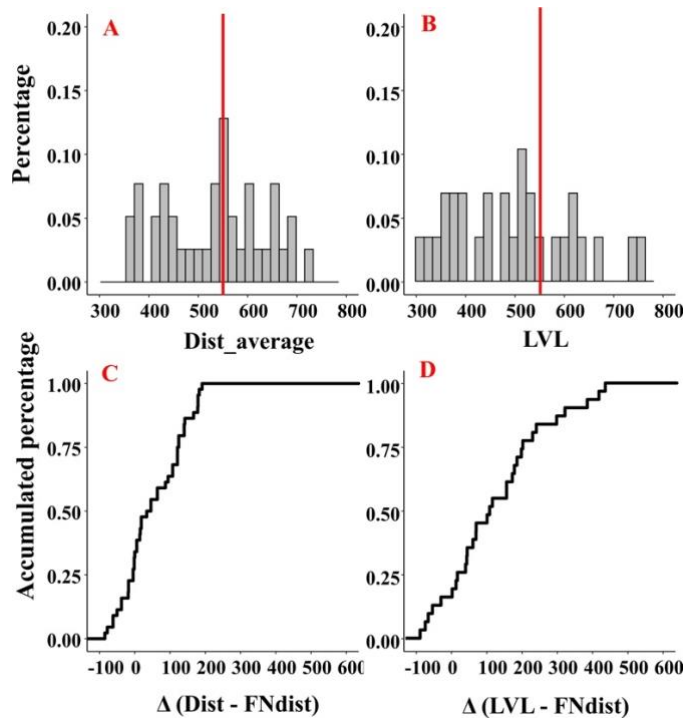
Figure 43. Distribution of distance of FN indicated in the dances and in the vector flight. The vertical red line indicated the **FN$_{dist}$** = 550 m.
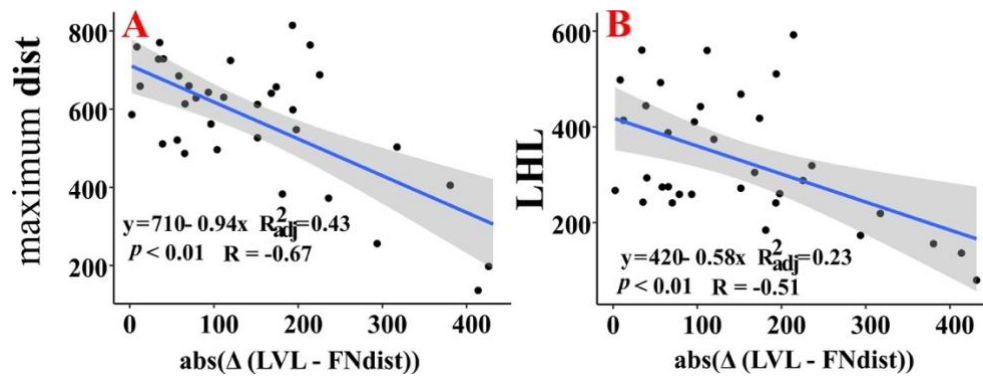
Figure 44. Impact of the accuracies of LVL on the search and homing fights. 'maximum **dist**', '**LHL**' and 'abs (Δ (**LVL** - **FN$_{dist}$**)' represent the maximum distance of foraging flight, the regression distance from the start of homing flight to the hive and the difference between the regression distance of the vector flight from the hive to FN, respectively.
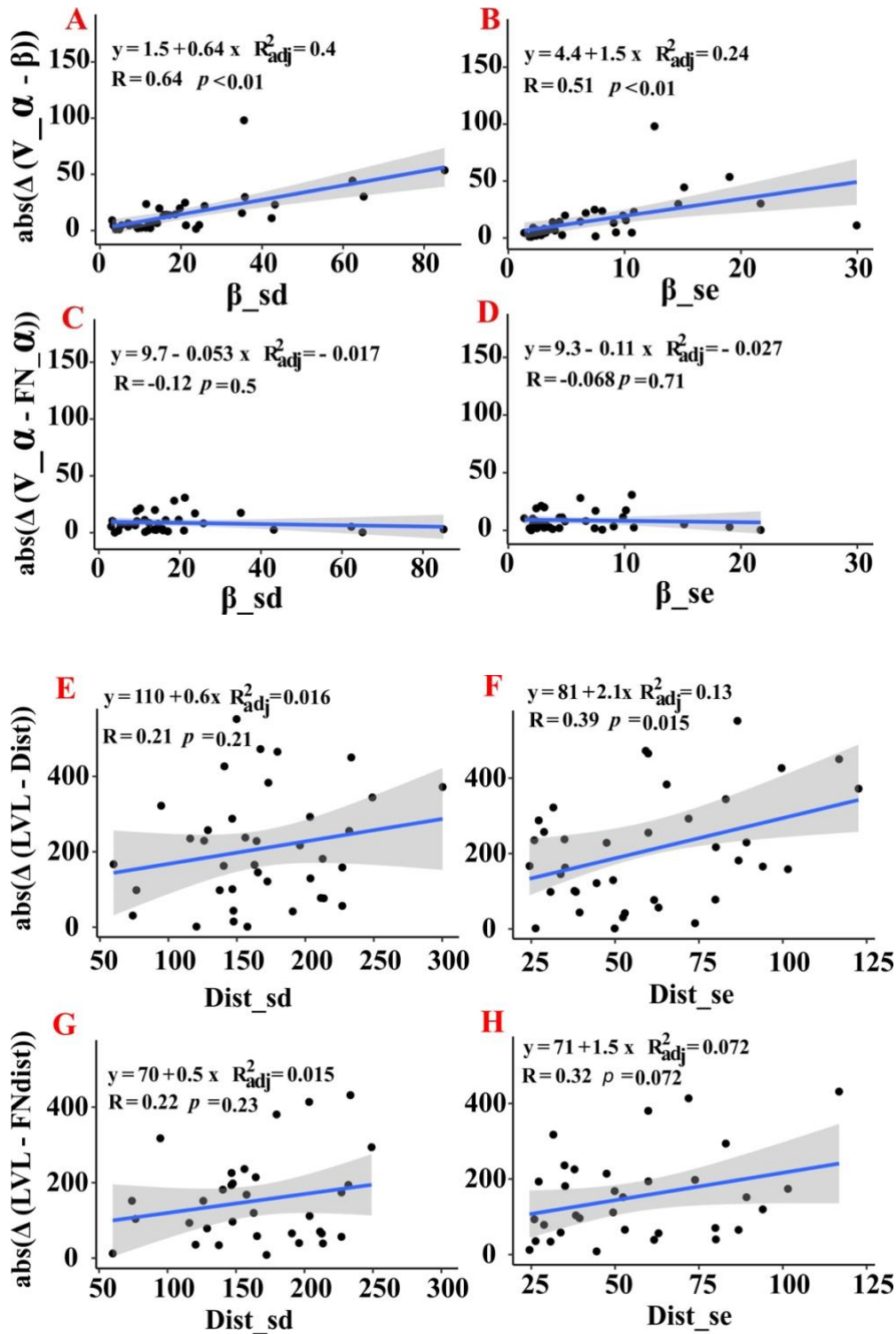
Figure 45. Impact of the variation of direction and distance indicated in the dances on the bees' preference to follow the dance information and the accuracy of vector flight toward FN. 'abs(Δ (**v_α** - **β**))' and 'abs(Δ (**LVL** - **Dist**))', 'abs(Δ (**v_α** – **FN_α**))' and 'abs(Δ (**LVL** - **FN$_{dist}$**))' represent the absolute values of differences between the direction and distance of the vector flight and the respective average direction and distance indicated in the dances, between the direction and distance of the vector flight and the respective direction and distance of FN. '**β**_sd' and '**β**_se', '**Dist**_sd' and

'**Dist**_se' denote the standard deviations and errors of the direction and distance indicated in the dances, respectively.

6.4.3 Individuality of flight performance in orientation and foraging flights

Here I will address the question of whether bees used the dance information differently on an individual level. This question was motivated by the finding that bees showed individual differences in performing their flights (Figure 41, 42). For example, bees 216, 244, 262, 267 and 271 showed individual patterns of foraging flight trajectories (Figure 41, 216C, 244C, 262C, 267C, 271C). The above analyses had revealed that the distance code in the dances did not significantly correlate with the average distances of foraging flights. This discrepancy might result from individual differences in receiving the distance information from particular attended dancing bees (Figure 46, 47).

Indeed, the distribution of dance encoded distance to **FN** ('**Dist**') experienced the bees 216, 244, 262, 267 and 271 differed considerably (Figure 46A). For example, the **Dist** for bee 216 as extracted from the different waggle runs during one dance were rather close to **FN**$_{dist}$, the accurate distance. For bee 244, these values were mostly around 430 m and some close to **FN**$_{dist}$ and a few between 600 ~ 700 m. For bee 262, the corresponding parameter were around 570 m, for bee 267 mostly around 350 ~ 470 m and a few between 400 ~ 730 m, and for bee 271 mostly between 475 ~ 530 m. However, the distributions of **LVL** for individual bees in the vector flight (Figure 46B), the maximum **dist** in the search flights (Figure 47B) and **LHL** in the homing flights (Figure 47A) did not fully match. For example, **LVL** of bee 216 was mostly close to 430 m and none beyond **FN**$_{dist}$, of bee 244 **LVL** was mostly within 510 ~ 590 m and a few within 320 ~ 400 m and > 730 m, of bee 262 **LVL** was around 425 m, of bee 267 **LVL** was mostly between 510 ~ 620 m and a few between 320 ~ 480 m and > 725 m, and of bee 271 **LVL** was mostly around 570 m and a few around 370 m and 600 ~ 680 m. The differences between the distance of **FN** encoded in the dances and **FN**$_{dist}$, and between **LVL** and distance **FN**$_{dist}$ for these five bees also showed that bees 271 and 267 were better in extracting the distance code in the dances, followed by bees 244 and 216 (Figure 46C). Similar results were observed in the search and homing flights among these bees (Figure 47). The maximum foraging flight distance of bee 271 was beyond 600 m, of bee 267 mostly beyond 600 m and a few between 470 ~ 600 and 680 ~ 770 m, of bee 216 mostly

around 500 m and a few between 320 ~ 400 m and one around 570 m, of bee 244 mostly around 520 m and a few within 400 m and between 570 ~ 760 m. **LHL**s of homing flights of bees 267 and 271 were both within 450 m, of bee 244 mostly between 450 ~ 570 m and a few within 400 m and beyond 600 m, of bee 216 within 510 m. Although bee 262 performed only one foraging flight and **LVL** was shorter than the distance code in the dances that was very close to **FN$_{dist}$**, she tended to search beyond **FN** (Figure 47B) and then switched to homing flight close to **FN** (Figure 47A).

Following the above results, the patterns of loops in the foraging flights were compared. Bee 271 had no loop in the search flights while bee 216 had the most loop (the 10th, 11th, 24th, 26th and 27th flights had 2, 3, 2, 3 and 4 loops, respectively) and followed by bees 244 (the 11th, 12th, 13th and 18th flights had 1, 2, 2 and 1 loops, respectively) and 267 (the 3rd, 12th and 16th flights had correspondingly 1, 3 and 3 loops). Bee 262 had 1 loop. These results were corresponding the difference of calibrating information of distances of **FN** indicated in the dances. Whether if the variation and dispersion of vector information indicated in the dances impact bees' loops were analyzed. However, no stronger evidence supported this hypothesis (Figure 49).

