DISSERTATION

LIVING IN THE SLOW OR FAST LANE: COGNITIVE PHENOTYPES IN HONEYBEES

Submitted by

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ABSTRACT

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The evolution and maintenance of cognitive variation is a question of fundamental interest in animal behavior because differences in cognition are predicted to underlie differences in behavior. The correlation between behavioral and cognitive variation has largely been conceptualized in terms of the speed-accuracy trade-off driving alternative cognitive strategies where ‘fast’ individuals are superficial learners that make inaccurate, risk-prone decisions relative to ‘slow’ individuals. My research has explored the factors that select for different cognitive abilities across species and the mechanisms that maintain variation in cognitive ability within species. To address these questions, I have identified how individuals of four honeybee species (*Apis mellifera, A. cerana, A. dorsata, A. florea*) differ in performance on multiple cognitive tasks and explored how such variation translates to behavioral outcomes and is shaped by ecology. In chapter one, I tested for the presence of variation in two different learning abilities in honeybee foragers and whether any component of learning influenced wing damage, an indicator of survival. My results demonstrated considerable interindividual variation in different types of learning abilities such that landmark and olfactory learning were negatively correlated. Additionally, I found that olfactory learning was positively correlated with maneuverability performance during flight, a measure which in turn positively influenced wing damage, a proxy for survival. This experiment demonstrated that individuals differ considerably in how they perform on two cognitive tasks and that cognitive ability has important implications for behaviors associated with survival. This work was further explored in chapter 2, where I studied how differences in learning preference relate to

decision making during foraging. I measured individual latency to learn on a solitary foraging task and latency to learn on a social foraging task and found that individuals that perform well in a solitary learning task perform poorly in a social learning task. These findings suggest that honeybees specialize in one type of learning strategy when making foraging decisions, and such differences may have important implications for how individuals provision their colony. The first two chapters focused on how differences in performance on cognitive tasks may represent a trade-off that correlates to different behaviors. In the latter half of my dissertation, I first used multiple cognitive traits to define a cognitive phenotype in an individual and then investigated how such differences might impact performance on multiple behaviors and life history traits to determine functional consequences of cognitive variation. I then expanded this research to determine how differences in ecology shape cognitive phenotypes. In chapter three, I tested for the presence of distinct cognitive phenotypes in *A. mellifera* foragers by measuring multiple cognitive traits and determining whether these traits covary to produce distinct slow and fast cognitive phenotypes. I then compared performance on multiple behavioral and life history tasks to see if there were functional differences between these cognitive types. My results indicate the presence of two cognitive phenotypes that meet the predictions of the speed-accuracy trade-off and that are conserved across colonies. Compared to slow bees, fast bees were described by high associative learning, high preference for novelty and high preference for variance, bees which also engage in more nursing behavior and transition to becoming a forager at an earlier age. In chapter four, which explored how ecological and life history differences shape cognitive phenotypes between closely related honeybee species, I tested for differences in the cognitive phenotype in four honeybee species, each of which occupied a unique ecological niche that was correlated to their position on the slow-fast life history axis. My results indicate that a set of cognitive traits consistently covary

within each species, resulting in slow and fast cognitive phenotypes that meet the predictions of the speed-accuracy tradeoff. I also found that the four species do not align on a slow-fast cognitive axis due to known differences in their life history and nesting ecology. Rather, cognitive differences among the species appear correlated to their brain size, which may be driven by differences in foraging range. Taken together, this work indicates that cognitive variation at the individual level has important behavioral and life history outcomes that may impact how the individual interacts with their environment and how the colony performs. At the species level, cognitive variation appears to be driven by a complex relationship with the species unique environment as well as underlying trade-offs associated with costs of cognition.

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DEDICATION

I dedicate this work to my friends who supported me, my family, who did not understand what I was doing but encouraged me all the same, and my mother, who unfortunately could not be here to see me finish this work.

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CHATPER 1

Interindividual variation in learning ability in honeybees

INTRODUCTION

Cognition is central to questions about functional explanations of behavior as it outlines the various mechanisms by which individuals acquire, process, store and act on information from the environment (Shettleworth, 2009). Nonetheless, the ecological forces that contribute to the evolution of specific cognitive abilities remain poorly understood. In humans, performance on different cognitive tasks tends to be strongly positively correlated, a phenomenon described as "general intelligence" or "*g*" (Thornton and Lukas, 2012). However, demonstrations of this in other animals are relatively rare and ecological theories of cognition instead posit selection for specific cognitive domains and resulting tradeoffs among them in response to different environmental challenges (Shettleworth, 2009). Any such trade-offs are however often difficult to verify because studies traditionally focused on interspecific comparisons of cognitive capacity are generally confounded by various other factors. This has led to a recent surge of interest in intraspecific variation in cognitive ability with the expectation that it might offer better insights into the evolution of cognitive ability and its impact on fitness (Thornton et al., 2014; Cauchoix et al., 2018).

It has been proposed that interindividual cognitive differences within a species can translate to alternative behavioral strategies with different fitness consequences (Sih and Giudice, 2012). This suggests that such interindividual differences in cognitive abilities are especially likely to be fostered in group living species due to forces related to either competition or mutual benefit.

Social insect colonies are prime examples of cooperative group living in which interindividual variation and the resulting adaptive diversity with respect to various traits is considered to be the major underlying force for their ecological success (Oster and Wilson, 1978; Jeanson and Weidenmüller, 2014). However, the patterns of such variation with respect to cognitive traits are less clear (Burns and Dyer, 2004; Raine et al., 2006; Muller and Chittka, 2012; Smith and Raine 2014; Klein et al., 2017). Learning, the ability to adjust behavior through experience, is often considered to be fundamental to all cognitive mechanisms because it allows animals to adaptively respond to environmental contingencies (Dickinson, 2012; Heyes, 2012). In honeybees, one of the classic models of animal cognition, a large body of work has demonstrated how learning relates to cognition and how a variety of learning abilities is crucial to their performance (Menzel, 2012; Giurfa, 2015). However, there is little empirical data regarding if there is interindividual variation in these abilities and if any, whether it has any influence on performance.

In spite of the intuitive and obvious relevance of learning on fitness, actual empirical demonstrations of a positive relationship between the two have been relatively rare (Dukas, 1999; Cole et al., 2012; Maille and Schradin, 2016). In honeybees and other social insects, the performance of individuals is generally measured in terms of their work capacity, most commonly in terms of foraging. The extent of foraging activity performed by an individual is known to be reflected in the amount of wing damage, which results from collisions with foliage incurred during foraging (Foster and Cartar, 2011) and has a strong negative impact on survival and lifespan (Cartar, 1992). We therefore measured the amount of natural wing damage seen in an individual to test a relationship between learning and forager performance. The study comprises of a set of three experiments to examine interindividual differences in different learning abilities that are critical to foraging in honeybees and the relationship between learning ability and wing damage, which as a measure of individual forager survival acts as a proxy for fitness at the colony level.

METHODS

Bees from five colonies were trained to a setup for assaying the landmark learning ability of individuals. The subjects evaluated for their landmark learning were collected at the end of this assay and subjected to an olfactory learning assay.

a) Landmark Learning Assay: The assay consisted of a maze, configured from an array of acrylic boxes with white, opaque walls and clear tops and placed on a platform 50 m from the hives, which the bees had to negotiate in order to reach a reward of 30% sucrose solution (Zhang et al., 1996). There were two types of boxes that constituted the maze, decision and non-decision boxes (Fig. A1). Decision boxes had three holes (4 cm diameter), each at the center of a different wall; a bee flew into such a box through one of these holes and it had to choose between the other two holes, one of which led into the next box in the correct path to the reward and the other which led to a dead end. Non-decision boxes had two holes and only required the bee to fly through one and exit through the other into the next box. The holes leading through the correct path in both decision and non-decision boxes were marked with a piece of blue tape to provide landmark cues. We used two different maze configurations during the experiment, 22 bees negotiated a maze with two decision boxes and 31 bees negotiated one with three.

Training consisted of placing a feeder filled with 30% sugar solution inside the first decision box and leaving it for 45 minutes to allow the bees to learn the landmark cue to enter the box. After 45 minutes, the feeder was moved into the second decision box in the path and so on for each decision box until the bees had learned the correct path through the maze to reach the reward placed in the final box. Bees were allowed to enter the maze freely, but were released from the top after acquiring the reward. Only bees that learned the entire path through the maze to the reward and were therefore foraging during the final 45 minutes of the training phase were individually marked and participated in the following test. The number of training trials each individual experienced during this time varied between 5 and 7, but was not controlled for.

Testing took place immediately after training. During the test, only one bee at a time was allowed to enter the maze to ensure that decisions were independent to each individual. A landmark learning score was assigned for each run through the maze to quantify its performance such that a score of 1 meant that the bee did not complete the maze within a maximum assigned time of five minutes, a score of 2 meant that the bee made one or more wrong turns, a score of 3 meant that the bee retraced its path but did not make any wrong turns, and a score of 4 meant that the bee negotiated the entire maze without making any mistakes. After a bee was tested on the maze thrice, it was collected for the olfactory learning assay.

b) *Olfactory Learning Assay:* Each bee was fed to satiation with a 30% sucrose solution and then starved for 18h at 27° C inside an incubator to increase their motivation for appetitive learning. Each bee was then tested for its olfactory learning ability using the Proboscis Extension Reflex (PER) assay (Bitterman et al., 1983). The assay consisted of presenting an individual bee with an odor (Conditioned Stimulus or CS) followed by a sucrose reward (Unconditioned Stimulus or US) in 6 consecutive trials with an inter trial interval of 11 minutes and recording the extension of the proboscis by the bee for the reward. A bee was considered to have learned the association between the odor and the sucrose reward and was given a score of 1 in a trial if it showed a conditioned response (CR) by extending its proboscis to the odor prior to the sucrose reward being provided. If the bee did not extend its proboscis at all or extended it only at the presentation of the sucrose reward, it was given a score of 0 in that trial. The total number of CRs for a bee was defined as its olfactory learning score.

c) Maneuverability Assay: The assay consisted of placing an individual bee in a flight arena (an acrylic box measuring 41.5 x 26.5 x 17 cm) containing an array of obstacles comprised of wooden pegs stuck to the floor of the box (Mountcastle et al., 2016). The walls of the box were covered with a floral pattern and the box was placed under an overhead white light to stimulate flight. The box was placed on an orbital shaker rotating at 3 rpm to simulate the moving obstacles in the foliage that a bee might naturally encounter while foraging. Each subject was allowed to fly in the arena for five minutes and its behavior was recorded using a digital video camera (Sony HDV 1080i). The flight behavior of a bee was analyzed in terms of two parameters: (a) number of landings, defined as the events when a bee settled on a peg or the floor, its feet touching first, and (b) number of collisions, defined as the events when the bee hit a peg, a wall, or the ceiling and could not maintain flight elevation and crashed to the floor. Following successful completion of the flight assay, individuals were subjected to the PER assay as described in the first experiment.

d) Wing wear: Newly emerged bees from two source colonies were marked and fostered in the colony, following which they were collected from the entrance of the colony as described above. These bees were subjected to the maneuverability assay as described above and following it each subject was euthanized and its wings were collected and analyzed for existing damage in the following manner. The two forewings were removed at the wing joint, their images were scanned into the computer and the area and perimeter of each wing was measured using ImageJ software (Foster and Cartar, 2011).

Statistical Analysis

As we were specifically interested in the relationship between different learning abilities and their relationship with performance, we only included learners or bees with an olfactory learning score ≥ 1 in our data analysis for the first two experiments. There was no significant difference in the landmark learning score between bees in the two maze types ($F_{1, 51} = 0.01$, $P = 0.91$), so data from the two were pooled. The maneuverability performance of a bee was measured by calculating the ratio of the number of landings to that of crashes (landings/crashes), where a higher value represents a higher performance. The relationship between maneuverability performance and olfactory learning was examined by using a linear mixed model with colony of origin and age of the bee as random effects and the significance of random effects were tested using likelihood ratio tests. Wing damage of a bee was determined by calculating the ratio of area to perimeter and then averaging that measure across the two wings, where a smaller value represents a higher damage (Foster and Cartar, 2011). Due to the relatively small number of bees for each specific age, bees were divided into two discrete groups: young bees (age 12-17 days) and old bees (20-24 days) and a linear mixed model was used to examine the influence of maneuverability and age on wing damage with colony of origin as a random effect. While some of the data showed a better fit with non-linear models, we chose linear models for biological parsimony. All statistical analyses were performed in R version 3.4.1.

RESULTS

There was significant interindividual variation observed in the different learning abilities assayed in each experiment. The distribution of the scores for each assay was significantly different from a normal distribution (Shapiro-Wilk test, Landmark learning: $W = 0.48$, $P < 0.001$; Olfactory learning: $W = 0.9$, $P < 0.001$; Maneuverability: $W = 0.94$, $P = 0.02$, Fig. 1.1A-C). There was a significant negative association between the landmark and olfactory learning performance of an individual bee (Pearson's Correlation; $r = -0.17$, $N = 31$, $P = 0.01$; Fig. 1.2). There was a significant positive association between olfactory learning and maneuverability performance ($F_{1,36} = 4.81$, $P < 0.001$, Fig. 1.3). There were no significant effects of either source colony or age (Colony: $\chi^2 < 0.001$, $P = 0.9$; Age: $\chi^2 = 1.98$, $P = 0.15$) on this relationship.

Maneuverability performance of an individual had a significant independent effect on the observed damage on its wings ($F_{1,31} = 5.10$, $P = 0.03$; Fig. 1.3), such that bees with higher maneuverability showed less wing damage (Fig 1.4). While age did not have a significant independent effect on wing damage ($F_{1,31} = 2.18$, $P = 0.15$), it showed a significant interactive effect with maneuverability to influence wing damage such that older and less agile bees showed more wing damage ($F_{1,31} = 5.57$, $P = 0.02$). There was also a significant effect of colony of origin on the observed wing damage ($\chi^2 = 4.83$, $P = 0.02$).

DISCUSSION

Our results show that there are significant differences among honeybee individuals in terms of their performance across the three tasks such that most individuals have high landmark learning ability while individuals seem more variable in terms of olfactory learning and maneuverabilithy. This suggests landmark learning may be a more general cognitive skill that is common to all foragers while relatively fewer foragers in the colony exhibit enhanced olfactory learning or maneuverability skills that may be required for more specialized foraging tasks. There is evidence to suggest that scouts, a relative minority in the colony, perform better on olfactory learning tasks compared to recruits (Carr-Markell and Robinson, 2014; Cook et al., 2019). It is possible that the diversity of performance on different learning tasks may reflect distributions of such different behavioral phenotypes within the colony. The different aspects of learning that are crucial to foraging performance show both positive and negative correlations among them in an individual bee. Our first experiment demonstrated a negative association between landmark and olfactory learning while our second experiment demonstrated a positive association between maneuverability and olfactory learning. While it might be tempting to consider the positive association as evidence for the "g" factor, one should note that our maneuverability assay, requiring bees to avoid obstacles in flight and therefore also consisting of a motor component, is not purely a learning task.