All trajectories of foraging flights toward **FN** were between the hive and **FN**, except for the 11th flight of bee 271 whose flight first directed toward S and then back toward **FN**. This flight was performed without dance following indicating that bee remembered the experience from earlier foraging flights and/or dance following effects. Compared to the other bees with less orientation flights toward S, bee 271 had 6 out of 7 orientation flights toward S (Figure 41, 271B). The former foraging flight (10th) was directed toward **FN** about 1 hour before this flight was also performed without dance followings (Figure 41, 271A, C). Bee 271 also had 3 foraging flight toward **FN** with many dance followings indicating **FN** before this flight. Interestingly, bee 216 initiated the first foraging flight (9th) toward **FN** without dance following, although the direction of the vector flight deviated the accurate direction to **FN** by **2**1.4°(**FN_α** (Δ (**v_α** − **FN_α**) = 21.4°) and from the accurate distance by 27.4 m (Δ (**LVL** - **FN$_{dist}$**) = 27.4 m)). The 13th foraging flight was also performed without dance following. This flight was initially directed to **FN** (Δ (**v_α** − **FN_α**) = 12.8°, Δ (**LVL** - **FN$_{dist}$**) = 416.7 m) and then switched to W-SW. Bee 216 performed four foraging flights on the same day toward **FN** and toward NW before this flight. Similarly, bee 244 performed the 18th flight with a deviation of direction of 8.1° (Δ

($\mathbf{v\_\alpha} - \mathbf{FN\_\alpha}$) = 8.1°) and a deviation of distance of 193.8 m (Δ (**LVL** - **FN$_{dist}$**) = 193.8 m)). The respective parameters for the following flights were 20th flight, -0.6° and 38 m and 21st flight, -6.2°, -35 m. These flights were performed without additional dance following within 10 min. She also performed 9 foraging fight before these flight on the same day toward **FN** after multiple dance followings. Similar conditions were observed in bee 267. She performed the 11th flight with Δ = 13.2°, -235 m, 20th flight with Δ = -0.7°, -0.3 m and 21st flight with Δ = -0.4°, -70 m without dance followings. She also had performed several foraging flights directed to **FN** before these flights. Bee 244 performed the 22nd flight without dance following went toward S around 30 min before the 23rd flight that also directed toward S. Another interesting case is bee 216. She performed the 22nd flight toward NE after following 7 dances indicating food sources at SE and 1 dance indicating food sources in the SW sector. Before this flight, she flew multiple times toward **FN**. A further case was bee 267 that flew directly toward **FN** on the 8th foraging flight with Δ = 2.4°, -3 m after having followed one dance indicating pollen sources in the SE. She had 2 orientation flights at the SW on the same day and 3 orientation flights and then 3 foraging flights toward N around 1 ~ 2 days before.

Bess 257, 272 and 282 had some few dance followings over their lifetime (Figure 49, Table S7) but none of these bees performed flight. Comparing the similar aged bees (i.e., bee 251) or bees that were 1 ~ 2 days older (i.e., bees 201 ~ 215, 218, 243, 246) with bee 257, one would expect her to leave the hive and start orientation flights. Indeed, she first appeared at the hive entrance on her 7th day of age, 4 times on the following four days, 6 times on the 11th day and final appearance on the 12th day. These 12 appearances at the entrance occurred without dance followings during the 4 hours before, except for the 5th appearance on the 11th day after she had followed 4 dances indicating FN. However, she did not start to fly up and was brought back to the hive entrance. Unsuccessful flight starts falling down on the ground were observed 8 times. Similar case was observed with bee 272 that first appeared at the hive entrance 5 hours after her last dance following on her 8th day of age. She then appeared again 3 times on the 11th day without having followed a dance.

Bee 282 first appeared at the entrance on her 6th day of age and 10 times more on the following days. All these appearance at the hive entranced occurred without

dance following within 1 day. She did not start flying, although she appeared to be highly motivated.
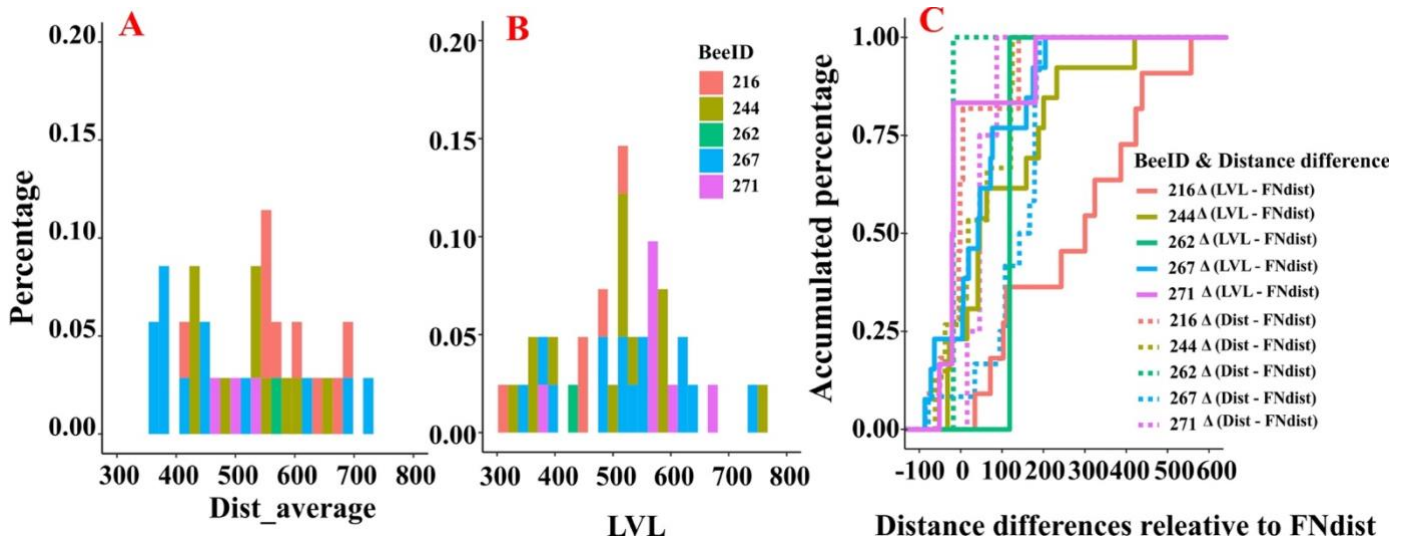
Figure 46. Individual differences in utilizing the vector information of distances about the dance indicated in the dances to guide the vector flight. '216 Δ (**LVL** - **FN**$_{dist}$)' and '216 Δ (**Dist** - **FN**$_{dist}$)' indicated the distance differences of bee 216 between **LVL** and **FN**$_{dist}$, and between **Dist** - **FN**$_{dist}$. Similar denotes for the other bees.
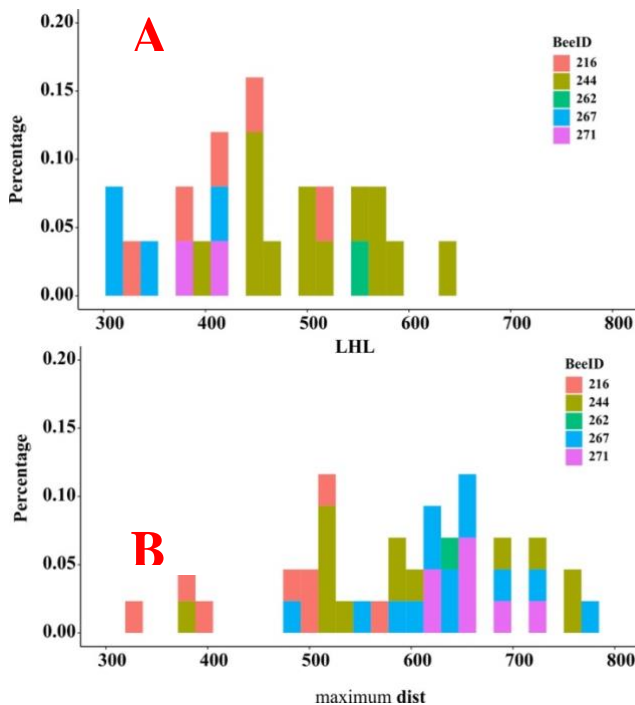
Figure 47. Individual differences in utilizing the vector information of distances about the dance indicated in the dances to guide the search flight and homing flight. '**LHL**' and 'maximum **dist**' represent the linear regression distance of homing flight and the maximum distance of search flight relative to the hive, respectively.
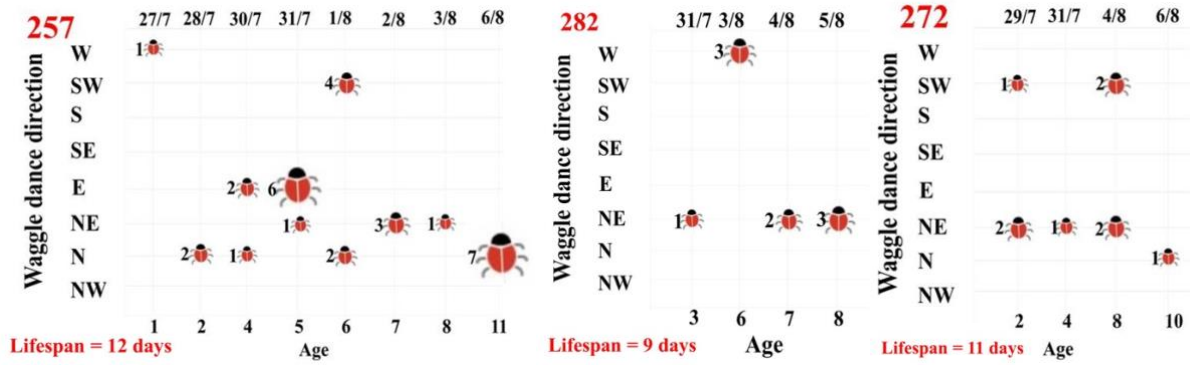
Figure 48. Dance followings of bees 257, 272 and 282 over lifetime without initiating flights.
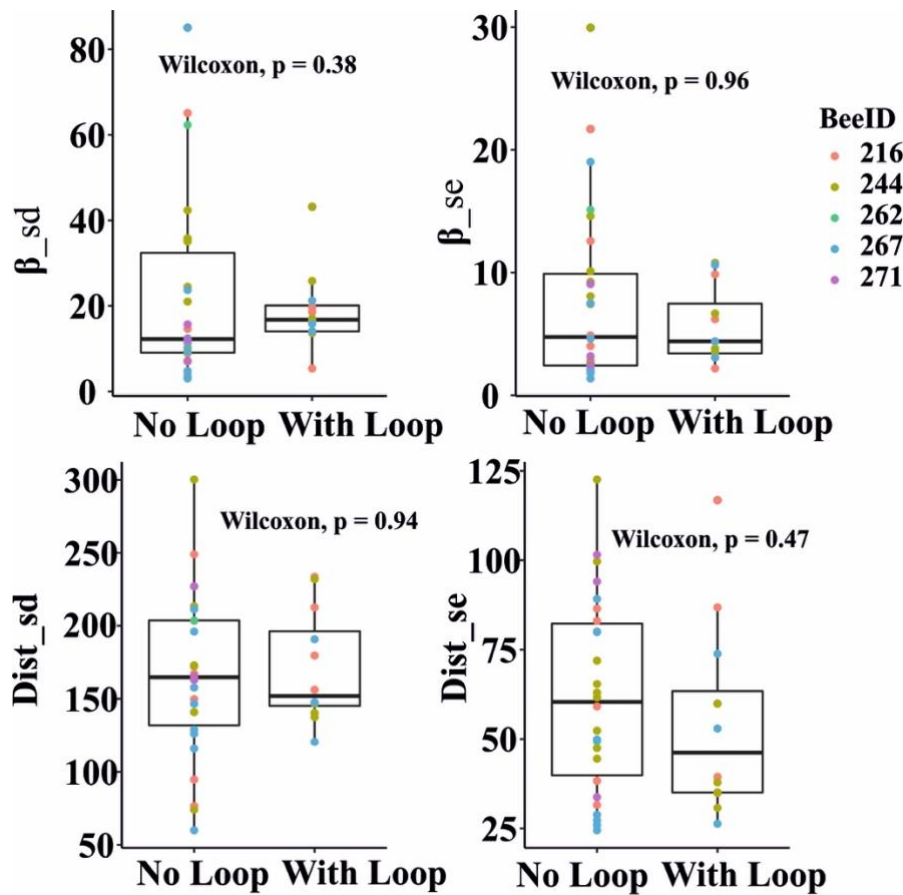
Figure 49. Impacts of variation and dispersion of direction and distance of dances indicating **FN** on the number of loops in foraging flights. 'β_sd' and 'β_se', '**Dist**_sd' and '**Dist**_se' denote the standard deviations and errors of the direction and distance indicated in the dances, respectively.

6.5 Summary and Discussion

Waggle dance is known to convey the vector information between the hive and food sources. The effect of dance communication was examined on the level of number of dance followings, and the variation and dispersion of distance and direction codes. Overall, bees performed more orientation and foraging flight after having followed more dances. The effect is stronger on foraging flight than on orientation flight. The numbers and direction of the waggle runs convey the vector information of distance and direction of food sources, respectively. Dances play an important role in motivating bees to perform both orientation and foraging flights. This was reflected by the numbers of dances bees followed, variations and dispersions of the distances and directions indicated in the dances. However, the motivational impacts of dance followings on the orientation and foraging flights differed. Overall, bees had more orientation and foraging flights with more dance followings, however, bees relied more on the dances in the foraging flights than in the orientation flights. The orientation flight rate was mainly influenced by the directions encoded in the dances, while the frequency of foraging flight relied on both the direction and distance codes. Higher variation of the directions indicated in the dances motivated bees to to perform more orientation flights while higher variation of both the direction and distance codes reduced rates of foraging flight. These opposing results could be explained by the bees' different motivations to perform orientation and foraging flights. Orientation flights help bees to acquire and establish knowledge about the sun compass and landscape features around the hive. Although bees need to be quite familiar with the sun compass system and calibrated the odometer calibrated before they can precisely decode the dance message, it is quite likely that they are able to apply innate estimates of vector decoding allowing them not only to safely guide their homing but also read basic features of potential food source availability (Menzel 2019). Thus, the variability of the direction code may indicate to the recruits a wide distribution of food sources and motivate them to perform more and further ranges of orientation flights. Foraging flights are directed to an indicated location. In my study, most of the foraging flights were directed to **FN** and a few were toward natural pollen food sources. All the foraging flight directing to **FN** was not successful since none of the bees landed at **FN**. Bees returning from an unsuccessful foraging flight may seek for further and possibly more reliable information. For another aspect, the influence of colony size on the benefits of dance communication was also discussed (Dornhaus et al. 2006, Schürch & Grüter 2014). In my experiment, the

colony size was tiny, the numbers of foragers were limited to a small group and the artificial feeder in the north **FN** was the main food source around natural environment. Bees thus may rely more strongly on the accurate dance information encoding the most favorite food source **FN** and spend less efforts in explore new but less favorite natural food sources. Although dances motivated bees to perform both orientation and foraging flights, bees dependent less on the dances as they had performed more orientation and foraging flights.

Additionally, the correlations between the accuracies of direction indicated in the dances and bees' motivation in extending flight distances in the vector, searching and homing flights were studied. However, no correlations were found. There was also no correlation between the accuracies of direction in the vector flights and bees' motivation to extending the flight distances in the search and homing flights. These results indicated that direction and distance were two independent vector systems. Except for a motivational role, dances served as a vector component in instructing bees to fly in certain directions and distances. The analysis revealed that the average directions and distances indicated in the dances that bees followed within 10 min before the current flight and after the former flight was more important for guiding bees to fly in the certain directions and distances during the long-distance orientation flights, although the differences between the maximum distances of orientation flight and that indicated in the dances remained large. However, the variations of vector information conveyed in the dances that bees followed during this time interval did not influence bees' tendencies to make use of the vector information in the dances for their flights, compared to the influences of the corresponding parameters in all the dances bees followed before the flight on bees' motivation to fly.

Compared to orientation flight, foraging flights concerned with a trade-off between waiting cost of following dances, locating target food sources (Dechaume-Moncharmont et al. 2005)) and yielding food sources. For one aspect, dances communicated the vector information of profitable food sources within a certain degree of errors. (Okada et al. 2014) suggested that precise information (0 ~ 5° errors) yielded great success in finding target foods, but decreased possibilities to find new food source, while errors ranging at 10 ~ 15° maintained a successful foraging trade-off. Some former studies also supported the benefits of spatial information in the dances depended on the spatiotemporal distribution of food sources that when food sources were at low densities (Beekman & Lew 2008,

Dechaume-Moncharmont et al. 2005, Schürch & Grüter 2014) or when food sources were stable (Schürch & Grüter 2014). In my studies, as the ranges of standard deviation and error of the direction were within ± 12° and ± 5°, respectively, there were not strong deviation between the preferred flight direction and the direction indicated in the dances. However, when the variation and error beyond these range, bees did not follow. This result indicated that honeybees could evaluate the values of dance information and determine whether to use their own experience to forage. My current results revealed that bees could average the vector information from the dances to guide their flights. However, I also addressed the question whether bees depended on the dances which were the most recently followed rather than average the vector information of multiple dances were further investigated. However, this hypothesis can not be confirmed due to the limited cases that only two orientation flights were performed after bees followed several dances indicating multiple directions, although these two flights showed that bees tended to fly toward the directions indicated in the dances with lower variation that they followed more (Figure 41, 216A). To further confirm this hypothesis, more data are required in future work. The flows of social information flow within a community were not evenly distributed among individuals. Bees' thresholds of transmitting and accessing to social information varied considerably (Grueter et al. 2016, Heyes 2012, Lotem & Halpern 2012). This raises questions on whether and how less informed and better informed bees via waggle dances behave differently in the outdoor performance. However, the mismatch of the five focal bees between the accuracies of vector information encoded in the dances and the regression distances of vector and homing flights and the maximum distance of search flight (Figure 46, 47) indicates that each bee's ability to decode and calibrate dance information is individually different. However, more data are required to enrich this observation.

The descriptive comparison between the direction/distance of former dance followings and flights, and the corresponding direction and distance of current flights indicates the influence of former experience of dance following and orientation/foraging flights on the future flight performance, however, this impact is still individually different. Except for physical reasons, what to explain the cases that bees 257, 272 and 282 did not perform any successful flight was that they may either followed the dances over 3 hours before the appearance at the hive entrance or they did not followed

dances. These results also further supported that bees rely on the vector information of the dances followed within 10 min before the flight.

Together, this study overcome the shortcomings of former studies and provide the first study to investigate how bees evaluate and utilize the dance information effectively to the orientation and foraging flights.

6.6 Reference

Ai, H., Kobayashi, Y., Matake, T., Takahashi, S., Hashimoto, K., Maeda, S., & Tsuruta, N. (2017). Development of honeybee waggle dance and its differences between recruits and scouts. *bioRxiv*. doi:https://doi.org/10.1101/179408

Al Toufailia, H., Couvillon, M. J., Ratnieks, F. L., & Grüter, C. (2013). Honey bee waggle dance communication: signal meaning and signal noise affect dance follower behaviour. *Behavioral Ecology and Sociobiology, 67*(4), 549-556.

Beekman, M., & Lew, J. B. (2008). Foraging in honeybees—when does it pay to dance? *Behavioral Ecology, 19*(2), 255-261.

Biesmeijer, J. C., & Seeley, T. D. (2005). The use of waggle dance information by honey bees throughout their foraging careers. *Behavioral Ecology and Sociobiology, 59*(1), 133-142.

Bracis, C., Gurarie, E., Van Moorter, B., & Goodwin, R. A. (2015). Memory effects on movement behavior in animal foraging. *PLoS One, 10*(8), e0136057.

Couvillon, M. J., Riddell Pearce, F. C., Harris-Jones, E. L., Kuepfer, A. M., Mackenzie-Smith, S. J., Rozario, L. A., ... Ratnieks, F. L. (2012). Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding. *Biology open, 1*(5), 467-472.

Dawson, E. H., Avarguès-Weber, A., Chittka, L., & Leadbeater, E. (2013). Learning by observation emerges from simple associations in an insect model. *Current Biology, 23*(8), 727-730.

De Marco, R. J., Gurevitz, J. M., & Menzel, R. (2008). Variability in the encoding of spatial information by dancing bees. *Journal of Experimental Biology, 211*(10), 1635-1644.

Dechaume-Moncharmont, F. X., Dornhaus, A., Houston, A. I., McNamara, J. M., Collins, E. J., & Franks, N. R. (2005). The hidden cost of information in collective foraging. *Proceedings of the Royal Society B: Biological Sciences, 272*(1573), 1689-1695.

Dornhaus, A., Klügl, F., Oechslein, C., Puppe, F., & Chittka, L. (2006). Benefits of recruitment in honey bees: effects of ecology and colony size in an individual-based model. *Behavioral Ecology, 17*(2), 336-344.

Dukas, R. (2008). Life history of learning: performance curves of honeybees in the wild. *Ethology, 114*(12), 1195-1200.

Dyer, F. C. (2002). The biology of the dance language. *Annual review of entomology, 47*(1), 917-949.

Farina, W. M., Grüter, C., & Arenas, A. (2012). Olfactory information transfer during recruitment in honey bees. *Honeybee neurobiology and behavior* (pp. 89-101): Springer.

Gilley, D. C., Miller, N., Courtright, T. J., & Thom, C. (2018). Behavioral Activity of Hydrocarbons Emitted by Honeybee Waggle Dancers. *Journal of Insect Behavior, 31*(1), 83-90.

Grueter, C., & Keller, L. (2016). nter-caste communication in social insects. *Current Opinion in Neurobiology, 38*, 6-11.

Heyes, C. (2012). What's social about social learning? *Journal of Comparative Psychology,, 126*(2), 193.

von Frisch K. (1965). *Tanzsprache und Orientierung der Biene*: Springer.

von Frisch K. (1967). *Dance language and orientation of bees*. Cambridge, MA.: Harvard University Press.

Klein, S., Pasquaretta, C., He, X. J., Perry, C., Søvik, E., Devaud, J. M., ...Lihoreau, M. (2019). Honey bees increase their foraging performance and frequency of pollen trips through experience. *Scientific reports, 9*(1), 1-10.

Lotem, A., & Halpern, J. Y. (2012). Coevolution of learning and data-acquisition mechanisms: A model for cognitive evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences,, 367*(1603), 2686-2694.

Menzel, R. (2019). The Waggle Dance as an Intended Flight: A Cognitive Perspective. *Insects, 10*(12), 424.

Menzel, R., Fuchs, J., Kirbach, A., Lehmann, K., & Greggers, U. (2012). Navigation and communication in honey bees. In *Honeybee Neurobiology and Behavior* (pp. 103-116). Dordrecht: Springer.

Menzel, R., Kirbach, A., Haass, W. D., Fischer, B., Fuchs, J., Koblofsky, M., ...Greggers, U. (2011). A common frame of reference for learned and communicated vectors in honeybee navigation. *Current Biology, 21*(8), 645-650.

Menzel, R., Manz, G., Menzel, R., & Greggers, U. (2001). Massed and spaced learning in honeybees: the role of CS, US, the intertrial interval, and the test interva. *Learning & memory, 8*(4), 198-208.

Okada, R., Ikeno, H., Kimura, T., Ohashi, M., Aonuma, H., & Ito, E. (2014). Error in the honeybee waggle dance improves foraging flexibility. *Scientific reports, 4*(1), 1-9.

Preece, K., & Beekman, M. (2014). Honeybee waggle dance error: adaption or constraint? Unravelling the complex dance language of honeybees. *Animal Behaviour, 94*, 19-26.

Price, R. A., Dulex, N., Vial, N., Vincent, C., & Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. *Science advances, 5*(2), eaat0450.

Riley, J. R., Greggers, U., Smith, A. D., Reynolds, D. R., & Menzel, R. (2005). The flight paths of honeybees recruited by the waggle dance. *Nature, 435*(7039), 205-207.

Schippers, M. P., Dukas, R., Smith, R. W., Wang, J., Smolen, K., & McClelland, G. B. (2006). Lifetime performance in foraging honeybees: behaviour and physiology. *Journal of Experimental Biology, 209*(19), 3828-3836.

Schürch, R., & Grüter, C. (2014). Dancing bees improve colony foraging success as long-term benefits outweigh short-term costs. *PLoS One, 9*(8), e104660.

Schürch, R., Ratnieks, F. L., Samuelson, E. E., & Couvillon, M. J. (2016). Dancing to her own beat: honey bee foragers communicate via individually calibrated waggle dances. *Journal of Experimental Biology, 219*(9), 1287-1289.

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, 186*(9), 813-819.

Tanner, D. A., & Visscher, P. K. (2008). Do honey bees average directions in the waggle dance to determine a flight direction? *Behavioral Ecology and Sociobiology, 62*(12), 1891-1898.

Tanner, D. A., & Visscher, P. K. (2010). Does imprecision in the waggle dance fit patterns predicted by the tuned-error hypothesis? *Journal of Insect Behavior, 23*(3), 180-188.

Tanner, D. A., & Visscher P. K. (2010). Adaptation or constraint? Reference-dependent scatter in honey bee dances. *Behavioral Ecology and Sociobiology, 64*(7), 1081-1086.

Thom, C., Gilley, D. C., Hooper, J., & Esch, H. E. (2007). The scent of the waggle dance. PLoS biology, 5(9), e228. *PLoS biology, 5*(9), e228.

Towne, W. F., & Gould, J. L. (1988). The spatial precision of the honey bees' dance communication. *Journal of Insect Behavior, 1*(2), 129-155.

WeidenmuÈller, A., & Seeley, T. D. (1999). Imprecision in waggle dances of the honeybee (*Apis mellifera*) for nearby food sources: error or adaptation? *Behavioral Ecology and Sociobiology, 46*(3), 190-199.

Wray, M. K., Klein, B. A., & Seeley, T. D. (2012). Honey bees use social information in waggle dances more fully when foraging errors are more costly. *Behavioral Ecology, 23*(1), 125-131.

7 Supplementary material

S1. Appendix. Model diagnostics

Diagnostic tools provided by the DHARMa package in R (Hartig, 2018) were used to evaluate the model fits. DHARMa simulates quantile residuals from a fitted (G)LMM that are standardized to values between 0 and 1. For a correctly specified model, these residuals should have a uniform distribution regardless of the underlying model structure. The package includes statistical tests on the residuals to check for zero inflation, dispersion, outliers and uniformity. Details are available from https://cran.r-project.org/web/packages/DHARMa/index.html.