While the notion of cognitive specialization is generally based on poor and good performers on a single learning task (Carr-Markell and Robinson, 2014; Cook et al., 2019), to the best of our knowledge this is the first time a specialization based on a negative correlation between the performance in two different types of learning tasks has been demonstrated in individual honeybees. This observed negative association between two types of learning in individuals has important implications for division of labor in social insect colonies, the models for which generally require individuals showing negative correlations between their response to different stimuli (Beshers and Fewell, 2001). Since previous findings documenting positive correlations between learning performance across different sensory modalities and sucrose responsiveness (Page et al., 2006) are somewhat at odds with such models, our results demonstrating a negative correlation between certain types of learning provide support regarding how individuals might show different proficiencies in performing different tasks. The observed negative correlation between performance in these learning tasks therefore also suggests that it is not likely an outcome of differences at the sensory level but rather due to a difference at a higher cognitive level.

Our findings support the idea that investment costs related to learning (Mery and Kawecki, 2004, 2005; Jaumann et al., 2013) may necessitate a tradeoff between performance in different learning modalities, leading to individual specialization. The fact that such learning specialization has not been observed in bumblebees (Muller and Chittka, 2012; Smith and Raine, 2014) may have to do with their lower degree of sociality compared to honeybees. The relationship between behavioral specialization and colony size or sociality has long been debated and there is evidence supporting the idea that larger and more eusocial colonies tend to have more specialized workers (Amador-Vargas et al., 2015; Kamhi et al., 2016). Studies also show that an increase in social complexity is accompanied by an increase in the capacity for distributed cognition mediated by more social communication and specialized brains (Lihoreau et al., 2012; O'Donnell et al., 2015). In smaller or less social groups such as bumblebees, individuals may need to maintain capacity in a broader range of learning modalities to allow them to be successful generalists while large honeybee colonies through specialization can profit from maintaining a cognitively diverse workforce.

Our results showing a positive relationship between olfactory learning and maneuverability and the influence of the latter on wing wear also for the first time suggest a possible consequence of interindividual variation in learning ability on performance in honeybees. It has been well established that in flying insects such as honeybees, wing damage has several functional consequences that include increased mortality resulting from factors such as increased vulnerability to extrinsic elements and increased energy expenditure (Cartar, 1992; Burkhard et al., 2002; Higginson and Barnard, 2004; Combes et al., 2010**;** Dukas and Dukas, 2011). A significant contributor to wing damage is the number of collisions bees encounter with vegetation while foraging (Foster and Cartar, 2011), which means that individuals with higher maneuverability, by better avoiding collisions, should gain in terms of survival and their foraging lifespan. While maneuverability is likely a product of both cognitive and motor skills and our experimental design is unable to distinguish between the two, it nevertheless underlines the point that variation in learning ability, by influencing individual foraging performance and lifespan, can have a significant influence on colony performance. While wing damage must initially arise from poor maneuverability, it is likely that maneuverability and wing damage exhibit a positive feedback loop, whereby a decreased maneuverability leads to further wing damage.

It is interesting to ask if there is any selection at the colony level to maintain interindividual variation and individual specialization in different learning modalities in large social groups such as the honeybee colony. If differences in learning ability correlate to different social roles, a colony may benefit from maintaining learning specialization and cognitive diversity (Burns and Dyer, 2004). For example, the two behavioral phenotypes among honeybee foragers, dancers that gather information and followers that use that information, may require different types of cognitive skills. Our earlier work has shown dancers and followers to be functionally equivalent to producers and scroungers, respectively (Katz and Naug, 2016), behavioral phenotypes that are known to be driven by differences in their learning ability (Katsnelson et al., 2011). Our future research directions therefore include examining if different behavioral phenotypes in a honeybee colony correlate to distinct cognitive phenotypes and if colonies with different distributions of these phenotypes show colony level differences in performance.

FIGURES

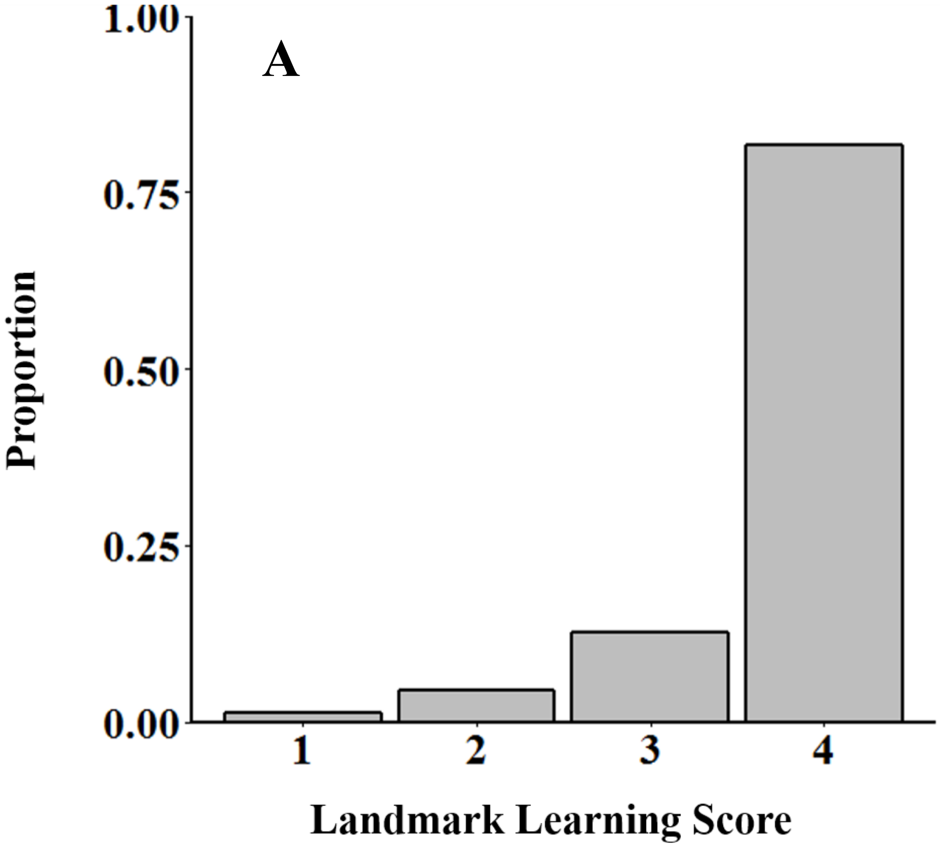

Figure 1.1A: Frequency distributions of landmark learning observed across all bees in the different experiments.

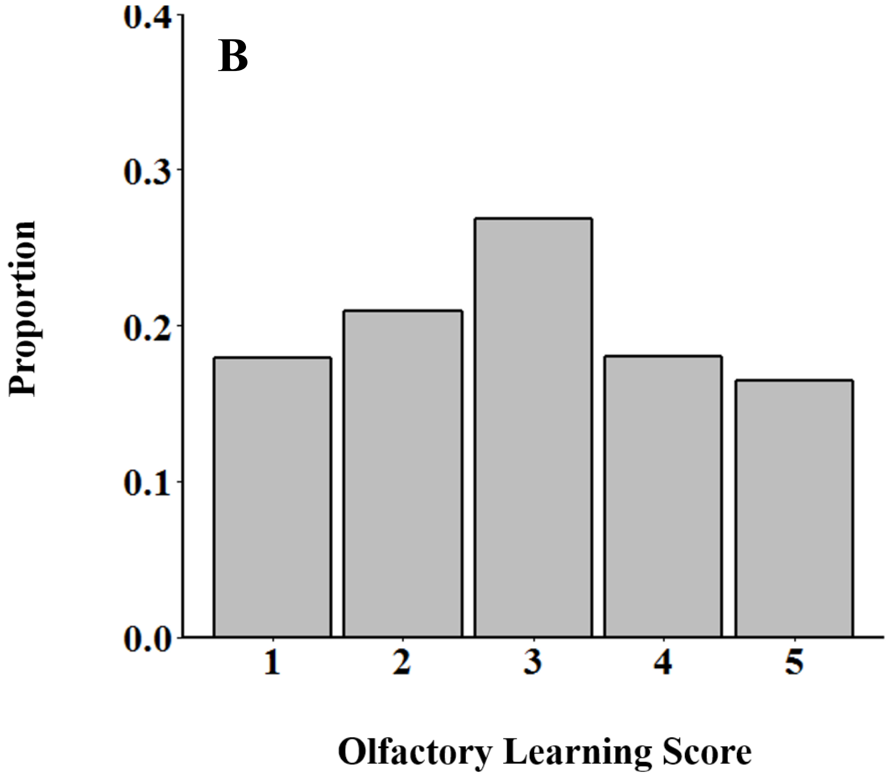

Figure 1.1B: Frequency distributions of olfactory learning observed across all bees in the different experiments.

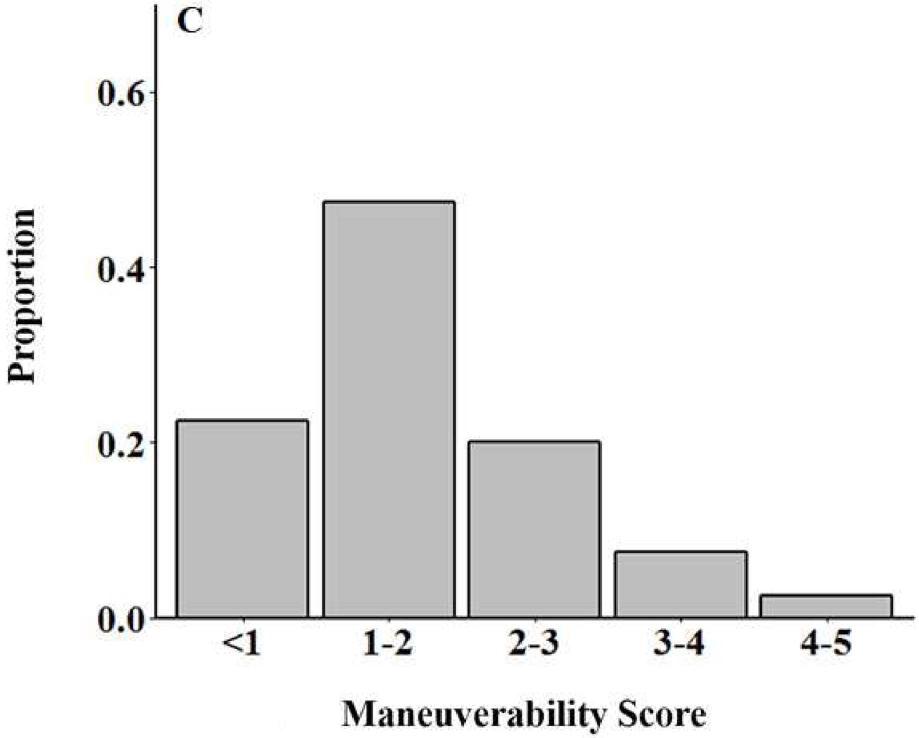

Figure 1.1C: Frequency distributions of maneuverability scores observed across all bees in the different experiments.

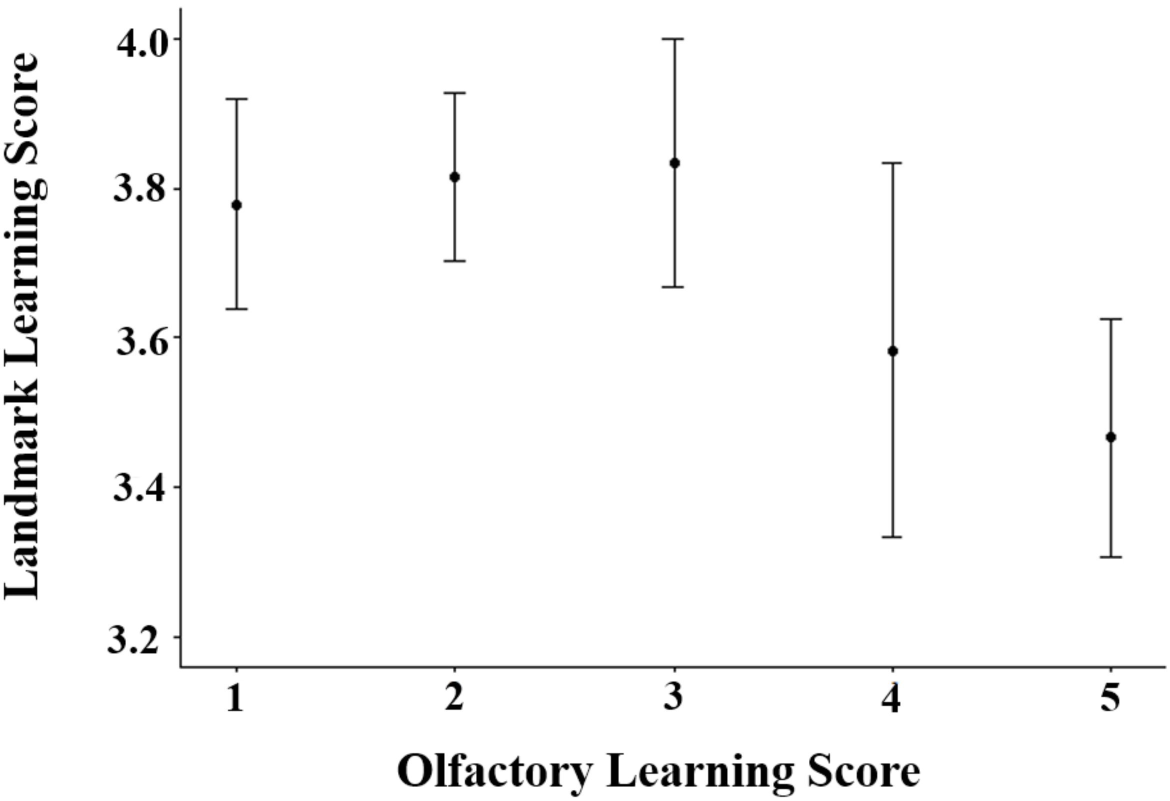

Figure 1.2: Correlation between landmark and olfactory learning performance of individual bees (N = 31) with data representing mean ± standard error across bees.