Table S1 Diagnostics for models on the influence of non-social factors on bees' behavior of leaving the colony to hive exit

Model	ZeroInflation	Dispersion	Outliers	Uniformity
Out ~ (1|BeeID)	0.85	0.85	0.16	0.76
Out ~ (Age|BeeID)	0.26	0.27	0.02	0.28
Out ~ (Time|BeeID)	< 0.01	< 0.01	0.64	< 0.01
Out ~ Age + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (Age|BeeID)	0.06	0.07	< 0.01	0.26
Out ~ Age + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (1|BeeID)	0.26	0.40	< 0.01	0.51
Out ~ Age + Temp + Wind + Empty cell + Worker + Time + (Age | BeeID)	0.20	0.26	0.06	0.68
Out ~ Age + Temp*Worker + Wind + Empty cell + Time + (Age | BeeID)	0.17	0.23	0.04	0.68

p-value calculated for each model was shown in the tables. *p*-value < 0.01 for 'ZeroInflation' and 'Dispersion' tests showed strong evidence of zero-inflation and over-dispersion in the residuals, respectively *p*-value < 0.01 for 'Outlier' showed strong evidence for data that outside of the range of simulated values; *p*-value < 0.01 for 'Uniformity' showed a lack of fit with the overall distribution failed to conform to expectation.

Table S2 Diagnostics for models on the influence of non-social factors on bees' switch behavior between 'LR' and 'OW'

Model	ZeroInflation	Dispersion	Outliers	Uniformity
Out_$_{LR/OW}$ ~ (1 | BeeID)	0.73	0.74	1	0.76
Out_$_{LR/OW}$ ~ (Age | BeeID)	0.82	0.84	1	0.73
Out_$_{LR/OW}$ ~ (1 | BeeID/Age)	0.62	0.62	1	0.83
Out_$_{LR/OW}$ ~ (NoOut | BeeID)	0.58	0.59	1	0.47
Out_$_{LR/OW}$ ~ (1 | BeeID/NoOut)	0.70	0.71	1	0.73
Out_$_{LR/OW}$~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + Time + (1 | BeeID)	0.65	0.62	1	0.92
Out_$_{LR/OW}$~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + (1 | BeeID)	0.66	0.61	1	0.83
Out_ $_{LR/OW}$~Temp + Empty cell + Worker + Age + NoOut + (1 | BeeID)	0.53	0.08	1	0.53

Table S3 Diagnostics for models on the influence of non-social factors on bees' switch behavior between 'OW' and 'FL'

Model	ZeroInflation	Dispersion	Outliers	Uniformity
Out_ OW-FL ~ (1 | BeeID)	0.22	0.22	1	< 0.01
Out_ OW-FL ~ (Age | BeeID)	0.92	0.92	1	0.82
Out_ OW-FL~ (1 | BeeID/Age)	0.22	0.22	1	0.03
Out_OW-FL ~ (NoOut | BeeID)	0.90	0.90	1	0.74
Out_ow ~ (1 | BeeID/NoOut)	0.23	0.23	1	0.07
Out_ OW-FL~Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Age + NoOut + Time + (1 | BeeID)	0.41	0.31	1	0.11
Out_ OW-FL~Wind + Worker + Age + (1 | BeeID)	0.45	0.35	1	0.23

Table S4 Diagnostics for models on the influence of non-social factors on bees' switch behavior between orientation flight and foraging flight

Model	ZeroInflation	Dispersion	Outliers	Uniformity
Flight ~ (1 | BeeID)	0.16	0.16	1	< 0.01
Flight ~ (Age | BeeID)	0.18	0.15	1	< 0.01
Flight ~ (NoF | BeeID)	0.14	0.14	1	< 0.01
Flight ~ (1| BeeID/Age)	0.21	0.20	1	< 0.01
Flight ~ (1| BeeID/NoF)	0.18	0.18	1	< 0.01
Flight ~ NoF + Temp + Wind + Weather + Brood + Honey cell + Empty cell + Worker + Time + (1 | BeeID/Age)	0.57	0.98	1	0.82
Flight ~ NoF + Temp + Honey cell + Worker + (1 | BeeID/Age)	0.66	0.66	1	0.92
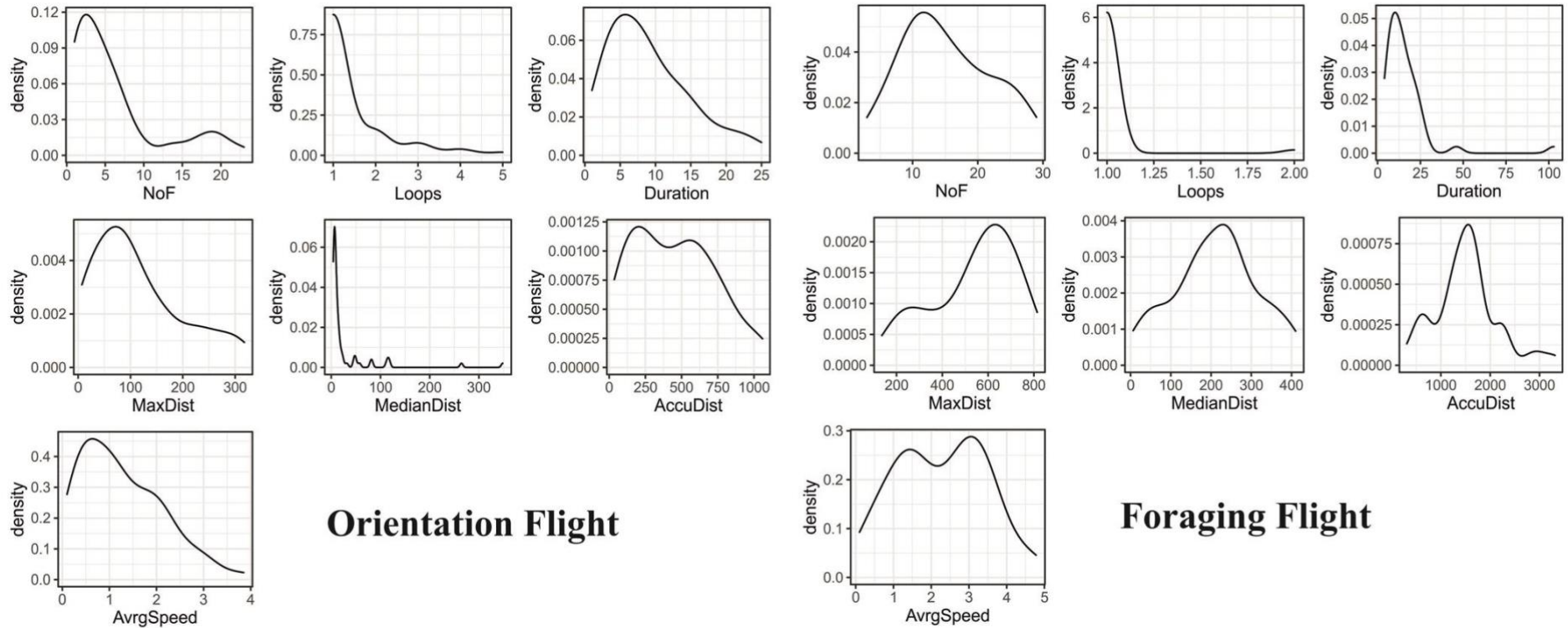
Figure S1. Density of parameters of flight performance within orientation and foraging flights. 'NoF': numbers of flights, 'Loops': numbers of loops within a single flight, 'Duration': flight duration (min) in a single flight, 'MaxDist' (m): maximum distance of a single flight relative to hive, 'MedianDist' (m): median distance of a single flight relative to hive, 'AccuDist'(m): accumulated distance of a single flight, 'AvrgSpeed'(m/s): average flight speed
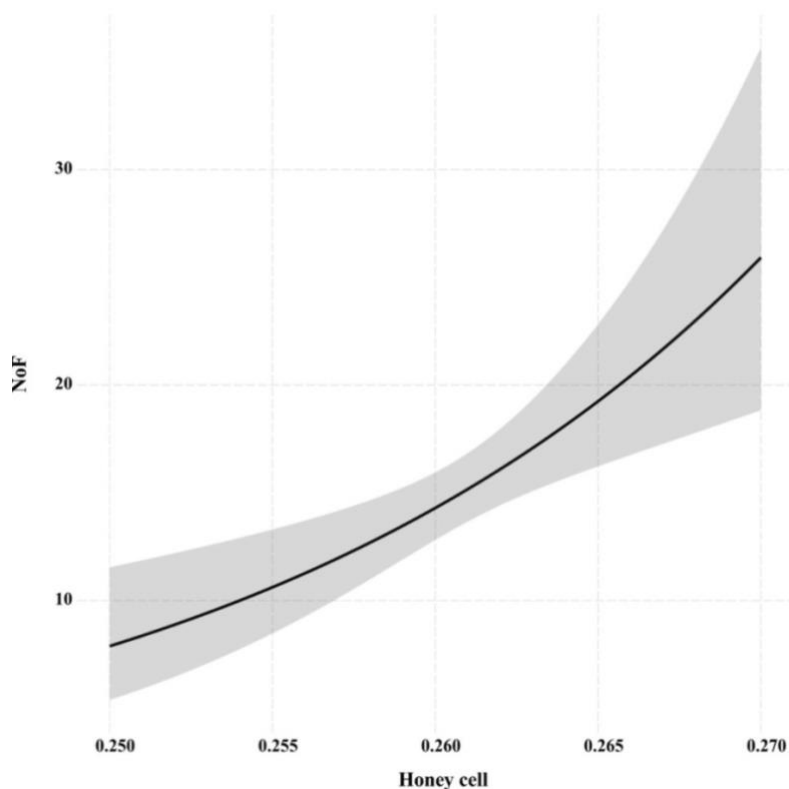
Figure S2. Predicted probabilities of frequency of trips during foraging flight against the number of honey cells
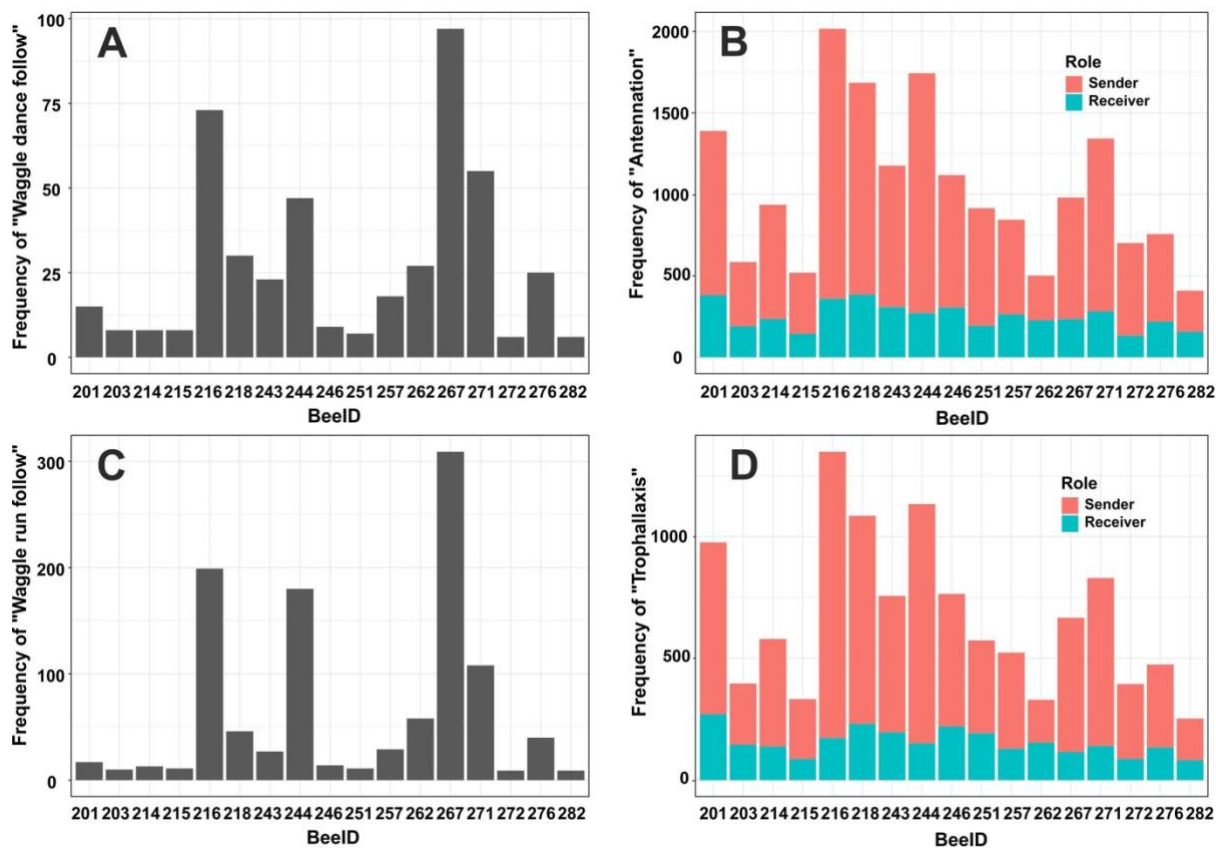
Figure S3. Frequency of indoor behaviors 'Waggle dance following', 'Waggle run follow', 'Antennation' and 'Trophallaxis' among 17 focal bees over their life spans.
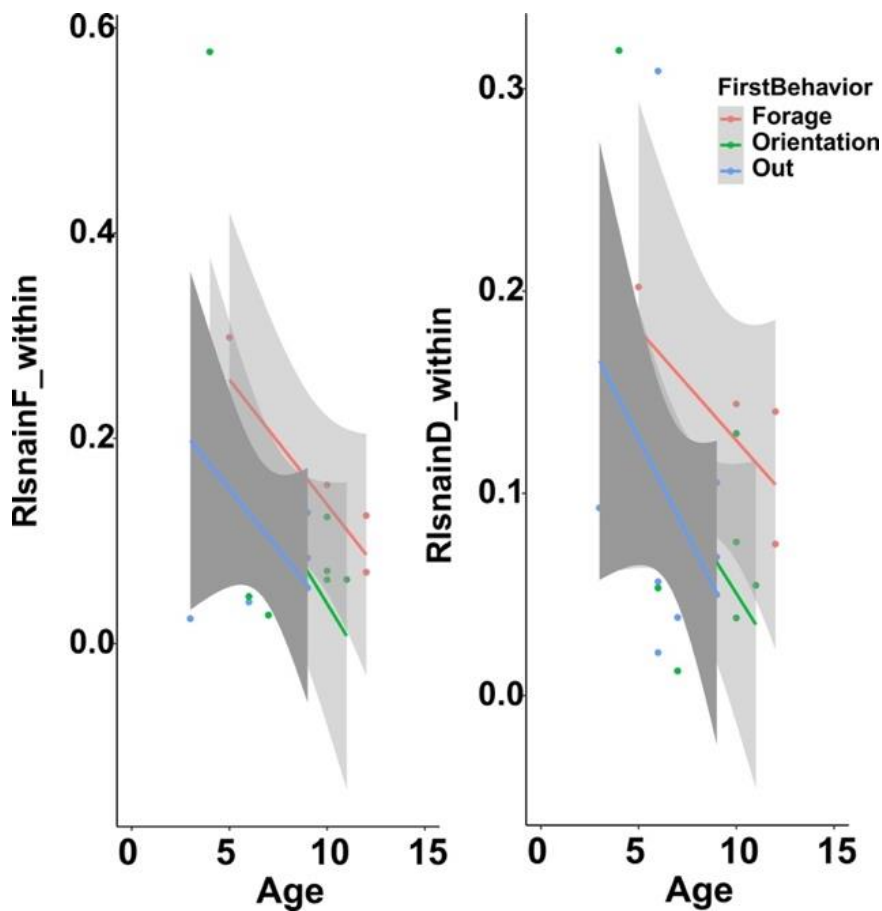
Figure S4. Relative intensity of 'sna' in both frequency and duration on the days when bees initiated different outdoor behaviors. 'RIsnainF_within' and 'RIsnainD_within' denote the relative intensity of 'sna' in frequency and duration at within-individual levels.