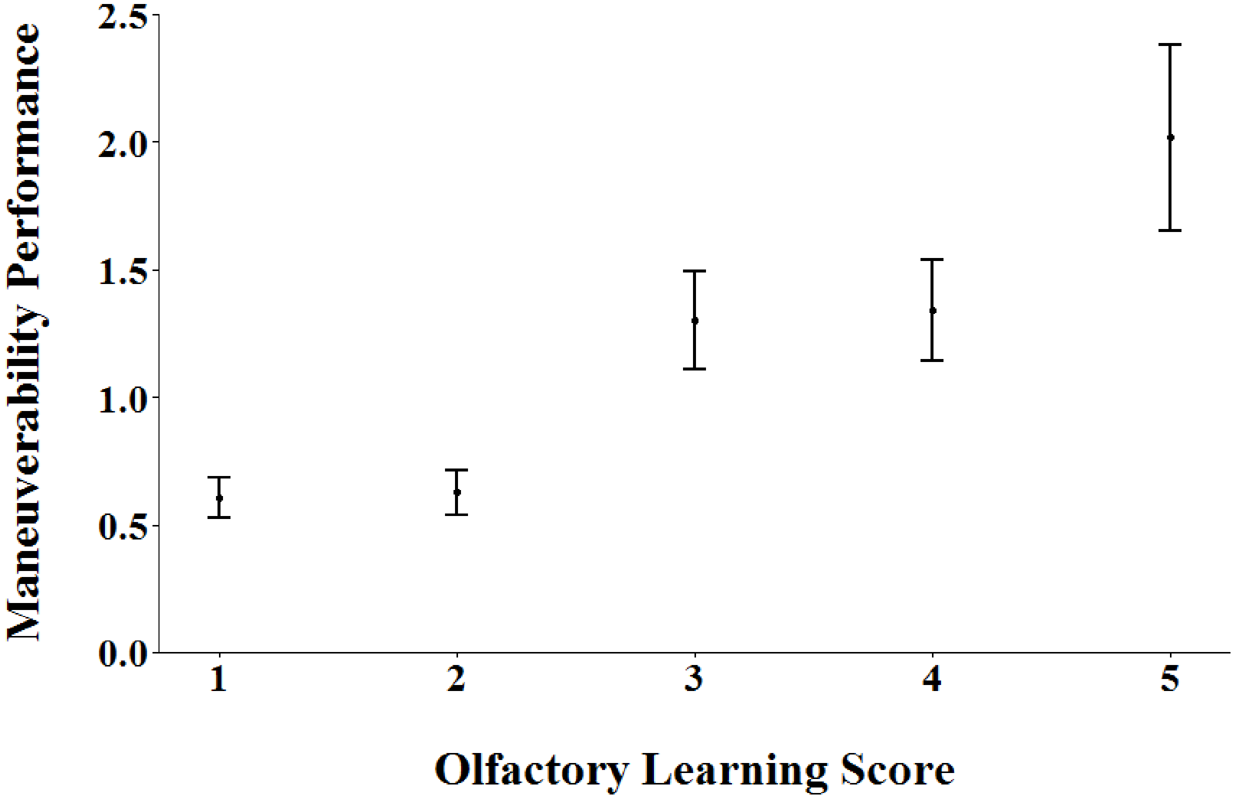

Figure 1.3: Correlation between olfactory learning and maneuverability with data representing mean ± standard error across bees (N = 37).

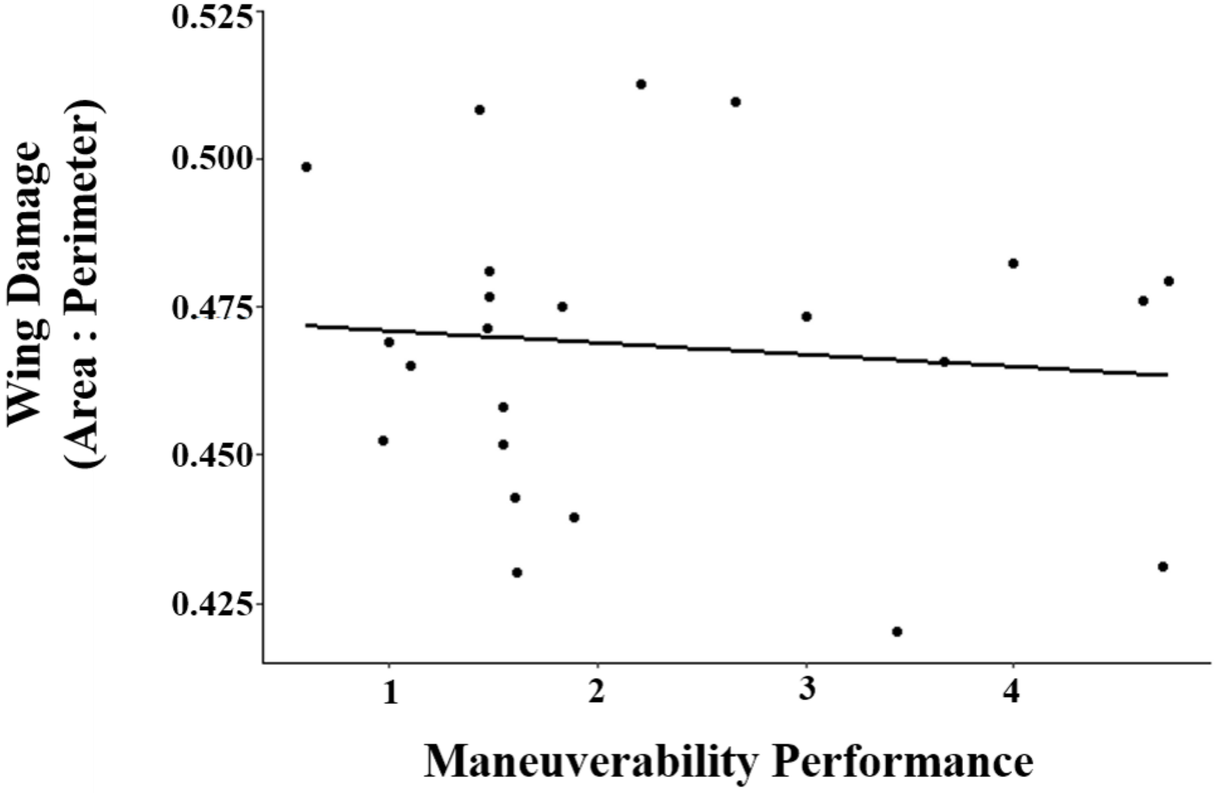

Figure 1.4: Average wing damage in individual bees (N = 34) as a function of their maneuverability score. Each point represents an individual bee and the solid line represents the regression ($y = 0.007x + 0.47$) between maneuverability and wing damage.

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CHATPER 2

Cognitive phenotypes and their functional differences in honeybees

INTRODUCTION

The existence of interindividual variation in behavioral traits has long been widely recognized, but the evolution and maintenance of such variation within a population has only recently become a topic of great interest (Sih et al., 2004; Réale et al., 2010; Stamps & Goorthuis, 2010). While the majority of these studies has focused on behavioral traits, such as boldness, exploration, aggression etc. (Réale et al., 2010), relatively less attention has been devoted to understanding the individual differences in cognitive traits that are hypothesized to underlie these observed behavioral differences (Carere & Locurto, 2011; Sih & Del Giudice, 2012). Although a connection between behavioral and cognitive variation has been demonstrated in a few studies (Guillette et al., 2011; Amy et al., 2012), separating these two levels of variation is challenging because the two are inextricably connected and cognitive traits can only be indirectly inferred by measuring some behavioral outputs. Using multiple cognitive traits to define individual differences in cognition (Griffin et al., 2015) and then investigating how such differences might determine performance on behaviors that have clear functional consequences can be a first step in this direction.

The correlation between behavioral and cognitive variation has largely been conceptualized in terms of the speed-accuracy tradeoff that can drive alternative cognitive strategies (Chittka et al., 2009). Most behaviors are predicted to show a risk–reward tradeoff such that the higher expression of a behavior can bring more rewards, but at the cost of a higher risk, and this tradeoff leads to a direct link between behavioral types on a fast-slow gradient and cognitive styles based on the speed–accuracy tradeoff (Sih & Del Giudice, 2012). According to this model, the fast-slow behavioral axis is an outcome of an underlying speed-accuracy tradeoff that produces a cognitive axis in which fast individuals spend less time gathering information to make rapid but somewhat more inaccurate decisions compared to those individuals who are slow. This in turn leads to the prediction that fast individuals would show faster associative learning and higher levels of impulsivity, neophilia and risk-proneness, but lower levels of sampling and retention. There is some evidence that individuals within a species may indeed vary in terms of their cognitive styles that meet the predictions of a speed accuracy tradeoff (Mazza et al., 2018; Jones et al., 2020) and that such cognitive differences can lead to significant differences in behavior with functional consequences (Keagy et al., 2009; Amy et al., 2012). However, most of these studies that have examined the relationship between cognition and behavior have seldom measured this covariance across multiple traits at these two different levels.

Social insect colonies are hotbeds of interindividual behavioral and cognitive variation, a diversity which is considered functionally critical to their division of labor and ecological success (Jeanne, 1988**;** Jeanson & Weidenmuller, 2014). The behavioral variation is reflected in not only individual differences in task performance, but it also translates to differences at the level of the whole colony (Wray et al., 2011; Bengston & Dornhaus, 2014). In honeybees and bumblebees, a significant amount of interindividual variability has also been recorded in terms of cognitive performance, measured mostly in terms of their associative learning abilities (Smith & Raine, 2014; Tait et al., 2019). The difference in learning abilities has also been shown to be correlated to differences in foraging performance (Raine & Chittka, 2008) and important life history traits such as age of first foraging (Pankiw & Page, 2001; Page et al., 2006). However, none of these previous studies simultaneously examined whether individuals covary in terms of a whole suite of cognitive, behavioral and life history traits in the framework of a slow-fast phenotypic gradient. In this study, we therefore set out to test the hypothesis that multiple cognitive traits covary among individuls such that they meet the predictions of a slow-fast cognitive axis and that individuals at different positions of such an axis also show differences among them in terms of behavior and life history traits along a similar slow-fast axis (Sih & Del Giudice, 2012).

METHODS

Brood frames with pupae were collected from 3 source colonies of the honeybee, *Apis mellifera*, one day prior to adult emergence and kept in an incubator set at 32° C. Upon emergence, individual adult bees were tagged with a unique number tag and introduced into an observation hive. The observation hive consisted of two brood frames, a full honey frame, a laying queen and workers. The hive was located in a dark room and was connected to the outside through a tube. The front glass pane of the observation hive was marked with a grid to assist in the behavioral sampling of tagged bees.

a) Behavioral Assay: Behavioral observations were conducted on the tagged bees when they were 7-14 day old using instantaneous scans. The hive was scanned every 15 minutes for two hours in the morning (0800-1000), afternoon (1300-1500) and evening (1600-1800) for 2 days, resulting in 48 scan samples spread over 12 hours of observations. The scans consisted of selecting each square in the grid in a systematic fashion and recording the behavior of any tagged bee in that square. Using these scan samples, the proportion of time spent by each tagged bee in brood care (an individual with its head inside a cell located on a brood frame), and social behavior (an individual engaged in trophallaxis or allogrooming), was calculated. As all tagged bees could not be located in each scan and the number of scan samples differed across bees, a bootstrapping procedure, consisting of selecting 10 random scan samples (with replacement) from the total number of scans available for a bee and repeating this procedure 100 times, was used to calculate the average proportion of time spent by an individual bee in each behavior. The behavioral profile was therefore not calculated for bees that had fewer than 10 scan samples. The age of first foraging (AFF) of an individual was estimated by monitoring the entrance of the colony every other day for two hours in the morning (0800-1000) and afternoon (1300-1500) and recording the first instance of a tagged individual spending at least 10 minutes outside the hive.

b) Cognitive Assay: Forager bees were collected at the hive entrance at least 3 days following a first foraging trip, immobilized on ice, and harnessed within a 4.5 cm long plastic drinking straw with a small wire around her thorax. Each bee was fed to satiation with 30% sucrose solution and then kept starved for 24 hours inside an incubator set at 27° C to increase her motivation for appetitive learning before performing the following series of assays.

a. Associative Learning: The associative learning ability of an individual bee was determined using the Proboscis Extension Reflex (PER) assay. It consists of presenting a bee with an odor (Conditioned Stimulus, CS) followed by a sucrose reward (Unconditioned Stimulus, US) in a series of trials (Bitterman et al., 1983). Our PER assay consisted of presenting each bee with six such trials with a 5-minute inter trial interval (ITI), with hexanol, octanone, geraniol and linalool as the CS odors used in different replicates. A bee is considered to have learned the association between the CS and the US when it extends its proboscis (a Conditioned Response, CR) to the CS prior to the US delivery, and the total number of CR is used as a measure of its learning ability.

b. Sampling and Preference for Novelty: Thirty minutes following the associative learning assay, each bee was subjected to a forced-choice proboscis extension response assay (Shafir et al., 1999, Mayack & Naug, 2011). This assay consists of presenting two different odors in four alternating 0.2 s pulses to the bee on either side of its head. Based on the orientation of its head and the extension of its proboscis at the end of the four odor pulses, a choice for one of the two odors is scored for the bee. Using the odor that was paired with the reward during the associative learning assay as one of the two odors and a novel odor as the other odor, preference for novelty is recorded as a binary score (1 or 0) and the number of times an individual turned its head toward each odor during the entire sequence of the four odor pulses provides a measure of sampling (Katz & Naug, 2015). The pairing of each odor as the novel odor and the direction of each odor was balanced across experimental replicates to account for any possible odor and side biases.

c. Retention (Memory): Each bee was fed to satiation following the novelty preference assay and then kept in the incubator for 24 hours. Each bee was then presented once with the CS odor that was associated with the sucrose reward in the associative learning assay the previous day and whether or not the bee extended its proboscis to the odor was recorded, which gave a binary score (1 or 0) for retention.

d. Preference for Variance (Risk): Thirty minutes following the retention assay, a PER assay was used to train the bees to associate two different odors with two different reward distributions. This consisted of pairing one odor with a variable reward and another with a constant reward and presenting these pairings to each bee in a predetermined pseudorandom sequence (ABBABABBABABAABAABAB) in a series of 20 trials with an ITI of 5 min (Shafir, et al., 1999, Mayack & Naug, 2011). In a trial in which the bee was presented with the odor that was paired with the variable reward, it received either a high reward of 0.4 µl or no reward (0 µl) in a predetermined pseudorandom sequence such that the overall probability of obtaining each reward type was 0.5. In a trial in which the bee was presented with the odor paired with the constant reward, it always received a 0.2 µl reward. Therefore, both the constant and the variable reward distribution had a mean of 0.2, but the variable one had a coefficient of variation (CV) of 100. The preference for variance for an individual was calculated as the number of times it extended its proboscis to the variable reward, divided by the total number of times it responded to the two rewards.

After the completion of the cognitive assays, the bees were returned to the observation hive and the entrance of the colony was monitored as described above and the last day a bee was recorded as entering or exiting the colony was used to calculate its lifespan.

Statistical Analysis

Generalized linear models were first used to test if the age of the individual, its colony of origin, odor used during the assay, and direction of odor presentation when applicable, had any effect on each cognitive measure, and the results largely ruled out any significant effect of these factors on the cognitive measures (Table A2.1). Only bees which had a CR score ≥ 1 in the associative learning assay were included in the analysis since the assays of the other cognitive measures were contingent upon the ability of a bee to learn a conditioned odor in the PER assay. The associations among the different cognitive traits were first assessed using Pearson correlations. A principal component analysis (PCA) was then performed (R package factoextra) to determine how the covariance among the different cognitive traits could define a cognitive axis. This was followed by a cluster analysis (R package NbClust) with squared Euclidean distances (k-means method) to group individuals based on their scores on the first two principal components. t-tests were then used to test whether these groups differed in their behavioral (proportion of time spent in nursing and social behavior) and life history (age of first foraging and survival) traits.

In order to compare the consistency of the cognitive axis, defined by the covariance among different cognitive traits, in bees from different colonies, linear models (R package lmertest) were constructed for those cognitive traits that showed high loadings on the first principal component axis. For each of these models, one cognitive trait was used as a response variable and another was used as a covariate, with colony as a fixed factor. In this approach (Michelangeli et al., 2019), a significant colony x covariate interaction indicates that the direction and magnitude of the correlation between a pair of cognitive traits differed among the colonies, while a non-significant interaction but a significant covariate indicates that the magnitude and direction of the correlation was similar among the colonies. For all analyses, data were mean centered due to the different units and variances of the cognitive measures. All analyses were performed in R version 3.4.1.

RESULTS

A Pearson correlation analysis revealed that associative learning ability had a significant positive association with both preference for novelty and preference for variance, which in turn were positively correlated with each other (Table 2.1).

The PCA found two principal components with eigenvalues greater than 1 describing the covariation among the different cognitive traits, with the first two principal components describing 40% and 21% of the total variance, respectively. Based on the loadings, the first principal component is described mainly by low associative learning, low preference for novelty and low preference for variance while the the second principal component is largely described by high sampling and high retention (Table 2.2).

The cluster analysis using the two PC scores for each bee revealed two distinct clusters of individuals (Kruskal-Wallis rank sum test: $\chi^2_{(1,36)} = 24.98$, $P < 0.001$), largely separated along the first principal component axis (Fig. 2.1). Cluster I, comprised of 22 (61%) individuals, therefore describes individuals with high associative learning, high preference for novelty and high preference for variance, while Cluster 2, comprised of 14 individuals (39%), describes those with low values for each of these three cognitive traits. Individuals in Cluster 1 and 2 therefore respectively conform to the descriptions of fast and slow cognitive phenotypes according to the model of Sih & Del Guidice (2012).

The t-tests comparing the differences in behavioral and life history traits between the two cognitive phenotypes showed that fast bees spent more time engaged in nursing behavior ($t_{(30.3)} = 4.78$, $P < 0.001$, Fig. 2.2A), but did not differ from slow bees in their display of social behavior ($t_{(27.4)} = 0.67$, $P = 0.50$, Fig. 2.2B). Fast bees also had a lower age of first foraging ($t_{(28.1)} = 2.08$, $P = 0.04$, Fig. 2.2C), but did not differ from slow bees in their lifespan ($t_{(29.8)} = 0.15$, $P = 0.88$, Fig. 2.2D).

There was no statistically significant colony-level differences in the covariation structure among the different cognitive traits, and in all the linear models, only the cognitive traits were significant covariates and no significant interaction effect was found between colony and any of these covariates (Table 2.3). This indicates that the magnitude and direction of each significant association between a pair of cognitive traits are similar across the colonies.

DISCUSSION

Our results indicate the presence of a suite of covarying cognitive traits, a cognitive axis, in honeybee foragers that is largely characterized by differences in associative learning, preference for novelty and preference for variance (risk). To the best of our knowledge, this is the first time that such a slow-fast cognitive axis has been demonstrated using multiple cognitive traits. Foragers differed in their expression of this cognitive axis, resulting in two distinct cognitive phenotypes, those that showed high associative learning and a high preference for novelty and risk (fast bees) and those that showed low associative learning and a low preference for novelty and risk (slow bees). These two different cognitive phenotypes also showed significant differences in their behavioral and life history traits such that the fast bees spent more time nursing and transitioned to becoming a forager at an earlier age. The consistency in the expression of the cognitive axis across colonies, albeit limited to three, strengthens our assertion about the presence of such distinct cognitive phenotypes in honeybee colonies.

The cognitive axis demonstrated here strongly meets the predictions of one based on a speed-accuracy trade-off (Sih & Del Giudice, 2012). However, two of the cognitive traits we measured - sampling and retention – were not found to be a part of this cognitive axis. Although both these traits loaded strongly on the second principal component axis, individuals did not show any substantial separation along this axis and therefore the contribution of these two traits to the cognitive axis could not be determined in this study. Among honeybee foragers, there are two well-known behavioral phenotypes, scouts that gather information and recruits that use that information (Seeley, 1983). Scouts are known to perform better on both non-associative learning (Cook et al., 2019) and reversal learning tasks (Carr-Markell & Robinson, 2014). While we did not measure it, reversal learning ability and differences in terms of it are likely to make a significant contribution to the cognitive axis and the resulting cognitive diversity within the colony (Dyer et al., 2014). We do not have information regarding the foraging behavior of our subject bees, but one could hypothesize that our fast bees are more likely to be the scouts while the slow bees act as recruits. It has been recently proposed that scouts and recruits are functionally equivalent to producers and scroungers (Katz & Naug, 2016), behavioral phenotypes that are known to be characterized by different learning abilities (Katsnelson et al., 2011). The results of this study are therefore consistent with the known behavioral differences among scouts and recruits in honeybees and adds to them by defining these two behavioral phenotypes in terms of two distinct cognitive phenotypes. A diversity in cognitive phenotypes within the colony that reflects differences in terms of a speed accuracy trade-off has been shown to make a positive contribution to colony performance (Chittka et al., 2003: Burns & Dyer, 2008).

Our results also indicate that the fast bees spent more time nursing and became foragers at an earlier age. These findings are consistent with the foraging syndrome described by Page et al. (2006), in which bees showing higher sensori-motor activity, higher associative learning, higher rates of behavioral development and earlier onset of foraging behavior are those which take up the task of pollen foraging. Although we did not identify our bees in terms of whether they were pollen or nectar foragers, it is possible that the cognitive phenotypes described here align with the foraging syndrome such that the fast bees are the ones who engage in pollen foraging. We could not find any existing data regarding whether pollen foragers also engage in more nursing at a young age, but the fact that pollen foragers perform better on associative learning tasks and have a higher sucrose sensitivity (Page et al., 2006), suggests that they may also have similarly high sensitivity to other stimuli such as brood pheromone and therefore do more nursing.

It was somewhat surprising that we did not detect a difference between the two cognitive phenotypes in terms of time spent in social behavior since one might expect fast bees with higher learning ability to act as scouts and be more likely to share information with other bees through trophallaxis – one of our measures of sociality. Similarly, the lack of a difference in survival between slow and fast bees was also unexpected, given that some recent work shows a positive association between associative learning and performance measures related to survival (Tait et al., 2019). However, since we did not measure foraging performance of our subject bees, we do not know if the foraging behavior of the two cognitive phenotypes in terms of foraging rates, foraging loads, etc. could elucidate the lack of difference in their survival. It is also possible that our description of cognitive phenotypes here based on multiple cognitive traits might obscure the earlier observed relationship between survival and associative learning alone.

It has been proposed (Sih & Del Guidice, 2012) that a cognitive axis defined by the speed-accuracy trade-off is a missing piece in the Pace-Of-Life Syndrome (POLS) hypothesis, a conceptual framework that predicts that individuals fall along a gradient of slow to fast life history that is integrated with suites of other traits at the physiological and behavioral levels (Ricklefs & Wikelski, 2002; Réale et al., 2010). Although our results provide mixed support for such a relationship between cognitive traits and other traits, our study is one of the first attempts in this direction. However, as the central premise of POLS hypothesis is based on slow individuals prioritizing future over current reproductive opportunities (Biro & Stamps, 2008**;** Réale et al., 2010), one probably needs to carefully consider how these different cognitive and behavioral traits measured at the individual level are linked to the expression of the same traits and performance at the colony level which is the unit of selection in social insects such as honeybees.

In summary, our results demonstrate that cognitive traits covary in honeybees such that it constitutes a cognitive axis that meets some of the predictions of the speed-accuracy tradeoff and that aligns with the POLS hypothesis. These cognitive phenotypes may reflect differences in forager social roles, and future research could directly compare scouts and recruits to determine how they differ in expression of the cognitive axis and related behavioral and life history traits. In addition, how these cognitive differences in individuals translate to group level decision making processes would represent an important extension of this work as well as determining the possible role of environment and ecology in shaping specific differences in this cognitive axis.

TABLES

Table 2.1. Pearson correlation matrix for all measured cognitive traits, with significant relationships shown in bold letters.

	Sampling	Novelty Preference	Retention	Variance Preference
Associative Learning Score	*r* = -0.22, *P* = 0.20	***r* = 0.36,** ***P* = 0.03**	*r* = 0.27, *P* = 0.12	***r* = 0.41,** ***P* = 0.01**
Sampling		*r* = -0.04, *P* = 0.83	*r* = 0.06, *P* = 0.73	*r* = -0.16, *P* = 0.34
Novelty Preference			*r* = 0.13, *P* = 0.47	***r* = 0.56,** ***P* < 0.001**
Retention				*r* = 0.07, *P* = 0.68

Table 2.2. The two principal components with eigenvalues > 1 with their rotated component loadings, eigenvalues, and the percentage of total variance explained.

Cognitive Trait	**PC1**	**PC2**
Associative Learning Score	-0.53	0.08
Sampling	0.22	0.66
Novelty Preference	-0.54	0.04
Retention Score	-0.22	0.72
Variance Preference	-0.57	-0.14
Eigenvalue	2.01	1.09
% Variance	0.40	0.21

Table 2.3. Generalized linear models testing the covariance between pairs of cognitive traits across colonies, with significant terms given in bold letters.

Model	**Term**	**df**	**Test Statistic**	**P-value**
Novelty Preference ~ Associative Learning Score	Associative Learning Score	1	$\chi^2 = 3.87$	**$P = 0.05$**
	Colony	2	$\chi^2 = 2.08$	$P = 0.35$
	Associative Learning Score x Colony	2	$\chi^2 = 1.06$	$P = 0.59$
Variance Preference ~ Associative Learning Score	Associative Learning Score	1	**$F = 6.99$**	**$P = 0.01$**
	Colony	2	$F = 0.14$	$P= 0.87$
	Associative Learning Score x Colony	2	$F = 1.17$	$P= 0.32$
Novelty Preference ~ Variance Preference	Variance Preference	1	$\chi^2 =15.07$	**$P < 0.001$**
	Colony	2	$\chi^2 = 3.60$	$P = 0.16$
	Variance Preference x Colony	2	$\chi^2 = 0.62$	$P = 0.73$

FIGURES

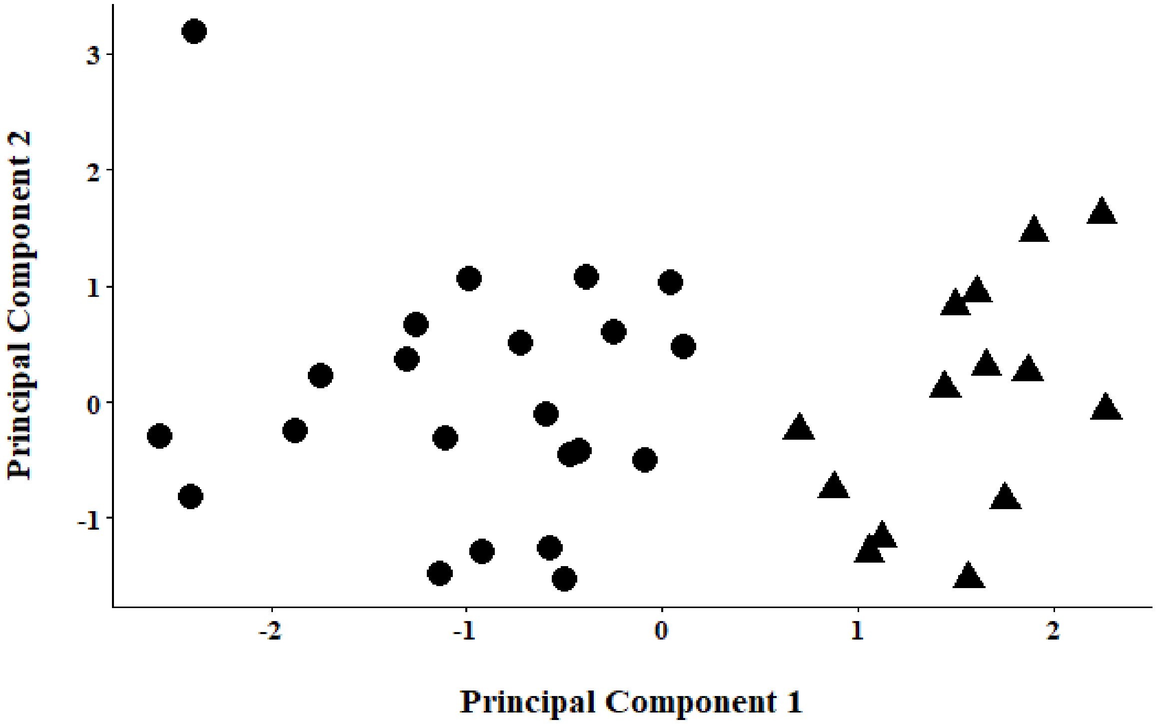

Figure 2.1. Cognitive phenotypes in honeybees defined by two clusters of individuals based on their positions in the coordinate space defined by the first two principal components, in which circles refer to Cluster 1 or Fast cognitive phenotypes and triangles refer to Cluster 2 or Slow cognitive phenotypes.