Table S5a. Kruskal-Wallis test on the count and duration of indoor behaviors among 17 focal bees

	Waggle run follow		sna		Qinterest	
	Chi-sq statistic	*p*-value	*Chi-sq* statistic	*p*-value	*Chi-sq* statistic	*p*-value
Count	47.28	< 0.01	66.01	<0.01	49.98	< 0.01
Duration	\	\	30.6	<0.05	29.86	< 0.05

Table S5b. Tunn test on the count and duration of indoor behaviors among 17 focal bees

Waggle run follow_count																	
BeeID	201	203	214	215	216	218	243	244	246	251	257	262	267	271	272	276	282
201	1.00	0.77	0.01	0.66	0.01	0.20	0.66	0.00	0.29	0.34	0.27	0.75	0.00	0.01	0.03	0.17	0.02
203	0.77	1.00	0.10	0.90	0.01	0.19	0.94	0.02	0.56	0.62	0.53	0.61	0.04	0.16	0.13	0.39	0.10
214	0.01	0.10	1.00	0.13	0.00	0.00	0.05	0.00	0.19	0.17	0.23	0.04	0.00	0.00	0.92	0.38	0.88
215	0.66	0.90	0.13	1.00	0.01	0.14	0.94	0.05	0.67	0.72	0.63	0.53	0.25	0.12	0.17	0.47	0.13
216	0.01	0.01	0.00	0.01	1.00	0.04	0.00	0.42	0.00	0.00	0.00	0.11	0.12	0.25	0.00	0.00	0.00
218	0.20	0.19	0.00	0.14	0.04	1.00	0.09	0.00	0.02	0.03	0.02	0.56	0.02	0.84	0.00	0.01	0.00
243	0.66	0.94	0.05	0.94	0.00	0.09	1.00	0.00	0.05	0.61	0.51	0.02	0.00	0.08	0.09	0.35	0.07
244	0.00	0.02	0.00	0.05	0.42	0.00	0.00	1.00	0.00	0.01	0.00	0.31	0.43	0.71	0.00	0.00	0.00
246	0.29	0.56	0.19	0.67	0.00	0.02	0.05	0.00	1.00	0.93	0.95	0.28	0.07	0.02	0.27	0.73	0.20
251	0.34	0.62	0.17	0.72	0.00	0.03	0.61	0.01	0.93	1.00	0.88	0.31	0.08	0.03	0.24	0.67	0.18
257	0.27	0.53	0.23	0.63	0.00	0.02	0.51	0.00	0.95	0.88	1.00	0.26	0.06	0.02	0.31	0.78	0.23
262	0.75	0.61	0.04	0.53	0.11	0.56	0.02	0.31	0.28	0.31	0.26	1.00	0.71	0.48	0.06	0.19	0.04
267	0.00	0.04	0.00	0.25	0.12	0.02	0.00	0.43	0.07	0.08	0.06	0.71	1.00	0.68	0.01	0.04	0.00
271	0.01	0.16	0.00	0.12	0.25	0.84	0.08	0.71	0.02	0.03	0.02	0.48	0.68	1.00	0.00	0.01	0.00
272	0.03	0.13	0.92	0.17	0.00	0.00	0.09	0.00	0.27	0.24	0.31	0.06	0.01	0.00	1.00	0.47	0.81
276	0.17	0.39	0.38	0.47	0.00	0.01	0.35	0.00	0.73	0.67	0.78	0.19	0.04	0.01	0.47	1.00	0.35
282	0.02	0.10	0.88	0.13	0.00	0.00	0.07	0.00	0.20	0.18	0.23	0.04	0.00	0.00	0.81	0.35	1.00

Color in dark red and light red indicated the p-value < 0.01 and p-value < 0.05 respectively.

Table S5b. Tunn test on the count and duration of indoor behaviors among 17 focal bees

sna_count

BeeID	201	203	214	215	216	218	243	244	246	251	257	262	267	271	272	276	282
201	1.00	0.77	0.01	0.66	0.01	0.20	0.66	0.03	0.29	0.34	0.27	0.75	0.37	0.17	0.03	0.17	0.02
203	0.77	1.00	0.04	0.90	0.01	0.19	0.94	0.05	0.56	0.62	0.53	0.61	0.31	0.16	0.13	0.39	0.10
214	0.01	0.04	1.00	0.13	0.00	0.00	0.02	0.00	0.19	0.17	0.23	0.04	0.00	0.00	0.92	0.38	0.88
215	0.66	0.90	0.13	1.00	0.01	0.14	0.94	0.05	0.67	0.72	0.63	0.53	0.25	0.12	0.17	0.47	0.13
216	0.01	0.01	0.00	0.01	1.00	0.01	0.00	0.42	0.00	0.00	0.00	0.04	0.04	0.25	0.00	0.00	0.00
218	0.20	0.19	0.00	0.14	0.01	1.00	0.09	0.54	0.02	0.03	0.02	0.56	0.80	0.84	0.00	0.01	0.00
243	0.66	0.94	0.02	0.94	0.00	0.09	1.00	0.03	0.55	0.61	0.51	0.53	0.20	0.08	0.09	0.35	0.07
244	0.03	0.05	0.00	0.05	0.42	0.54	0.03	1.00	0.00	0.01	0.00	0.31	0.43	0.71	0.00	0.00	0.00
246	0.29	0.56	0.19	0.67	0.00	0.02	0.55	0.00	1.00	0.93	0.95	0.28	0.07	0.02	0.27	0.73	0.20
251	0.34	0.62	0.17	0.72	0.00	0.03	0.61	0.01	0.93	1.00	0.88	0.31	0.02	0.03	0.24	0.67	0.18
257	0.27	0.53	0.23	0.63	0.00	0.02	0.51	0.00	0.95	0.88	1.00	0.26	0.06	0.02	0.31	0.78	0.23
262	0.75	0.61	0.04	0.53	0.04	0.56	0.53	0.31	0.28	0.31	0.26	1.00	0.71	0.48	0.06	0.19	0.04
267	0.37	0.31	0.00	0.25	0.04	0.80	0.20	0.43	0.07	0.02	0.06	0.71	1.00	0.68	0.01	0.04	0.00
271	0.17	0.16	0.00	0.12	0.25	0.84	0.08	0.71	0.02	0.03	0.02	0.48	0.68	1.00	0.00	0.01	0.00
272	0.03	0.13	0.92	0.17	0.00	0.00	0.09	0.00	0.27	0.24	0.31	0.06	0.01	0.00	1.00	0.47	0.81
276	0.17	0.39	0.38	0.47	0.00	0.01	0.35	0.00	0.73	0.67	0.78	0.19	0.04	0.01	0.47	1.00	0.35
282	0.02	0.10	0.88	0.13	0.00	0.00	0.07	0.00	0.20	0.18	0.23	0.04	0.00	0.00	0.81	0.35	1.00

Table S5b. Tunn test on the count and duration of indoor behaviors among 17 focal bees

Qinterest_count

BeeID	201	203	214	215	216	218	243	244	246	251	257	262	267	271	272	276	282
201	1.00	0.04	0.00	0.26	0.01	0.05	0.02	0.39	0.25	0.54	0.30	0.48	0.75	0.39	0.01	0.08	0.27
203	0.04	1.00	0.78	0.02	0.00	0.65	0.90	0.40	0.36	0.03	0.38	0.05	0.07	0.33	0.88	0.74	0.02
214	0.00	0.78	1.00	0.00	0.00	0.31	0.86	0.18	0.10	0.00	0.14	0.01	0.01	0.11	0.88	0.44	0.00
215	0.26	0.02	0.00	1.00	0.87	0.02	0.01	0.10	0.06	0.53	0.07	0.73	0.45	0.09	0.01	0.02	0.92
216	0.01	0.00	0.00	0.87	1.00	0.00	0.00	0.02	0.00	0.30	0.01	0.57	0.26	0.02	0.00	0.00	0.76
218	0.05	0.65	0.31	0.02	0.00	1.00	0.48	0.57	0.52	0.02	0.55	0.05	0.08	0.47	0.45	0.90	0.01
243	0.02	0.90	0.86	0.01	0.00	0.48	1.00	0.27	0.20	0.01	0.24	0.02	0.03	0.20	0.98	0.60	0.00
244	0.39	0.40	0.18	0.10	0.02	0.57	0.27	1.00	0.96	0.19	0.97	0.20	0.32	0.94	0.25	0.53	0.10
246	0.25	0.36	0.10	0.06	0.00	0.52	0.20	0.96	1.00	0.10	1.00	0.14	0.22	0.88	0.18	0.48	0.05
251	0.54	0.03	0.00	0.53	0.30	0.02	0.01	0.19	0.10	1.00	0.13	0.82	0.84	0.19	0.01	0.03	0.58
257	0.30	0.38	0.14	0.07	0.01	0.55	0.24	0.97	1.00	0.13	1.00	0.16	0.26	0.89	0.22	0.52	0.07
262	0.48	0.05	0.01	0.73	0.57	0.05	0.02	0.20	0.14	0.82	0.16	1.00	0.71	0.20	0.02	0.06	0.79
267	0.75	0.07	0.01	0.45	0.26	0.08	0.03	0.32	0.22	0.84	0.26	0.71	1.00	0.32	0.02	0.09	0.49
271	0.39	0.33	0.11	0.09	0.02	0.47	0.20	0.94	0.88	0.19	0.89	0.20	0.32	1.00	0.18	0.45	0.09
272	0.01	0.88	0.88	0.01	0.00	0.45	0.98	0.25	0.18	0.01	0.22	0.02	0.02	0.18	1.00	0.57	0.00
276	0.08	0.74	0.44	0.02	0.00	0.90	0.60	0.53	0.48	0.03	0.52	0.06	0.09	0.45	0.57	1.00	0.02
282	0.27	0.02	0.00	0.92	0.76	0.01	0.00	0.10	0.05	0.58	0.07	0.79	0.49	0.09	0.00	0.02	1.00