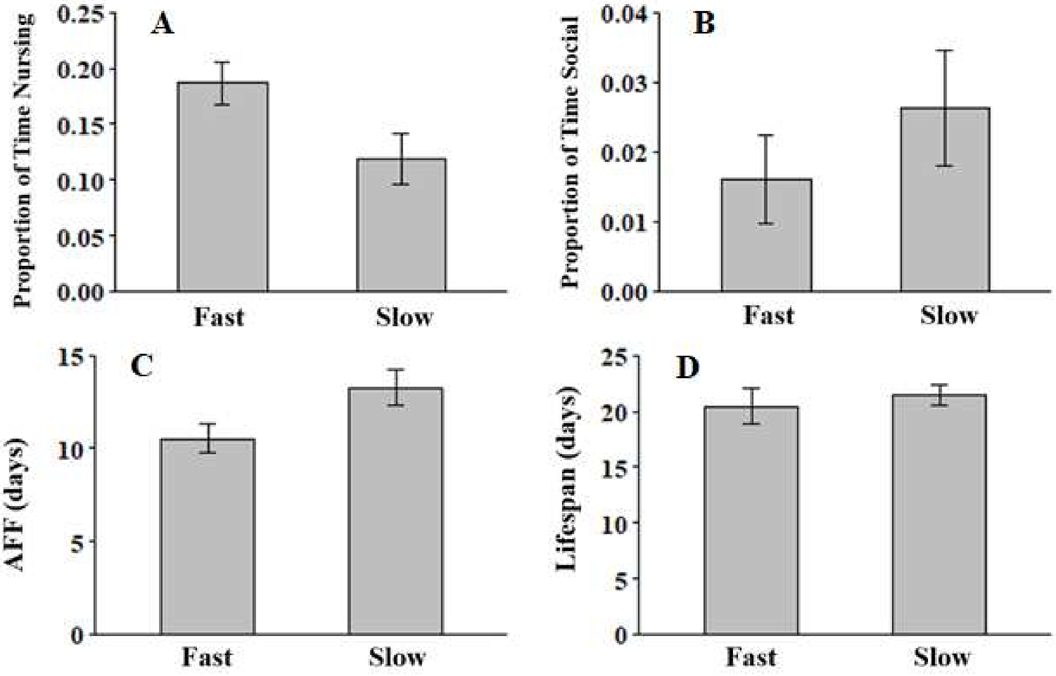

Figure 2.2. Behavioral and life history differences between fast (*N* = 22) and slow (*N* = 14) cognitive phenotypes in terms of A) nursing behavior, B) social behavior, C) age of first foraging, and D) lifespan. Bars represent mean ± SE.

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CHATPER 3

Nesting ecology does not explain cognitive differences among species

INTRODUCTION

The evolution of cognitive variation and how it determines differences in behavior is a question of fundamental interest in animal behavior (Dukas, 2004). There is strong evidence that cognitive traits evolve as a function of differences in ecology and life history and factors such as the complexity of the physical environment, social structure, diet and mating behavior have been identified as possible explanations for cognitive differences among species (Macphail & Bolhuis, 2001; Bolhuis, 2005; Healy et al., 2009; Chittka et al., 2012; Cauchoix & Chaine, 2016). Although comparative studies have greatly enhanced our understanding of the ecological factors that drive variation in cognition, these studies are generally limited to looking at variation in a single cognitive trait – learning ability. This limits our understanding regarding how ecological differences might drive variation in the overall cognitive phenotype which comprises of multiple other cognitive traits such as risk sensitivity, neophilia, sampling tendency, impulsivity, etc. (Sih & Del Giudice, 2012). It has been suggested that measuring how multiple cognitive traits covary to influence behaviors may provide a more complete understanding of the link between cognitive and behavioral variability (Griffin et al., 2015). While some recent studies have used this approach at the intraspecific level (Keagy et al., 2009; Mazza et al., 2018; Tait & Naug, 2020), measuring such covariation patterns across related species is key to understanding how differences in ecology can shape cognitive differences.

Recently, both intra- and interspecific differences regarding a variety of phenotypic traits have been described with a theoretical framework known as the Pace-Of-Life Syndrome, in which individuals are placed along a slow-fast axis, each end of which is associated with a suite of physiological, behavioral and life history traits (Réale et al., 2010). It has been proposed that this slow-fast pace-of-life axis is aligned with a slow-fast cognitive axis described by the speed-accuracy tradeoff, which predicts fast individuals to show higher levels of learning, risk, sampling, neophilia and impulsivity, leading them to make more rapid but somewhat more inaccurate decisions, compared to slow individuals (Sih & Del Giudice, 2012). There is indeed some recent evidence at the intraspecific level that links slow-fast differences in behavior and life history with cognitive differences that correspond to slow and fast cognitive phenotypes (Tait & Naug, 2020; Amy et al., 2012). However, it has never been tested whether a slow-fast cognitive axis comprised of multiple cognitive traits is consistent across related species and if interspecific differences in cognitive traits fall along a slow-fast gradient that match similar differences in behavior and life history among them (Fig. 3.1). Interspecific comparisons of cognitive traits using such an approach can begin to shed light on the role of ecological factors in driving the evolution of slow-fast cognitive phenotypes.

Honeybees as a taxonomic group are an attractive prospect for such studies of comparative cognition due to the well-established status of *Apis mellifera* as a model with a rich cognitive repertoire that can be rigorously measured under both laboratory and field conditions (Menzel, 2012). The existence of interindividual cognitive variation is well-documented in *A. mellifera* (Raine & Chittka, 2008; Mayack & Naug, 2012; Smith & Raine 2014; Katz & Naug, 2015; Tait et al., 2019), variation that is known to have a significant influence on foraging performance and other life history traits (Tait & Naug, 2020; Page et al., 2006). However, our extensive knowledge regarding such cognitive variation is largely limited to *Apis mellifera*, with little known about the cognitive traits of other honeybee species such as *A. cerana, A. florea* and *A. dorsata* (Kaspi &

Sharoni, 2013; Tan et al., 2015), each of which has a distinct ecology related to their nesting behavior (Seeley, 1982). Of the tropical species, *A. cerana* nests in cavities similar to *A*. mellifera, while *A. dorsata* and *A. florea* nest in the open, utilizing a layer of bees-commonly referred to as the curtain-to protect the comb. Previous comparative research suggests that the need to maintain a large worker population in the open nesting species results in longer lived workers with a slower behavioral "tempo" than the closed nesting species. Workers of open nesting species are predicted to be more cautious and perform behavioral tasks (e.g. foraging) at a slower rate, ultimately reducing mortality (Dyer & Seeley, 1991; Bhagavan et al., 2016; Bhagavan & Brockmann, 2019). It has also recently been shown that a large number of such behavioral and life history traits covary to define slow–fast phenotypes in *A. mellifera* (Mugel & Naug, 2020). In this study, by measuring multiple cognitive traits in the four species of honeybees, we therefore test whether cognitive traits similarly covary to define a consistent slow-fast cognitive axis and distinct cognitive phenotypes in all the species and whether any cognitive differences among them are correlated to the known differences in their behavioral tempo, life history and nesting ecology.

METHODS

The cognitive assays with *Apis mellifera* were conducted with bees from colonies ($N = 3$) maintained in Fort Collins, Colorado. Capped brood was extracted from a colony one day prior to adult emergence and kept overnight in an incubator set at 32°C. Upon emergence, adults ($N = 36$) were transferred to a queen-right observation hive. The assays with the three other honeybee species were conducted with their colonies (2 colonies of *A. cerana*, 3 colonies of *A. florea* and 1 colony of *A. dorsata*) maintained in the campus of the National Center for Biological Sciences (NCBS) in Bangalore, India. Due to the general propensity of *A. dorsata* for nesting in difficult, often remote, locations, it was possible to train and collect foragers from only one colony of this species. Additionally, because the logistic difficulties of extracting and reintroducing brood to the open nesting species, none of the foragers were not age marked. All bees used for the assays were collected at a sucrose feeder away from the colonies, to ensure that they were foragers, immobilized on ice, and harnessed within a plastic tube. All bees (N = 36, *A. cerana*: N = 17, *A. dorsata*: N = 41, A. *florea*: N = 37) was fed to satiation with 30% sucrose solution and then starved for 24 hours in an incubator set at 27° C to increase their motivation for the following cognitive assays.

a) Associative Learning: The associative learning ability of an individual bee was determined using the Proboscis Extension Reflex (PER) assay. It consists of presenting a bee with an odor (Conditioned Stimulus, CS) for ten seconds and a sucrose reward (Unconditioned Stimulus, US) simultaneously after five seconds, resulting in an overlap of CS and US for five seconds, in a series of trials (Bitterman et al., 1983). Our PER assay consisted of presenting each bee with six such trials with a 5-minute inter trial interval (ITI), with hexanol, octanone, geraniol and linalool as the CS odors used in different replicates. A bee is considered to have learned the association between the CS and the US when it extends its proboscis (a Conditioned Response, CR) to the CS prior to the US delivery, and the total number of CR is used as a measure of its associative learning ability.

b) Sampling and Preference for Novelty: 30 minutes following the associative learning assay, each bee was subjected to a forced-choice proboscis extension response assay (Tait & Naug, 2020; Shafir et al., 1999). This assay consists of presenting two different odors in four alternating 0.2 s pulses to the subject on either side of its head, after which the individual is allowed an additional 30 seconds to sample the odors. Based on the orientation of its head and the extension of its proboscis at the end of the four odor pulses, a choice for one of the two odors is scored for the subject. Using the odor that was paired with the reward during the associative learning assay as one of the two odors and a novel odor as the other odor, preference for novelty is recorded as a binary score (1 or 0) and the number of times an individual turned its head toward each odor during the entire sequence of the four odor pulses provides a measure of sampling (Tait & Naug, 2020; Katz & Naug, 2015). The pairing of each odor as the novel odor and the direction of each odor was balanced across experimental replicates to account for any possible odor and side biases. Following the sampling and novelty preference assay, bees were fed to satiation and maintained in a dark incubator set at 27° C for 24 hours.

c) *Preference for Variance (Risk):* 24 hours after the novelty assay, a PER assay was used to train the bees to associate two different odors with two different reward distributions. This consisted of pairing one odor with a variable 30% sucrose reward and another with a constant 30% sucrose reward and presenting these pairings to each bee using a micro syringe in a predetermined pseudorandom sequence in a series of 20 trials with an ITI of 5 min (Tait & Naug, 2020; Shafir et al., 1999). In a trial in which the subject was presented with the odor that was paired with the variable reward, it received either a high reward of 0.4 µl or no reward (0 µl) in a predetermined pseudorandom sequence such that the overall probability of obtaining each reward type was 0.5. In a trial in which the subject was presented with the odor paired with the constant reward, it always received a 0.2 µl reward. Therefore, both the constant and the variable reward distribution had a mean of 0.2, but the variable one had a coefficient of variation (CV) of 100. The preference for variance for an individual was calculated as the number of times it extended its proboscis to the variable reward, divided by the total number of times it responded to the two rewards.

Statistical Analysis

Generalized linear models were first used to test for the effects of the different odors used and the direction of odor presentation wherever applicable. Generalized linear models were used to test for differences in each of the four cognitive traits across the four species. A principal component analysis (PCA) was then performed separately for each species to determine the covariance among the four different cognitive traits within each species and if such a covariance pattern could be used to define a cognitive axis (R package *stats*). All data were mean-centered and standardized for PCA analysis. A canonical variate analysis (CVA), which determines the association between multiple variables and can maximize the separation between *a priori* defined groups in a multivariate space, was then used to group the four species according to their performance on the four different cognitive traits (R package Morpho). The first two canonical variate components for all the individuals were then represented as a bivariate scatterplot and the centroid for each species was calculated as a geometric mean of all the individuals of that species. While both PCA and CVA are similar techniques that are used in multivariate analysis to reduce the number of variables, CVA maximizes the variance explained between groups (in this case, species) while PCA maximizes the variance explained among individuals (Carter & Feeney, 2012).

A multi-response permutation procedure (MRPP, R package vegan), a technique that calculates whether there is a difference between defined groups (species here) was used to determine the extent of cognitive dissimilarity among the four species. Groups that are clustered in multidimensional space have lower average distances, δ, to their group centroid than their inter-group centroids, and therefore are dissimilar to the other groups. To assess the fit of each individual to its *a priori* defined group, the group membership of each individual was randomized in each permutation and the resulting δ values (δ_{exp}) were compared to the observed δ (δ_{obs}) to calculate a p-value. Within group agreement, *A*, was calculated as 1-(δ_{obs})/ (δ_{exp}) to measure how well the individuals fit within their respective groups. If *A* is 1, all individuals within a group are identical, and it is 0 if all individuals behave randomly. All analyses were performed in R version 3.4.1.

RESULTS

An analysis of variance (ANOVA) revealed significant differences in associative learning ($F_{3,127} = 10.6$, $P < 0.001$) and sampling frequency ($F_{3,127} = 7.53$, $P < 0.001$), but not in preference for novelty ($\chi^2_{3,127} = 5.54$, $P = 0.13$) or preference for risk ($F_{3,127} = 1.77$, $P = 0.15$) among the four species (Fig. 3.2). In general, the *A. cerana* and *A. florea* were more similar to each across all four tasks than to *A. dorsata* or *A. mellifera.*

PCA Analysis

The variance explained by the first two principal components was similar in each of the four species (Table 3.1). The covariance structure among the different cognitive traits, defined by the positive or negative loadings that described the first principal component, was also largely similar. On average, preference for novely had the highest loading (0.56), followed by associative learning (0.48), sampling (0.43) and risk preference (0.38). The covariance among these three traits were also consistent in three of the four species, showing a positive covariance between preference for novelty and associative learning and their negative covariance with sampling. *A. dorsata* showed a different covariance structure with preference for novelty negatively covarying with both associative learning and sampling. The covariance structure among the traits in the second principal component was much more inconsistent across the four species.

CVA Analysis

The covariance structure revealed by the CVA analysis mirrored the one from the PCA analysis to some extent with the first canonical variate showing a positive covariance between preference for novelty and associative learning, but both showing a negative covariance with preference for risk (Table 3.2). Based on the first canonical variate, the largest differences between the species are defined by their preference for risk and their associative learning ability, resulting in two distinct clusters of species, with one group composed of *A. florea* and *A. cerana* showing higher risk preference and lower associative learning ability compared to the other group comprised of *A. dorsata* and *A. mellifera* (Fig 3.3A). The MRPP supported the presence of these two distinct groups of species (Fig. 3.3B) that differed significantly in term of their cognitive traits (MRPP: $A = 0.08$, observed $\delta = 3.97$, expected $\delta = 4.24$, $P < 0.01$).

DISCUSSION

Out results, albeit in only four species, indicate that there is evidence of a similar suite of covarying cognitive traits in the four honeybee species. The slow-fast cognitive axis observed here is based on a positive covariation between associative learning ability, preference for novelty and risk, which meets the predictions of a speed-accuracy tradeoff (Sih & Del Giudice, 2012), and results in ‘slow’ and ‘fast’ cognitive phenotypes that were also earlier seen independently in *A. mellifera* (Tait & Naug, 2020). In this cognitive axis, high associative learning and high preference for novelty and risk define a ‘fast’ cognitive phenotype, while low associative learning and low preference for novelty and risk define a ‘slow’ cognitive phenotype (Sih & Del Giudice, 2012). Although *A. dorsata* exhibited slight differences regarding this axis, the overall relationships between the cognitive traits remained surprisingly consistent across the different species. The repeated presence of slow and fast cognitive phenotypes in the different species lends support to the consistency of such a cognitive axis and indicates a likely adaptive basis to this phenotypic diversity. Among honeybee foragers, two behavioral phenotypes have been described: scouts that gather information and recruits that use that information (Seeley, 1983), which are thought to be functionally equivalent to producers and scroungers (Katz & Naug, 2020). These two foraging types are also characterized by differences in learning abilities such that scouts perform better on learning tasks (Carr-Markell & Robinson, 2014; Cook et al., 2019). Although we did not measure foraging behavior, it is possible that foragers with a fast cognitive phenotype more frequently adopt scout behavior than slow bees. This could have important implications for fitness as a positive effect of cognitive diversity have been reported in a foraging context in honeybees (Dyer et al., 2014)

We also found evidence in support of a scenario in which each honeybee species occupies a unique position on this cognitive axis such that some species are relatively slow and some are fast. However, it is interesting that the covariance structure at the within-species level was not fully consistent with the one at the between-species level where the strength of association between associative learning and risk preference was weaker. The higher importance of risk in driving the interspecific differences in cognition suggests that future research should closely examine the relationship between risk-preference and the ecology of these species, especially since honeybees have been found to modulate their foraging behavior in response to predation risk (Reader et al., 2006; Tan et al., 2013). While the relationship between risk-prone decision-making with regard to resource variability and risk-prone foraging has not been explored, it is interesting to consider that a similar cognitive mechanism may underlie these traits and further research is needed to better understand how individuals interpret risk in different contexts. The four species also did not align on the slow-fast cognitive axis as what would be predicted based on the known differences in behavior and life history among them, which are in turn correlated to their nesting ecologies.

Based on their longer worker lifespan that in turn is associated with lower mortality, it has been suggested that the open nesting *A. dorsata* and *A. florea*, are the two 'slow' species compared to the cavity nesting *A. mellifera* and *A. cerana*, the two 'fast' species (Dyer & Seeley, 1991). Our results however indicate that it is *A. cerana* and *A. florea* which cluster together as a group that is significantly more risk-prone than *A. mellifera* and *A. dorsata*. If nesting ecology is not correlated to the observed cognitive differences among the four species, it becomes important to consider other differences among them such as body size and metabolic rate that are considered important in driving slow-fast phenotypic differences (Réale et al., 2010; Biro & Stamps, 2008). However, metabolic rate is an unlikely explanation for the observed cognitive differences because differences in metabolic rate are also correlated to nesting ecology with the two cavity nesting species showing higher mass specific metabolic rate than the two open nesting species (Dyer & Seeley, 1991).

In terms of body size, *A. dorsata* and *A. mellifera* are significantly larger than *A. cerana* and *A. florea* (Dyer & Seeley, 1991). Related to the possible influence of these size differences, but one that is more pertinent to cognition, is an interesting difference among the four species in terms of brain size that is correlated to the observed difference in their cognition. Using the data of Gowda & Gronenberg (2019) collected at the Indian Institute of Science in Bangalore, India, and comprised of absolute brain volumes collected via dissection and imaging a cluster analysis of the absolute brain volume of each species (*A. dorsata*, N = 8, *A. florea* N = 8, *A. cerana,* N =

8, *A. mellifera*, N = 8) results in two distinct clusters where the two larger species have significantly larger brain size than the two smaller species (Kruskal Wallis rank sum test: $\chi^2_{1,\,31} = 22.51$, $P < 0.001$, Fig. 3.4). The two species with significantly larger brains, *A. mellifera* and *A. dorsata*, are the species which are more risk-averse and show higher associative learning, compared to *A. cerana* and *A. florea*, which have smaller brains. While differences in brain size among the four species can be attributed to isometric scaling related to their differences in body size, some recent evidence suggests that absolute brain size may be a better predictor of performance on cognitive traits than brain size corrected for body mass, especially among closely related species (Deaner et al., 2007; Herculano-Houzel, 2011; MacLean et al., 2014). While the relationship between brain size and specific cognitive traits is not well-known, the positive relationship between brain size and learning ability observed here has been widely reported (Reader & Laland, 2002; Buechel et al., 2018; but see Chittka & Niven, 2009; Lihoreau et al., 2012).

In social insects, spatial learning and sensory ecology related to foraging is known to have played a significant role in the evolution of brain size (Farris, 2016), and there is evidence that increased experience processing visual information increases the size of mushroom bodies, the neural regions responsible for higher order cognitive processing (Durst et al., 1994). In line with this hypothesis, there are large differences in foraging habit among the four honeybee species (Seeley, 1982; Dyer & Seeley, 1991). Of the two species with larger brains, *A. mellifera* inhabits a more ephemeral foraging environment, known to be correlated with increases in brain size (Roth & Pravosudov, 2009; Roth et al., 2010), while *A. dorsata* has a much larger foraging range relative to *A. cerana* and *A. florea*. It is therefore possible that the complexity of foraging environment has played a role in the observed differences in brain size and cognitive traits among the four honeybee species. Although we measured olfactory learning, not visual, it is intriguing to hypothesize that the demands of synthesizing multi-faceted environmental cues (visual, olfactory, tactile) may have impacted cognitive ability of these species. The role of ecological factors such as foraging in complex spatiotemporal environments on the evolution of brain size is a major question in cognitive ecology (Sherry et al., 1992; Rosati, 2017; Tello-Ramos et al., 2019), although we know little about such relationships in these various honeybee species and it would be interesting to investigate how foraging ecology shapes the specific traits comprising the slow-fast cognitive axis.

In summary, our results demonstrate that different cognitive traits covary consistently in the four honeybee species and largely meet the predictions of the speed-accuracy tradeoff. The covariance among these cognitive traits that result in slow-fast cognitive phenotypes within each species may reflect selection for cognitive diversity in colonies, and future research should explore how such differences relate to colony performance in these four species. We found partial support for the hypothesis that there are slow-fast cognitive differences among the four species, but these differences were not correlated to the known differences in behavior, life history and nesting ecology among them. Instead, we propose that these differences in cognitive traits among the four species may, in part, be explained by differences in their brain size and foraging ecology. Future research could explore the specific ecological factors that influence cognitive differences among the four species and how they translate to differences in their behavior and life history.

TABLES

Table 3.1. Principal components with eigenvalues ≈ 1 and their rotated component loadings, and the percentage of total variance explained for each species.

	A.mellifera		***A. cerana***		***A. dorsata***		***A. florea***	
Trait	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Associative Learning	0.51	-0.17	0.57	-0.23	-0.21	-0.76	-0.63	0.37
Sampling	-0.27	0.88	-0.54	-0.37	-0.65	0.13	0.28	0.55
Preference for Novelty	0.55	0.38	0.35	-0.78	0.65	0.08	-0.69	0.06
Preference for Risk	0.59	0.20	-0.48	-0.42	0.32	0.62	-0.16	-0.74
Variance explained	0.48	0.24	0.46	0.25	0.49	0.27	0.36	0.27
Eigenvalue	1.95	0.97	1.84	1.01	1.67	1.08	1.45	1.10

Table 3.2. The two canonical variates and their representative behaviors.

Cognitive Trait	**CV1**	**CV2**
Associative Learning	-0.52	-0.31
Sampling	0.19	-0.21
Preference for Novelty	-0.23	-1.26
Preference for Risk	1.39	-0.82
% Variance Explained	0.95	0.04

FIGURES

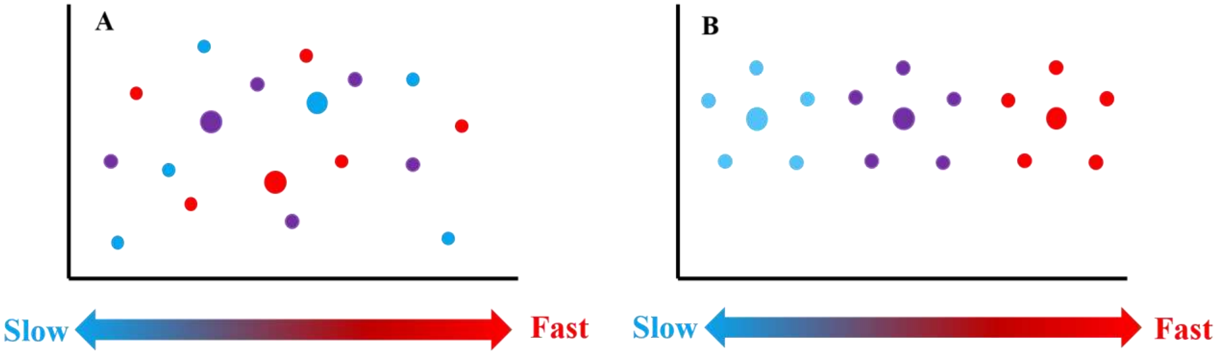

Figure 3.1. Possible alternative models describing the position of individuals from three hypothetical species along a slow-fast cognitive axis described in a two-dimensional cognitive trait space, where small dots of different colors represent individuals of different species and large dots represent the respective species means. Model A depicts a scenario where individuals of each species are similarly distributed along the entire slow-fast cognitive axis while model B depicts a scenario where individuals of each species occupy a unique position on the cognitive axis such that some species are relatively slow and some are relatively fast in terms of their cognitive phenotype.

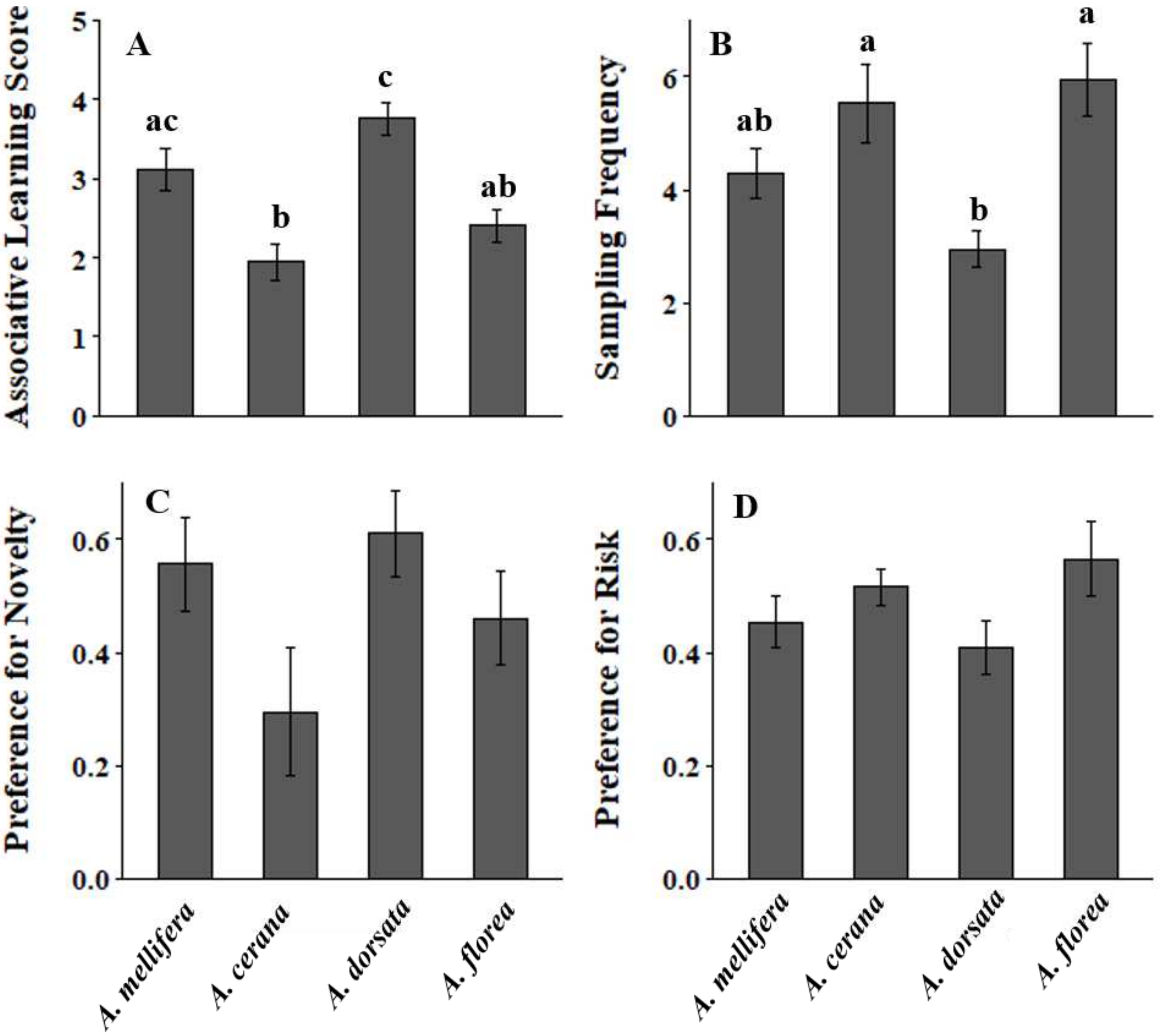

Figure 3.2 A-D. Cognitive differences among four honeybee species (*A. mellifera*: *N* = 36, *A. cerana*: *N* = 17, *A. dorsata*: *N* = 41, A. *florea*: *N* = 37) in terms of a) associative learning, b) sampling frequency, c) preference for novelty, and d) preference for risk. Bars represent mean ± SE with significant differences shown with different letters. (Pos-hoc comparisons in Table A3.1).

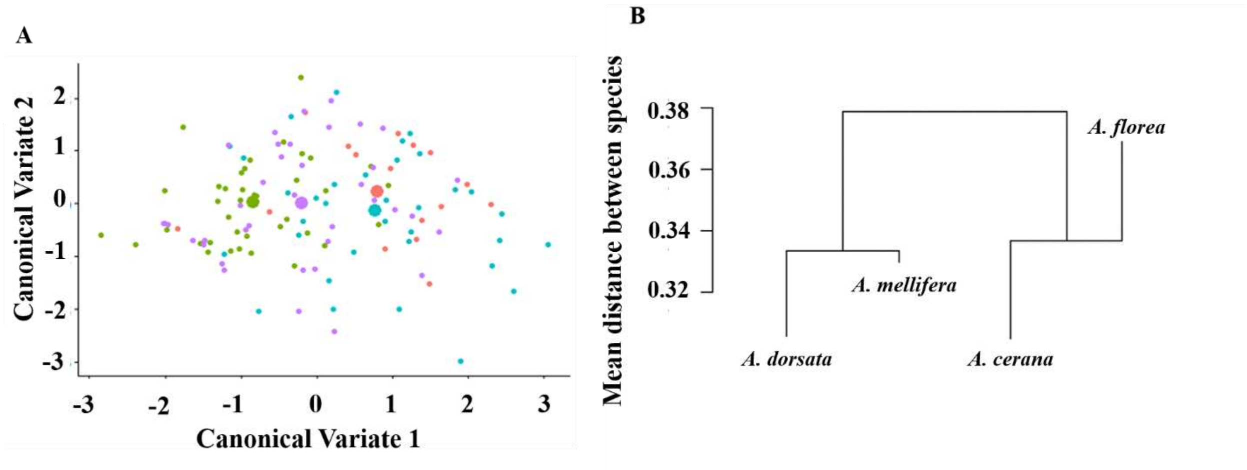

Figure 3.3. The position of the four honeybee species in a multivariate cognitive trait space defined by two canonical variates and represented as, (A) a bivariate plot where each smaller point represents an individual honeybee and the four larger points indicate the respective centroids for each species (*A. mellifera*: purple, *A. dorsata*: green, *A. florea*: blue, *A. cerana*: pink), and (B) a dendrogram using mean distances between species in the cognitive trait space, where horizontal lines indicate mean dissimilarity between species while length of termini indicate dissimilarity of a species within the cluster, with termini pointing upward indicating species that are more heterogenous than the combined cluster.