Table S5b. Tunn test on the count and duration of indoor behaviors among 17 focal bees

sna_duration

BeeID	201	203	214	215	216	218	243	244	246	251	257	262	267	271	272	276	282
201	1.00	0.27	0.48	0.92	0.04	0.15	0.89	0.36	0.24	0.94	0.41	0.61	0.05	0.11	0.29	0.66	0.50
203	0.27	1.00	0.60	0.54	0.01	0.05	0.35	0.10	0.70	0.38	1.00	0.20	0.03	0.03	0.92	0.54	0.71
214	0.48	0.60	1.00	0.53	0.00	0.03	0.47	0.14	0.70	0.41	0.76	0.31	0.01	0.03	0.71	0.81	0.97
215	0.92	0.54	0.53	1.00	0.06	0.41	0.88	0.39	0.39	0.80	0.90	0.53	0.13	0.29	0.39	0.73	0.58
216	0.04	0.01	0.00	0.06	1.00	0.31	0.03	0.48	0.01	0.01	0.01	0.25	0.88	0.64	0.00	0.02	0.01
218	0.15	0.05	0.03	0.41	0.31	1.00	0.18	0.83	0.03	0.14	0.10	0.80	0.53	0.61	0.03	0.12	0.08
243	0.89	0.35	0.47	0.88	0.03	0.18	1.00	0.34	0.36	0.98	0.59	0.57	0.08	0.13	0.36	0.75	0.58
244	0.36	0.10	0.14	0.39	0.48	0.83	0.34	1.00	0.13	0.27	0.10	0.85	0.60	0.69	0.05	0.18	0.13
246	0.24	0.70	0.70	0.39	0.01	0.03	0.36	0.13	1.00	0.32	0.85	0.18	0.01	0.02	0.89	0.64	0.86
251	0.94	0.38	0.41	0.80	0.01	0.14	0.98	0.27	0.32	1.00	0.93	0.38	0.04	0.12	0.41	0.82	0.64
257	0.41	1.00	0.76	0.90	0.01	0.10	0.59	0.10	0.85	0.93	1.00	0.28	0.04	0.03	0.76	0.77	0.98
262	0.61	0.20	0.31	0.53	0.25	0.80	0.57	0.85	0.18	0.38	0.28	1.00	0.51	0.58	0.17	0.36	0.28
267	0.05	0.03	0.01	0.13	0.88	0.53	0.08	0.60	0.01	0.04	0.04	0.51	1.00	0.86	0.01	0.04	0.03
271	0.11	0.03	0.03	0.29	0.64	0.61	0.13	0.69	0.02	0.12	0.03	0.58	0.86	1.00	0.02	0.07	0.05
272	0.29	0.92	0.71	0.39	0.00	0.03	0.36	0.05	0.89	0.41	0.76	0.17	0.01	0.02	1.00	0.56	0.77
276	0.66	0.54	0.81	0.73	0.02	0.12	0.75	0.18	0.64	0.82	0.77	0.36	0.04	0.07	0.56	1.00	0.80
282	0.50	0.71	0.97	0.58	0.01	0.08	0.58	0.13	0.86	0.64	0.98	0.28	0.03	0.05	0.77	0.80	1.00

Table S5b. Tunn test on the count and duration of indoor behaviors among 17 focal bees

Qinterest_duration

BeeID	201	203	214	215	216	218	243	244	246	251	257	262	267	271	272	276	282
201	1.00	0.58	0.34	0.02	0.58	0.48	0.36	0.38	0.15	0.05	0.89	0.29	0.80	0.66	0.35	0.63	0.61
203	0.58	1.00	0.99	0.02	0.46	0.82	0.90	0.84	0.78	0.04	0.48	0.17	0.73	0.44	0.92	0.82	0.55
214	0.34	0.99	1.00	0.00	0.05	0.32	1.00	0.40	0.81	0.00	0.23	0.06	0.51	0.04	0.90	0.57	0.31
215	0.02	0.02	0.00	1.00	0.14	0.00	0.00	0.02	0.01	0.46	0.04	0.33	0.03	0.11	0.00	0.01	0.73
216	0.58	0.46	0.05	0.14	1.00	0.28	0.13	0.13	0.04	0.23	0.63	0.59	0.44	0.89	0.13	0.29	0.76
218	0.48	0.82	0.32	0.00	0.28	1.00	0.48	0.34	0.17	0.02	0.59	0.18	0.92	0.19	0.60	0.94	0.39
243	0.36	0.90	1.00	0.00	0.13	0.48	1.00	0.74	0.82	0.01	0.28	0.08	0.54	0.11	0.90	0.61	0.36
244	0.38	0.84	0.40	0.02	0.13	0.34	0.74	1.00	0.78	0.01	0.20	0.06	0.37	0.19	0.62	0.42	0.42
246	0.15	0.78	0.81	0.01	0.04	0.17	0.82	0.78	1.00	0.00	0.15	0.04	0.37	0.03	0.67	0.40	0.17
251	0.05	0.04	0.00	0.46	0.23	0.02	0.01	0.01	0.00	1.00	0.10	0.69	0.07	0.23	0.01	0.03	0.69
257	0.89	0.48	0.23	0.04	0.63	0.59	0.28	0.20	0.15	0.10	1.00	0.37	0.73	0.85	0.33	0.57	0.70
262	0.29	0.17	0.06	0.33	0.59	0.18	0.08	0.06	0.04	0.69	0.37	1.00	0.27	0.81	0.10	0.18	1.00
267	0.80	0.73	0.51	0.03	0.44	0.92	0.54	0.37	0.37	0.07	0.73	0.27	1.00	0.35	0.61	0.87	0.71
271	0.66	0.44	0.04	0.11	0.89	0.19	0.11	0.19	0.03	0.23	0.85	0.81	0.35	1.00	0.13	0.27	0.94
272	0.35	0.92	0.90	0.00	0.13	0.60	0.90	0.62	0.67	0.01	0.33	0.10	0.61	0.13	1.00	0.69	0.37
276	0.63	0.82	0.57	0.01	0.29	0.94	0.61	0.42	0.40	0.03	0.57	0.18	0.87	0.27	0.69	1.00	0.70
282	0.61	0.55	0.31	0.73	0.76	0.39	0.36	0.42	0.17	0.69	0.70	1.00	0.71	0.94	0.37	0.70	1.00

Table S6. Metrics for principal component analysis on outdoor behavioral states among 17 focal bees

BeeID	Out	LR	OW	FR	F	O	FL
201	0.47	0.00	0.00	0.00	0.00	0.47	0.47
203	0.14	0.00	0.00	0.00	0.00	0.14	0.14
214	0.33	0.07	0.07	0.00	0.00	0.20	0.20
215	0.29	0.14	0.00	0.00	0.00	0.14	0.14
216	2.79	0.43	0.14	0.29	0.86	1.07	1.93
218	0.20	0.00	0.00	0.00	0.00	0.20	0.20
243	0.62	0.08	0.15	0.00	0.00	0.38	0.38
244	2.92	0.46	0.00	0.08	1.15	0.92	2.08
246	0.23	0.15	0.00	0.00	0.00	0.08	0.08
251	0.08	0.00	0.00	0.00	0.00	0.08	0.08
257	1.00	0.00	1.00	0.00	0.00	0.00	0.00
262	1.40	0.00	0.00	0.00	0.20	1.20	1.40
267	2.36	0.27	0.09	0.09	1.36	0.55	1.91
271	1.36	0.18	0.00	0.00	0.64	0.55	1.18
272	0.36	0.00	0.36	0.00	0.00	0.00	0.00
276	0.45	0.09	0.00	0.00	0.00	0.36	0.36
282	1.22	0.00	1.11	0.00	0.00	0.00	0.00

The metrics include the mean count for each outdoor state. Each mean is calculated by the total counts of behavioral events divided by the lifespan of each bee. 'Out', bees left the comb to the hive exit, a state preparing for outdoor behaviors; 'LR', bees appeared at the inside of hive exit, but immediately returned into the inside colony; 'OW', bees rushed out to the outside of the hive exit , but did not fly up after caught, attached with radar transponders and

released; 'FR', bees flew up from the hive exit with radar transponders, but returned immediately to hive exit; 'FL', successful flight after catch-release paradigm procedure; 'FL' was differentiated into 'O' and 'F', representing orientation flight and foraging flight.

Table S7. Numbers of flights, dances and standard deviations and errors of dances

Bee	Date	Out	Flight	Or	For	D	Lifespan	Out_m	Flight_m	Or_m	For_m	D_m	sd	se
201		7	7	7	0	15	15	0.5	0.5	0.5	0.0	1.0	74	18
203		1	1	1	0	8	7	0.1	0.1	0.1	0.0	1.1	84	26
214	24.07	5	3	3	0	8	15	0.3	0.2	0.2	0.0	0.5	34	11
215		2	1	1	0	8	7	0.3	0.3	0.1	0.0	1.1	75	23
216		39	27	14	13	76	14	2.8	1.9	1.0	0.9	5.4	86	6.1
218		3	3	3	0	30	15	0.2	0.2	0.2	0.0	2.0	95	14
243		8	5	5	0	24	13	0.6	0.4	0.4	0.0	1.8	94	18
244	26.07	38	26	10	16	43	13	2.9	2.1	0.8	1.2	3.3	78	5.3
246		3	1	1	0	9	13	0.2	0.1	0.1	0.0	0.7	80	23
251		1	1	1	0	7	12	0.1	0.1	0.1	0.0	0.6	78	23
257	27.07	12	0	0	0	18	12	1.0	0.0	0.0	0.0	1.5	68	13
262		7	7	6	1	26	5	1.4	1.4	1.2	0.2	5.2	55	8.6
267		27	23	6	17	75	11	2.5	1.9	0.5	1.5	6.8	46	3.1
271	28.07	16	13	6	7	57	11	1.5	1.2	0.5	0.6	5.2	38	3.1
272		4	0	0	0	5	11	0.4	0.0	0.0	0.0	0.5	97	33
276		5	4	4	0	25	11	0.5	0.4	0.4	0.0	2.3	87	14
282	29.07	11	0	0	0	6	9	1.2	0.0	0.0	0.0	0.7	80	27

'Flight' includes both orientation flight and foraging flight. 'Or', 'For' and D' indicated orientation flights, foraging flights and dances, respectively. 'Flight_m', 'Or_m', 'For_m' and 'D_m' are calculated by the total numbers of flight, orientation flight, foraging flight and dance followings divided by the shortest lifespans among these 14 bees. 'sd' and 'se' are the overall standard deviation and error of directions indicated in dances that bees follow. 'Date', the date when bees are emerged and introduced into the colony.
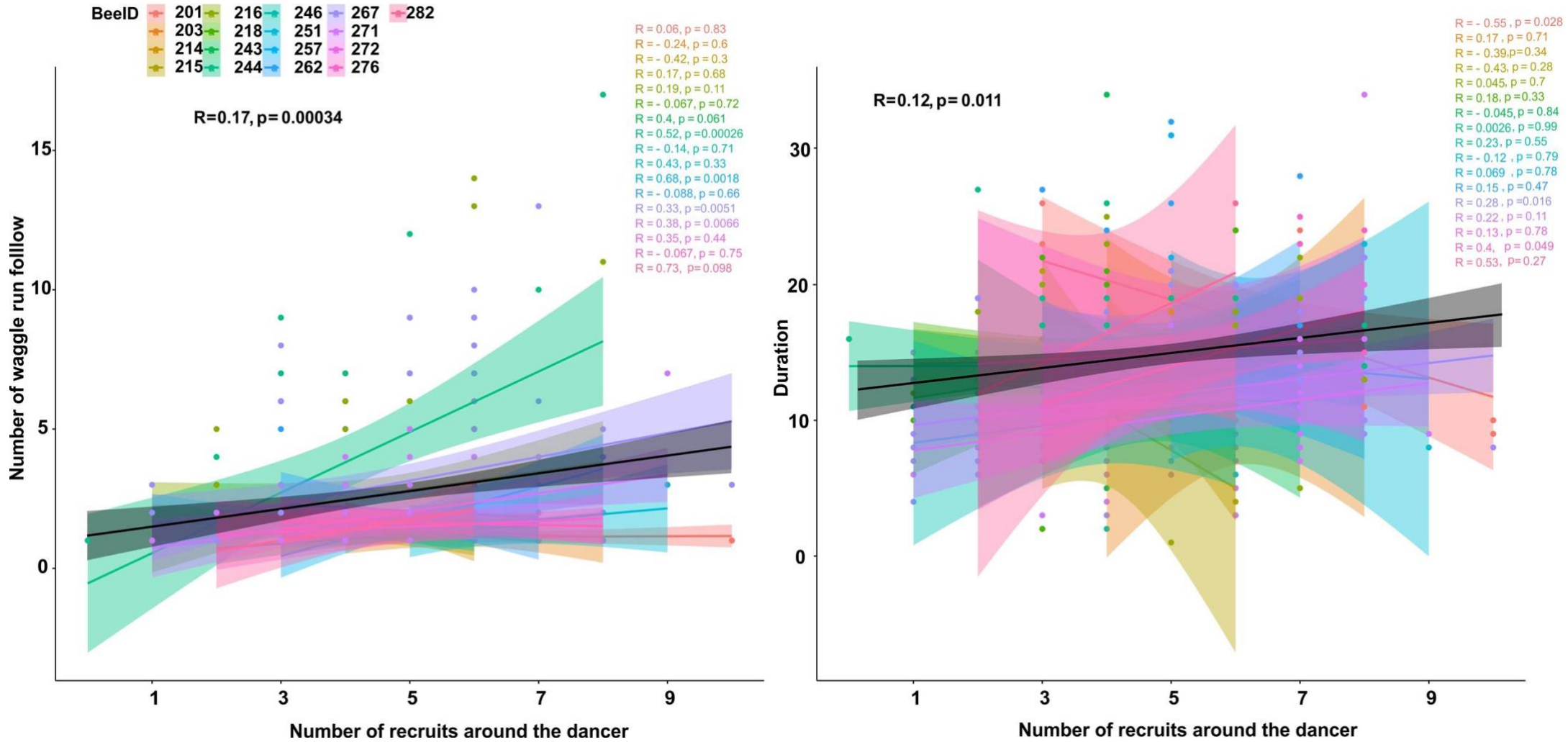
Figure S5. Correlation between the number of recruits around the dancer and the focal bees' tendency to follow the dance. Y axis in left and right figures represented the number and duration of waggle runs bees followed. The black line shows the overall