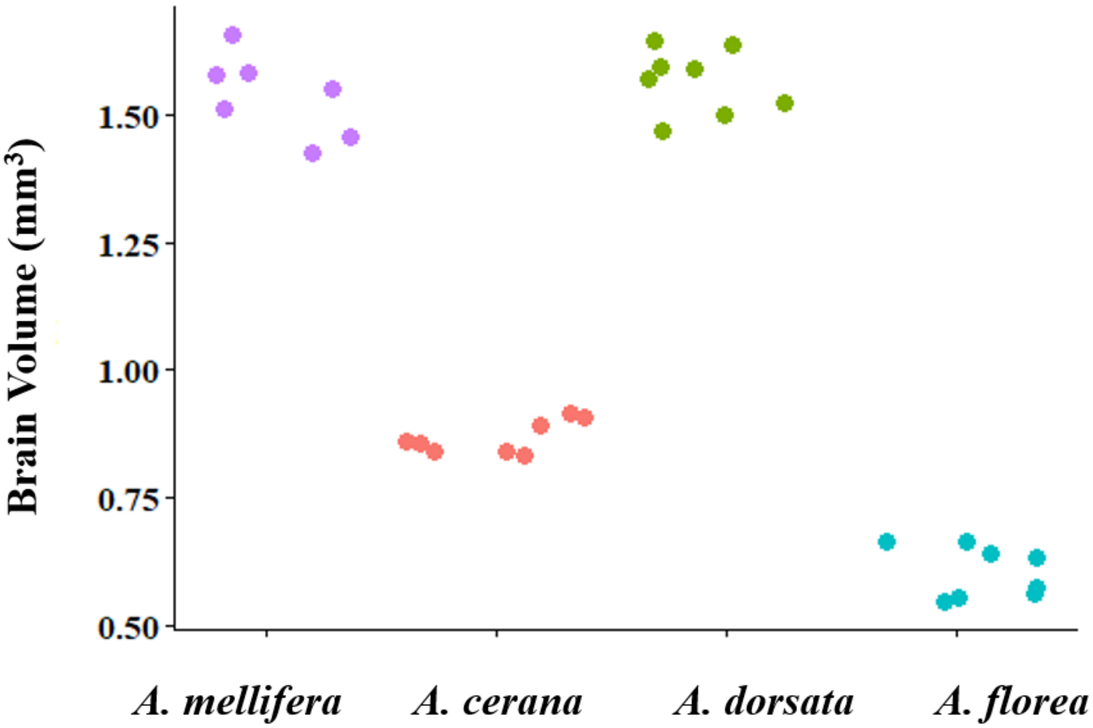

Figure 3.4. Brain volume differences among four species of honeybees, with points representing individuals. Brain volume for each species was derived from the dataset of Gowda & Gronenberg, (2019).

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CHATPER 4

Interindividual variation in use of social information on a learning task regulates the speed-accuracy trade-off in honeybee foragers

INTRODUCTION

Animals can acquire information about their environment through either personal experience (individual learning) or by observing their social conspecifics (social learning). The propensity of an individual to use these two types of information has largely been modeled based on an economic analysis of both (Kendal et al. 2005). Personal information, while being more reliable, is likely to be more costly to acquire than social information (Laland et al. 1993, Laland, 2004). However, social information may be scarce and conflict with personal information, reducing its value (Giraldeau et al., 2002, Rieucau & Giraldeau, 2011). While the research on social learning has mostly focused on whether animals possess the cognitive ability to learn from tutors, what is more often overlooked is that there could be individual differences in the propensity to use these two types of information.

The recent interest in interindividual variation in various levels of phenotypic traits in terms of a slow-fast axis (Sih et al., 2004; Réale et al., 2010) has led to the proposal that such an axis is largely derived from a speed-accuracy tradeoff in decision-making (Carere & Locurto, 2011; Dougherty & Guillette, 2018; Sih & Del Guidice, 2012). This is based on the idea that more immediate rewards can result from fast decisions even though they are subject to lower levels of accuracy while slower, more accurate decisions can lead to gains in the longer term at the cost of immediacy. In this framework, fast individuals are predicted to show higher performance on learning new tasks with lower levels of sampling, but which also leads to slower reversal learning and poor retention, relative to slow individuals (Sih & Del Guidice, 2012). While there is some recent empirical evidence that supports the existence of such fast and slow cognitive phenotypes and how they correlate to differences in other phenotypic traits (Guillette et al., 2009; Bebus et al., 2016; Mazza et al., 2018; Tait & Naug, 2020), whether these different cognitive phenotypes also differ in their ability to utilize personal and social information remains largely unknown. The usage of these two types of information, which have different costs associated with them, might also lead to differences in the dynamics of the speed-accuracy trade-off between individual and social learning, although, to the best of our knowledge, this has never been explicitly addressed.

The preferential use of social information is predicted to correlate to the propensity of an individual to engage in prosocial behavior, and a few studies have documented individual differences in the tendency to use either social or personal information (Rosa et al., 2012). However, whether these tendencies are also part of the differences between slow-fast cognitive phenotypes is poorly understood. Since behavioral traits that are associated with cognitive differences have been shown to impact social information use (Boogert et al., 2006), it is interesting to ask if there is interindividual variation in these two types of information use and if the parameters of individual and social learning differ in a manner that leads to differences in the speed-accuracy tradeoff between these two contexts. In social groups, the usage of these two types of information, and thereby two different modes of learning, is likely to be correlated to the phenotypic variation between producers and scroungers, leading individuals to occupy distinct social roles (Katsnelson et al., 2011; Aplin & Morand-Ferron, 2017).

In honeybees, the two well-known behavioral phenotypes, scouts and recruits, which are analogous to producers and scroungers, differ in their use of personal and social information when making foraging decisions (Katz & Naug 2016). More recently, it has been shown that these two behavioral phenotypes might also be correlated to the two cognitive phenotypes on the slow-fast axis that is associated with differences in learning abilities (Tait & Naug 2020). Honeybees are known to be adept at individually learning complex combinations of various parameters such as color, shape and patterns to discriminate rewarding and unrewarding flowers in a foraging context (Menzel & Giurfa 2001; Giurfa, 2015). Surprisingly, whether they show social learning in a similar context has not received as much attention. The use of social information during foraging in a patch is, however, well documented in many other social insects (Leadbeater & Chittka 2007a), even though there is little information regarding whether there is any individual-level variation in these two types of information usage and thereby two types of learning. In this study, by measuring the learning rate and accuracy of individual honeybee foragers on a discrimination task in both an individual and a social context, we therefore tested a) if there is a difference in the speed-accuracy trade-off between these two contexts, and b) if there is individual-level variation among honeybees in terms of how they differ in their individual and social learning abilities.

METHODS

The bees used in the experiment came from 4 source colonies of the honeybee, *Apis mellifera*. Brood frames with pupae were collected one day prior to adult emergence and kept in an incubator set at 32° C. Upon emergence, individual adult bees were marked with paint on their thorax and introduced into an experimental hive that consisted of two brood frames, a full honey frame, a laying queen and workers, and was located in a dark room, connected to the outside with a tube.

Individually marked foragers were trained to a small foraging arena within an enclosure constructed of PVC pipes and clear, plastic curtain on all sides with an entrance flap such that one bee at a time could be introduced and made to forage in the arena so that the performance of each bee could be tested without the influence of other bees. The foraging arena consisted of 20 randomly placed artificial flowers, each filled with 20μL of either 30% sucrose solution (rewarding flower) or water (unrewarding flower). The rewarding and unrewarding flowers were each assigned a different color (pink, blue, yellow, purple), which was randomized across different replicates to control for any color bias. Between successive foraging bouts, the flowers were puffed with pressurized air to clean them of any odor cues left by a foraging bee and were refilled using an automated mechanism.

For each foraging bout by a bee in the arena, its visits to the two types of flowers were recorded. A bee could visit as many flowers as it wanted in each bout and was allowed to end each bout when it chose to exit the arena to return to the hive. The first 20 visits to the different flowers by a bee were recorded, which usually required 3-5 foraging bouts, following which the bee was excluded from the arena, so that the choice behavior of different bees could be compared on a similar scale. Visits to the rewarding color flower were considered correct decisions while visits to unrewarding flowers were incorrect decisions.

Using this choice assay, each bee was sequentially presented with two different learning tasks, an individual learning task and a social learning task. In the individual learning task, the bee made the choice between the two flowers on its own while in the social learning task the bee made the choice aided by an additional social cue - a single model (dead) bee attached to one of the rewarding flowers, based on the experimental design of Leadbeater & Chittka (2007b). Once a bee completed one learning task, the colors of the rewarding and unrewarding flowers were both changed to two new colors and the bee was presented with the other learning task. A bee completed the two learning tasks over two days, one task per day, and the order of the two learning tasks was randomized across individuals.

Statistical Analysis

For every individual, the raw data consisted of 20 successive choices between rewarding and unrewarding flowers in each of the two learning tasks. From these data, a learning curve was created for each bee on each task by calculating an accuracy index for each of the 20 flower visits as the proportion of correct choices for the rewarding flowers in the 5 visits up to and including that visit (Leadbeater & Chittka, 2007b). A logistic function was then fit to this moving accuracy index against flower visit number (Prism 9, Graphpad software) to calculate the learning parameters for each bee on each task,

$$f(x) = \frac{Y}{1 + e^{(-kx)}}$$

where and Y is the maximum accuracy, x is the flower visit number, k is the slope of the learning curve, and $1/k$ is the inflection point, which we define here as a measure of latency to learn (see Fig 4.1. as an example).

Linear mixed models were used to test for any differences in the learning rate, latency and maximum accuracy between the individual and social learning tasks where one of the three learning parameters was the response variable, task (individual or social) was the predictor and bee identity, age and colony of origin were random effects. To determine how learning rate and latency affected maximum accuracy, two separate linear mixed models were used, one for individual learning and one for social learning, where maximum accuracy was the response variable with either learning rate or latency as the predictors and age and colony of origin as random effects. Finally, Pearson's correlations were used to determine whether performance on each of the three learning parameters were correlated between the individual and social contexts.

RESULTS

Linear mixed models revealed significant differences in maximum accuracy ($\chi^2 = 18.49$, $P < 0.001$), but there were no differences in learning rate ($\chi^2 = 0.92$, $P = 0.33$) or latency ($\chi^2 = 0.03$, $P = 0.84$; Fig. 4.2) between the individual and social learning tasks. Maximum accuracy was higher on the social learning task than the solitary learning task.

For the individual learning task, learning rate negatively affected maximum accuracy ($\chi^2 = 9.74$, $P = 0.001$, Fig. 4.3A) and latency had a positive effect on maximum accuracy ($\chi^2 = 4.04$, $P = 0.04$, Fig. 4.4A). However, for the social learning task, there was no significant relationship between learning rate and maximum accuracy ($\chi^2 = 1.02$, $P = 0.31$, Fig. 4.3B) or latency and maximum accuracy ($\chi^2 = 1.15$, $P = 0.28$, Fig. 4.4B).

There was a significant negative relationship in the maximum accuracy acquired in the two learning contexts ($r = -0.46$, $t_{1,43} = 3.42$, $P = 0.001$, Fig. 4.5), and bees that had high accuracy on the individual learning task had low accuracy on the social learning task and *vice versa*. None of the two other learning parameters showed a significant relationship between the individual and social contexts (Learning Rate: $r = 0.01$, $t_{1,43} = 0.06$, $P = 0.94$; Latency: $r = -0.03$, $t_{1,43} = 0.18$, $P = 0.86$).

DISCUSSION

Our results provide support for a context dependent speed-accuracy trade-off in individual honeybees that may also reflect the existence of two distinct cognitive phenotypes composed of individuals that prioritize different types of information during foraging. To the best of our knowledge, this is the first demonstration of interindividual variation in the expression of a speed-accuracy trade-off in two different learning contexts. Foragers differed in the rate of learning and latency to learn, which likely reflects sampling, such that those with a slow learning rate and more extended sampling had higher maximum accuracy than those with a fast learning rate and shorter sampling. This trade-off between learning rate and accuracy was however only expressed during the individual learning task and not during the social learning task. We also found that the maximum accuracy on the individual and social learning tasks constituted a trade-off such that foragers which acquired high accuracy on the individual learning task had low accuracy on the social learning task, and *vice versa*, and that maximum accuracy on the social learning task was greater than the individual learning task.

The relationship of learning rate and sampling with maximum accuracy demonstrated here meets the predictions of the speed-accuracy trade-off (Chittka et al., 2003). However, since this relationship was only observed for the individual learning task and not the social learning task, and that maximum accuracy was greater on the social learning task than the individual task, suggests that individual learning may be more difficult than social learning (Laland, 2004; Kendal et al., 2005). This increased difficulty, requiring more effort both in terms of time and energy, during individual learning leads to the steep trade-off observed here, while an environment with available social information seems to reduce the costs of learning, uncoupling the negative relationship between speed and accuracy. Previous work has demonstrated that as the complexity of foraging tasks increase, the costs associated with the use of personal information also increase, and foragers rely more heavily on any available social information (Saleh et al., 2006; Kawaguchi et al., 2007; Wray et al., 2012; Smolla et al., 2016; Baracchi et al., 2018). In scenarios of increasing complexity where social information is not available, individuals will either spend more time gathering information (Chittka et al., 2003) or suffer decreases in accuracy (Wang et al., 2018). We believe that the difficulty level between our two learning tasks was significantly different, and only during the more difficult challenge of the individual learning task did the behavior of the bees reflect a speed-accuracy trade-off. Future research should explore how honeybee foragers flexibly manage the speed-accuracy trade-off and in what circumstances they adjust their behavior to suit the challenge of the learning task.

The trade-off we observed in maximum accuracy on the two learning tasks suggests that individual honeybee foragers prioritize different types of information when making decisions. Such differences may reflect unique cognitive strategies among foragers and previous work in honeybees has identified two distinct cognitive phenotypes that are characterized by differences in several correlated cognitive traits. Fast individuals exhibit enhanced performance on associative learning tasks and are risk-prone and neophilic, relative to slow individuals (Tait & Naug, 2020). Although it is still unclear as to how sociality and social information use correlate to these different cognitive phenotypes (Sih & Del Guidice, 2012; Dougherty & Guillette, 2018), individuals that sample less and pay more attention to conspecific behavior are more likely to use social information while making decisions (Rosa et al., 2012). The prioritization of personal information on the other hand is predicted to be a consequence of more frequent and thorough exploration of the environment (Kurvers et al., 2010), which is likely to increase performance on individual learning tasks. The results from this study therefore suggests that fast cognitive phenotypes are more likely to prioritize personal information and engage in more individual learning while slow cognitive phenotypes will rely more on social learning.