regression while colored lines give the regression lines for each individual. Correlation coefficients and corresponding p-values at the overall level and individual level are shown in black and colors.
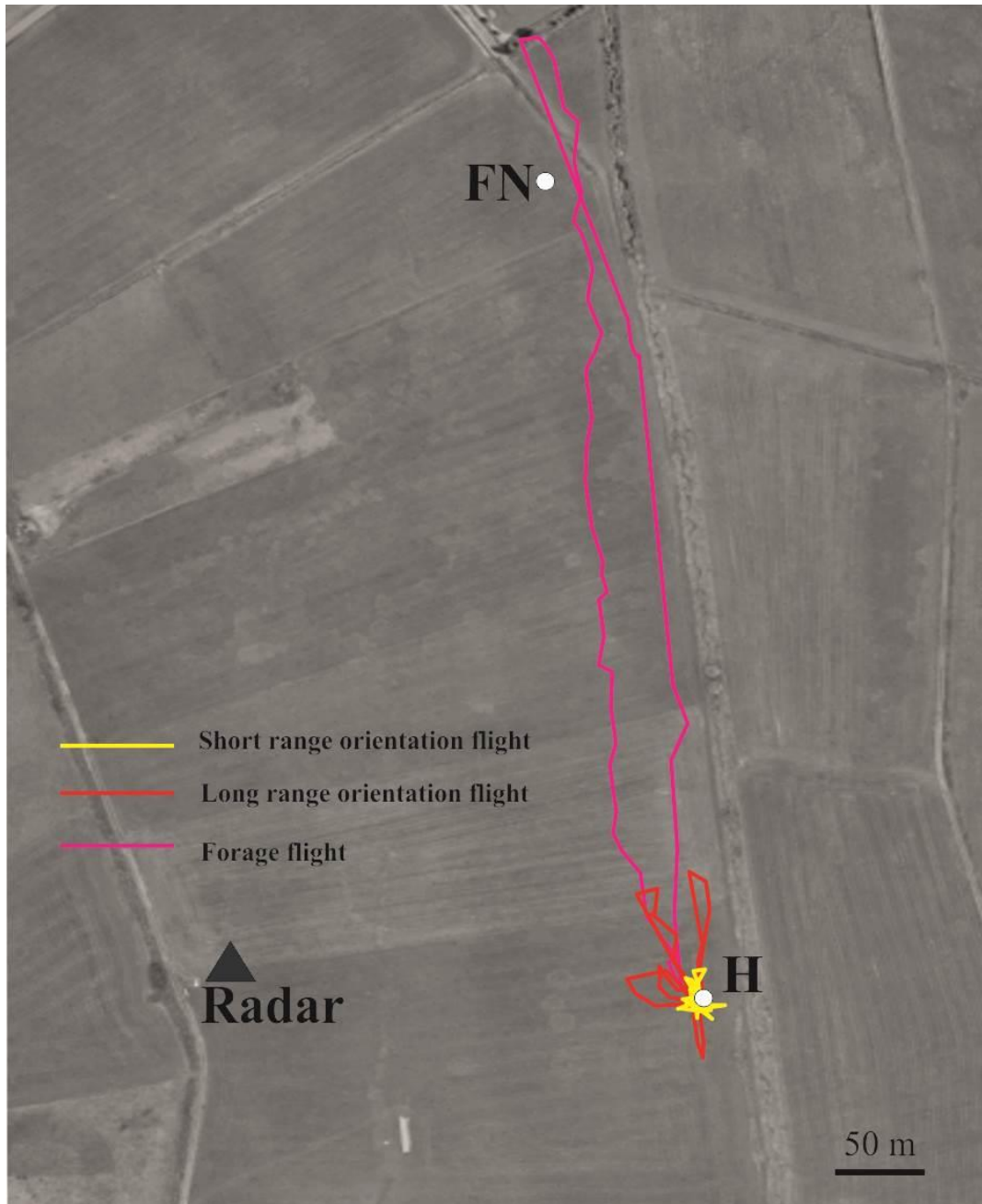
Figure S6. Sample of short/long -range orientation flight and foraging flight. 'FN', artificial feeder in north; 'H', bee colony; 'Radar', harmonic radar.
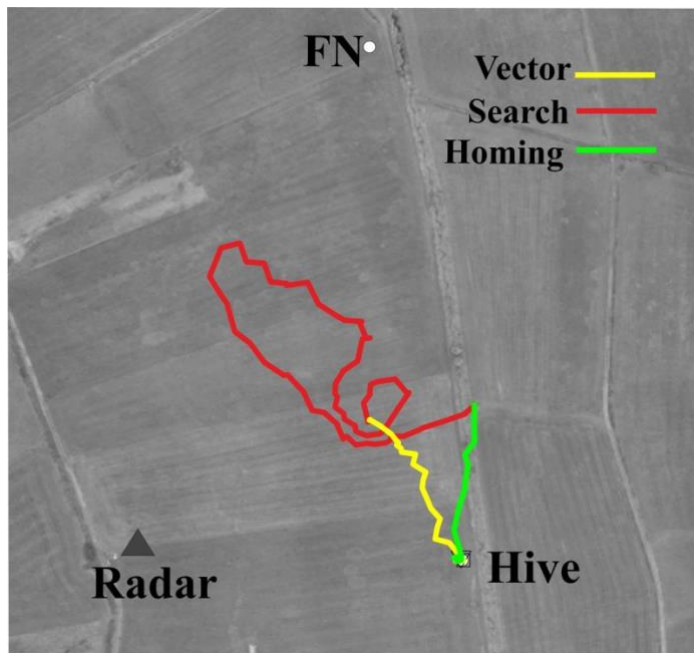
Figure S7. Segment of foraging flight into vector, searching and homing part
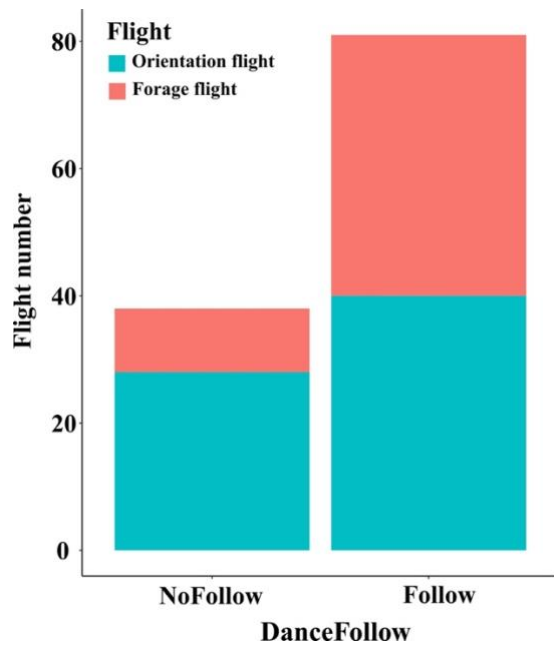
Figure S8. Total numbers of flights performed with or without dance following among 14 bees. 'NoFollow' and 'Follow' described that bees carried out the flights without following or with following dances before the flight.

Predicted probabilities of long orientation flights
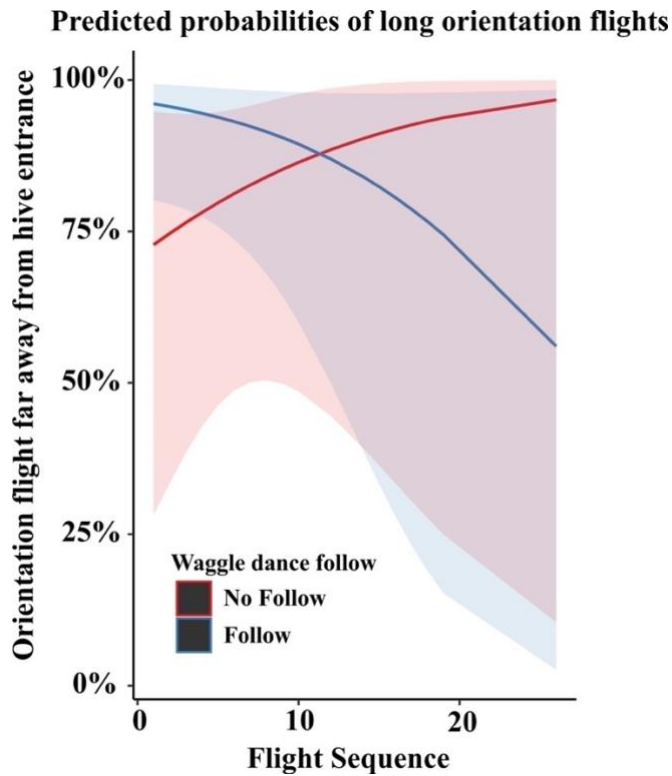
Figure S9. Co-influence of flight sequences and dances follows on the probabilities of orientation flight far away from entrance. 'No Follow' and 'Follow' described that bees carried out the flights without following or with following dances before the flight.
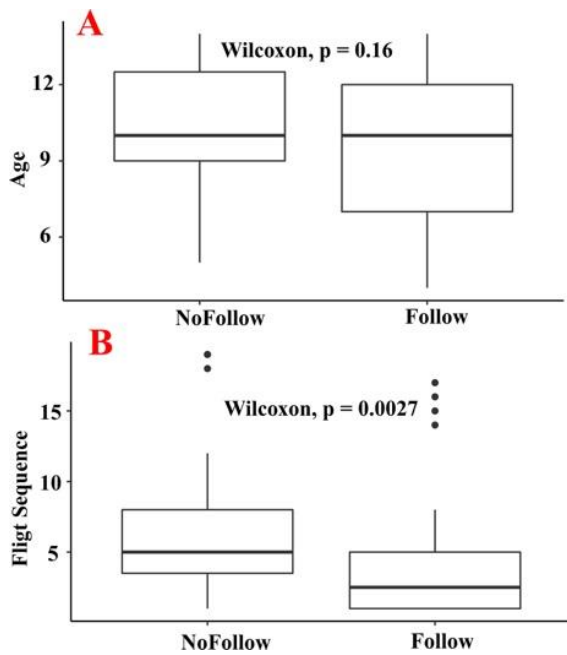
Figure S10. Impacts of ages and flight sequences on determining whether bees had long-distance orientation flights with or without dance following.
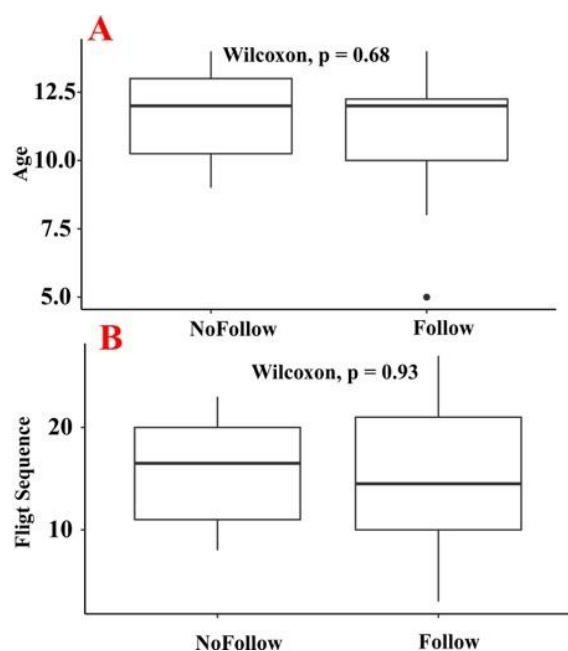
Figure S11. Impacts of ages and flight sequences on determining whether bees had foraging flights with or without dance following.
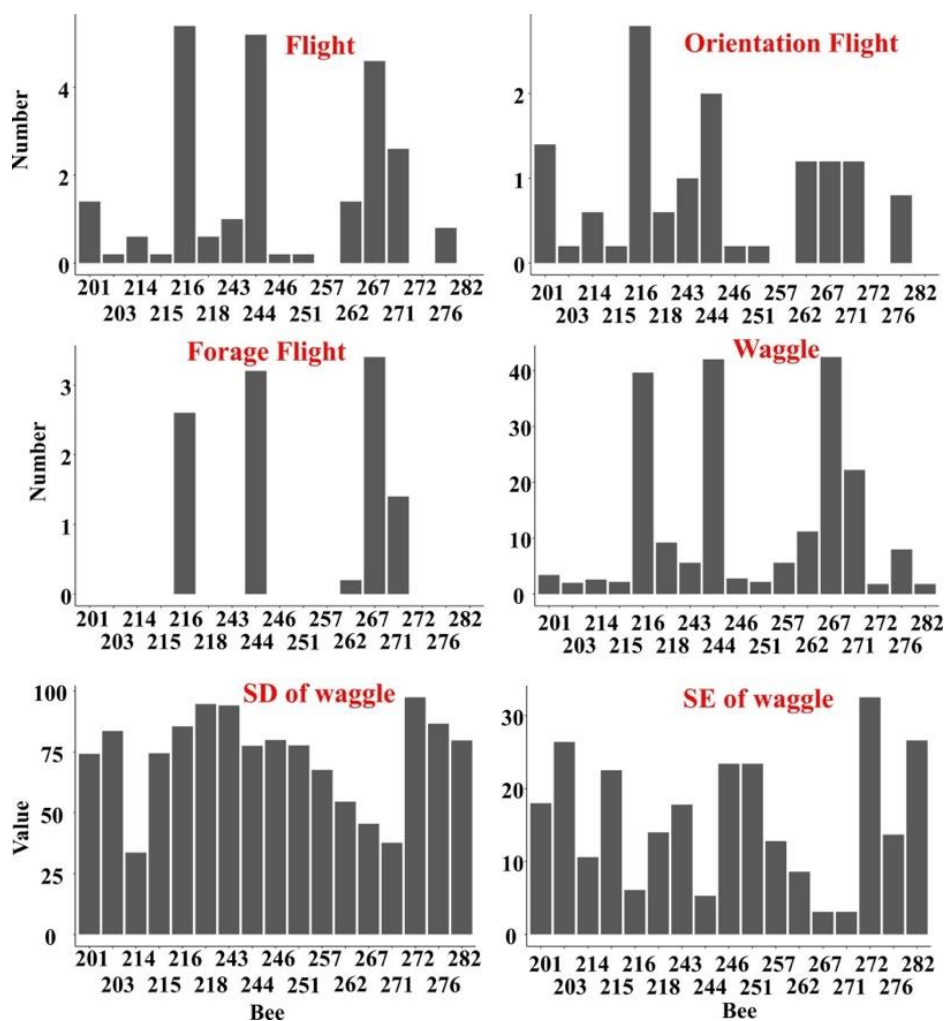
Figure S12. Numbers of flights, dances, standard deviation and errors of dances among14 bees. Due to bees' different life spans, the numbers of flight (orientation flight and foraging flight included) and dances followed were averaged.
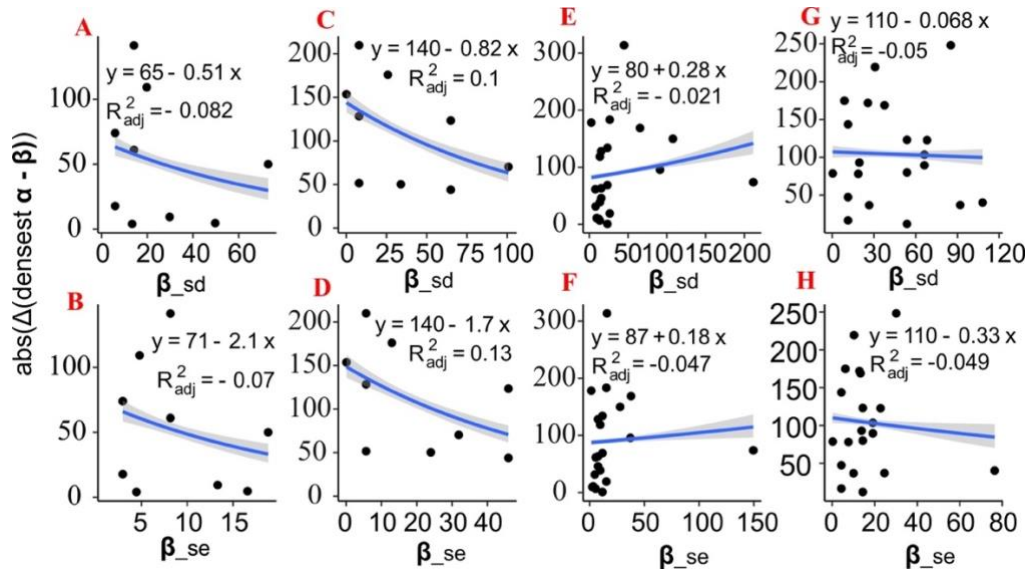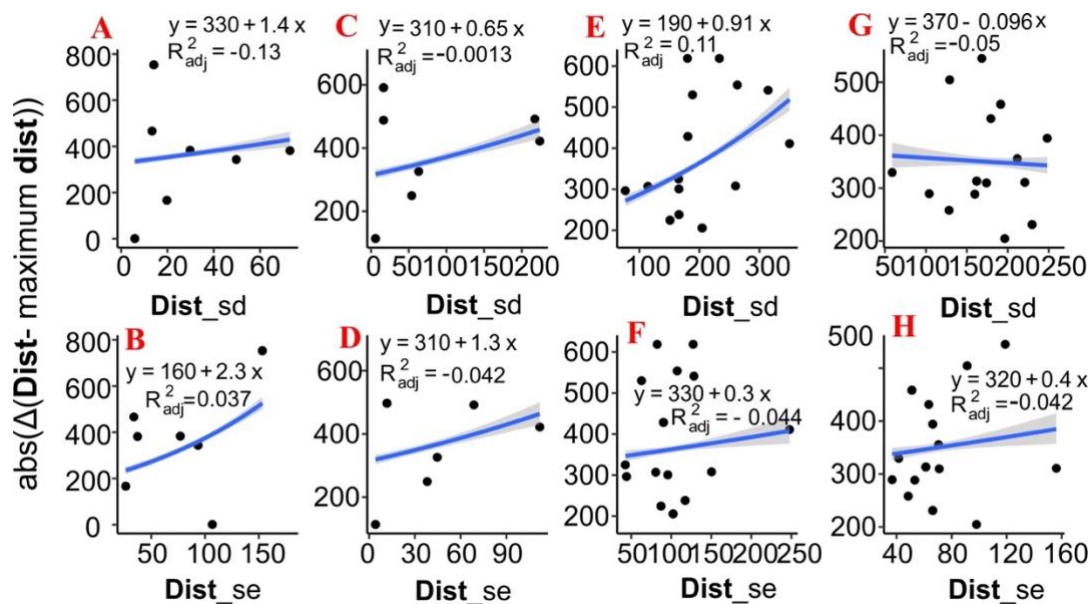

Figure S13a. The impact of variations and dispersions of directions indicated in the dances followed within different time intervals before the orientation flights on bees' orientation flight performance. 'β_sd' and 'β_se' represent the standard deviation and error of the direction indicated in the dances. abs (Δ (densest α - β)) denotes the absolute value of difference between the densest direction of orientation flight and the average direction indicated in the dances. A-B, C-D, E-F and G-H show the respective correlations in the time intervals of (0 ~ 15 min), (15 ~ 30 min), (30 min ~ 24 h) and (> 1 day).

Figure S13b. The impact of variations and dispersions of distance indicated in the dances followed within different time intervals before the the bees' orientation flight performance. '**Dist**_sd' and '**Dist**_se' represent the standard deviation and error of distance indicated in the dances. abs (Δ (**Dist**-maximum **dist**)) denote the absolute value of difference between the average distance indicated in the dances and the maximum flight distance. A-B, C-D, E-F and G-H show the respective correlations in the time intervals of (0 ~ 15 min), (15 ~ 30 min), (30 min ~ 24 h) and (> 1 day).
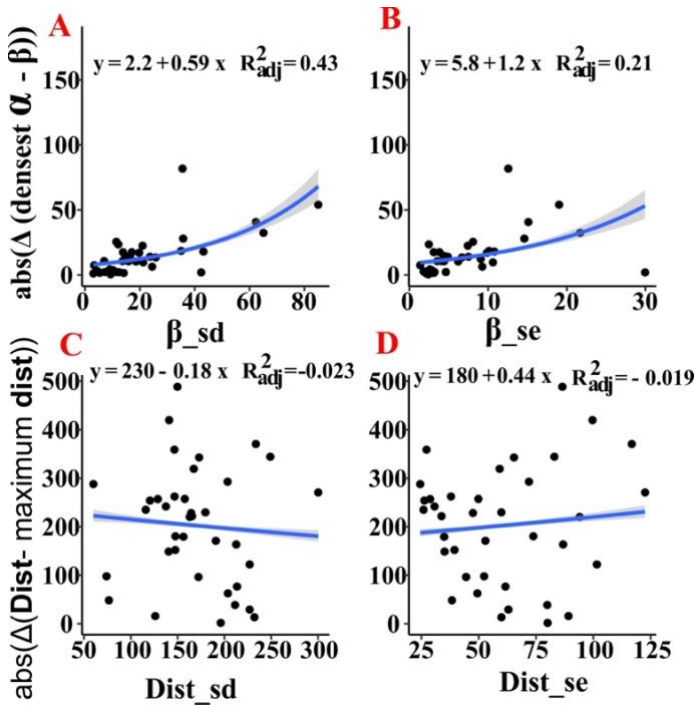

Figure S14a. The impact of variations and dispersions of direction and distance indicated in the dances followed before the bees' foraging flight performance. '**β**_sd' and '**β**_se', '**Dist**_sd' and '**Dist**_se' correspondingly represent the standard deviation and error of the direction and distance indicated in the dances. abs (Δ (densest **α** - **β**)) and abs (Δ (**Dist** - maximum **dist**)) denotes the absolute value of differences between the densest direction and maximum distance of the whole foraging flight and the corresponding average direction and distance indicated in the dances.
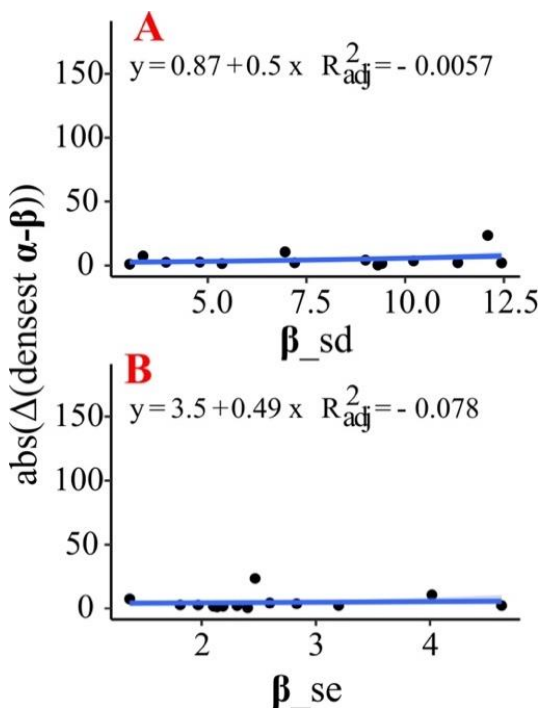

Figure S14b. The impact of smaller variations and dispersions of directions indicated in the dances followed before the bees' foraging flight performance. The ranges of SD and SE were <= 12 and <= 5, respectively.