In a social group such as the honeybee colony, the preferential use of one type of information may also relate more widely to distinct social roles. There are two well-documented behavioral phenotypes among honeybee foragers, scouts that explore their environment, gathering new information about resources, and recruits that use that information (Seeley, 1983; Biesmeijer & Vries, 2001), which are therefore functionally equivalent to producers and scroungers (Katz & Naug, 2016). A significant positive correlation between the performance of an individual on an individual learning task and its tendency to act as a producer is well documented (Katsnelson et al., 2011). Previous work in honeybees has demonstrated that scouts and recruits differ in performance on a variety of learning tasks (Cook et al., 2019; Carr-Markell & Robinson, 2014) though the performance of these two phenotypes on individual and social learning tasks has not been explicitly tested. Although this study did not identify the tested subjects in terms of whether they were scouts or recruits, one would predict that scouts would prioritize their own information and do better in individual learning tasks while recruits would do better in exploiting social information. It would also be interesting to ask how interindividual variation in using these two types of information, and therefore performance in these two types of learning, would influence performance at the group level. A more challenging or scarce environment may enhance the value of producing new information that rely on individual learning while a more benign or rich environment may lead to ample availability of social information and the opportunity to use it. In a social context such as that of a honeybee colony, the frequency of these two cognitive phenotypes would therefore interact with the resource environment to determine colony fitness.

At the proximate level, variation in social role among honeybee workers is known to be linked to differences in neurotransmitters. High concentrations of octopamine are reported to correlate to a higher propensity to engage in scouting, increased frequency of dance behavior and better performance on associative learning tasks (Scheiner et al., 2006; Barron et al., 2007; Liang et al., 2012) and it would be a logical next step to ask if octopamine is also involved in determining the prioritization of personal or social information and flexibly regulating it based on the social and physical environment of an individual. Since individual and social learning also seem to incur different amounts of energetic costs on the individual, it would be also interesting to determine if these two cognitive phenotypes differ in their rates of energy use, or metabolic rate, which is the other fundamental variable that has been found to underlie the differences between slow-fast phenotypes (Reale et al. 2010), including those in honeybees (Mugel & Naug 2020).

In summary, our results demonstrate that individuals experience a speed-accuracy trade-off while learning a discrimination task in a foraging context, but this relationship is context dependent, only occurring during individual learning, and not social learning. Honeybee foragers also significantly differed in whether they prioritized individual or social information, which may reflect the existence of two distinct cognitive strategies that contribute to a larger slow-fast cognitive axis. Insight into how individual differences in information usage translate to social roles in the honeybee colony would help connect the known interindividual variation in cognitive traits to important behavioral traits that determine colony performance and fitness.

FIGURES

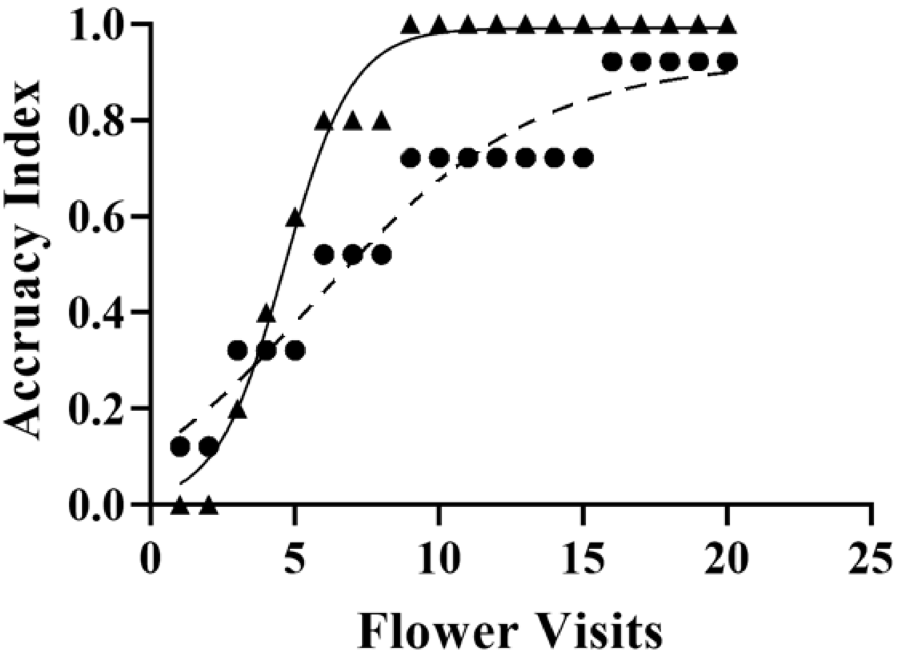

Figure 4.1. Example of individual learning curves for two different bees, where different shapes represent empirical data points and the lines represent the fitted logistic functions. Note the differences in slope (learning rate), inflection point (latency to learn), and maximum accuracy (asymptote) between the two bees.

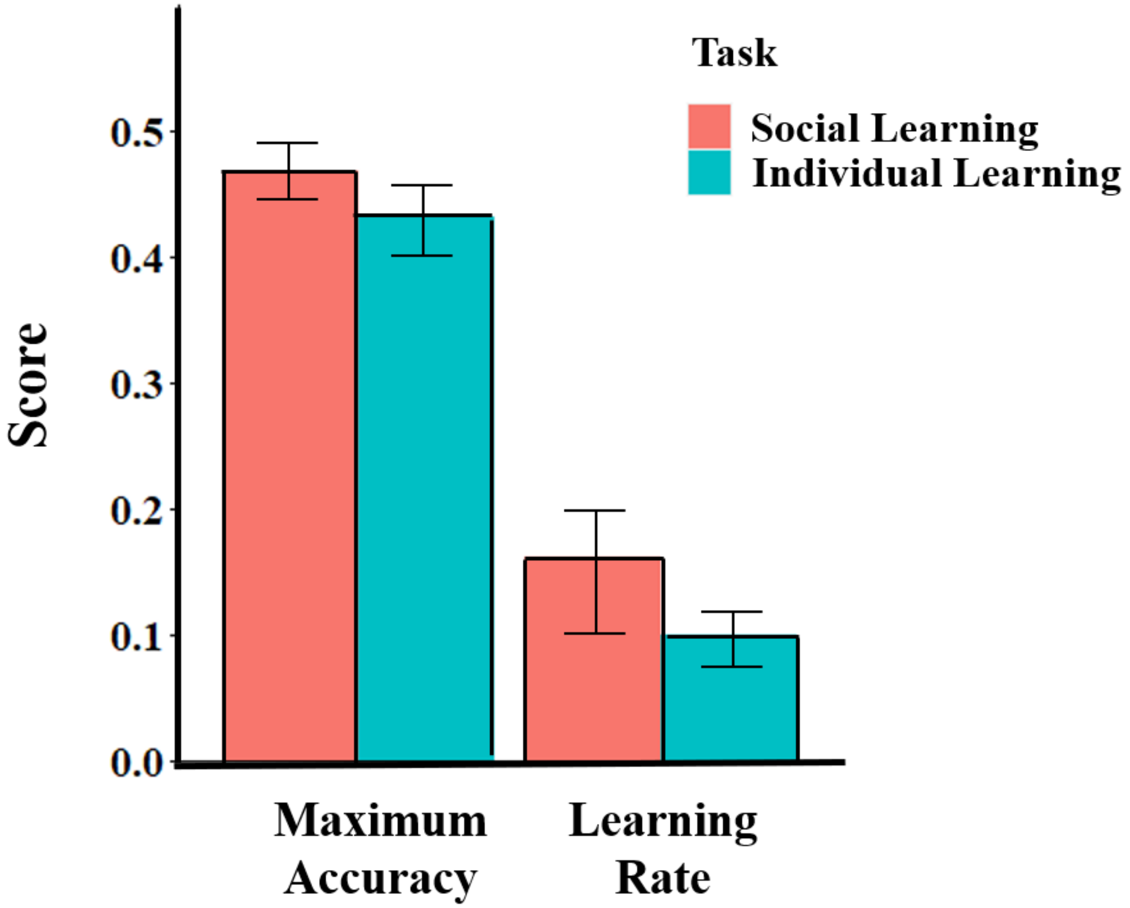

Figure 4.2. Maximum accuracy and learning rate on the individual and social learning task for all bees (*N* = 45). Bars represent means ± SE.

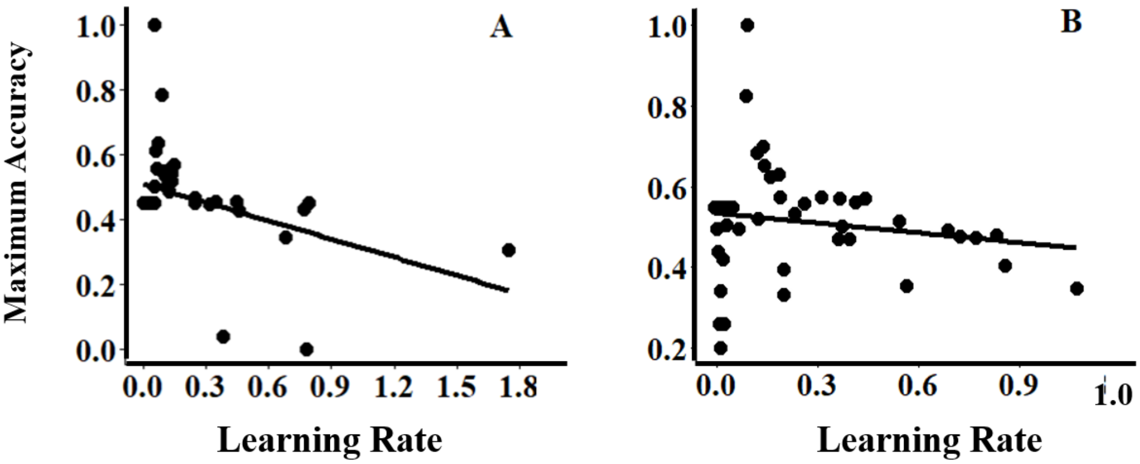

Figure 4.3. Relationship between learning rate and maximum accuracy for A) individual learning (y = 0.50 – 0.19x) and B) social learning (y = 0.53 – 0.07x) tasks, with dots representing data points for individual bees and the lines representing the regression equations.

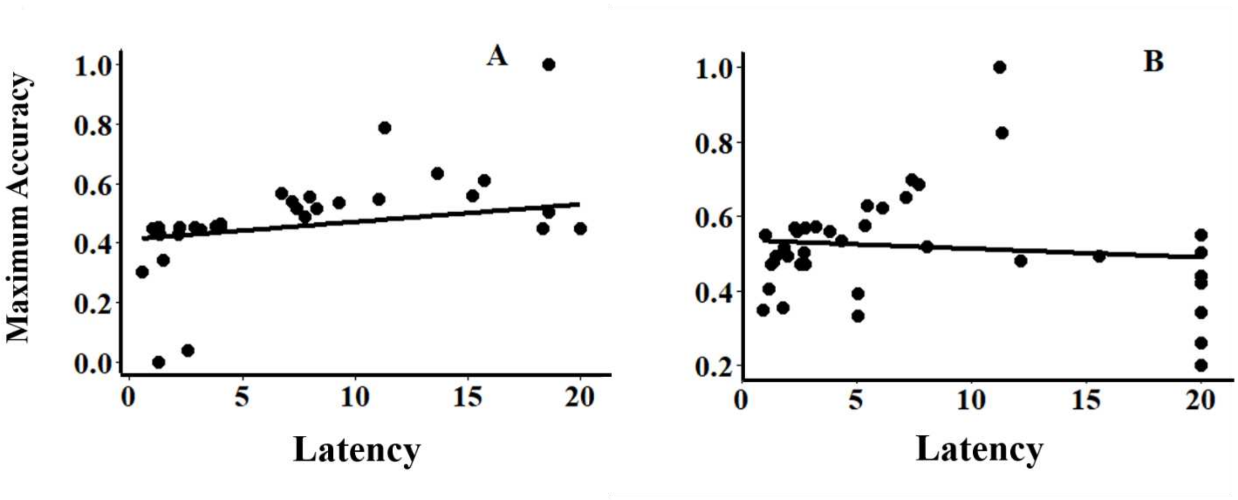

Figure 4.4. Relationship between latency and maximum accuracy for A) individual (y = 0.42 + 0.005x) and B) social learning (y = 0.55 - 0.002x) tasks, with dots representing data for individual bees and the lines representing the regression equations.

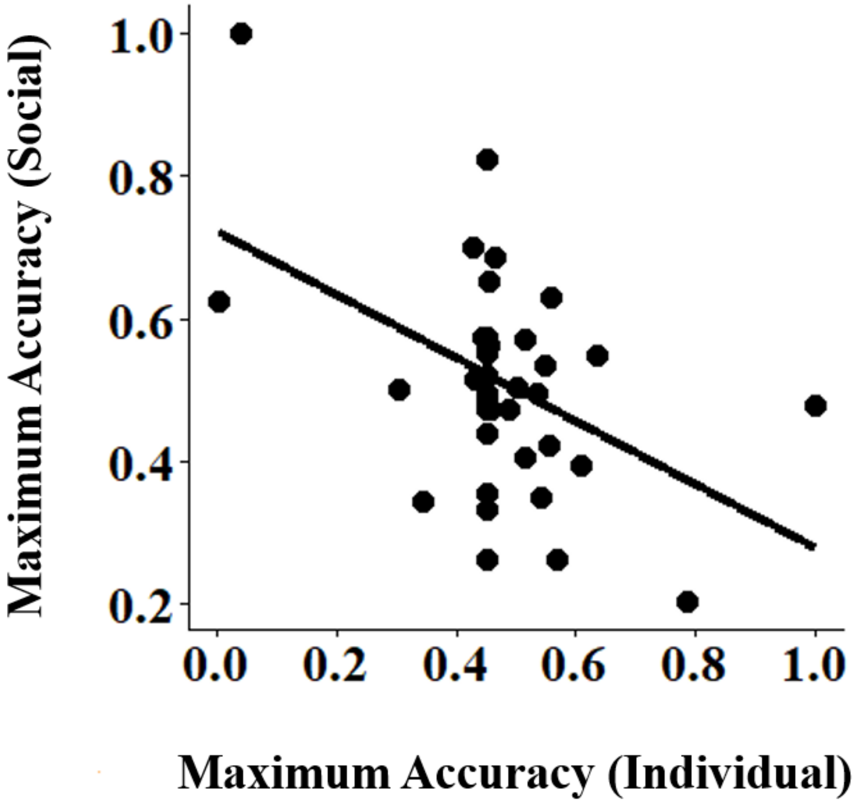

Figure 4.5. Relationship between maximum accuracy on an individual and a social learning task. Points represent individual bees and the solid line represents the direction of the correlation.

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APPENDIX

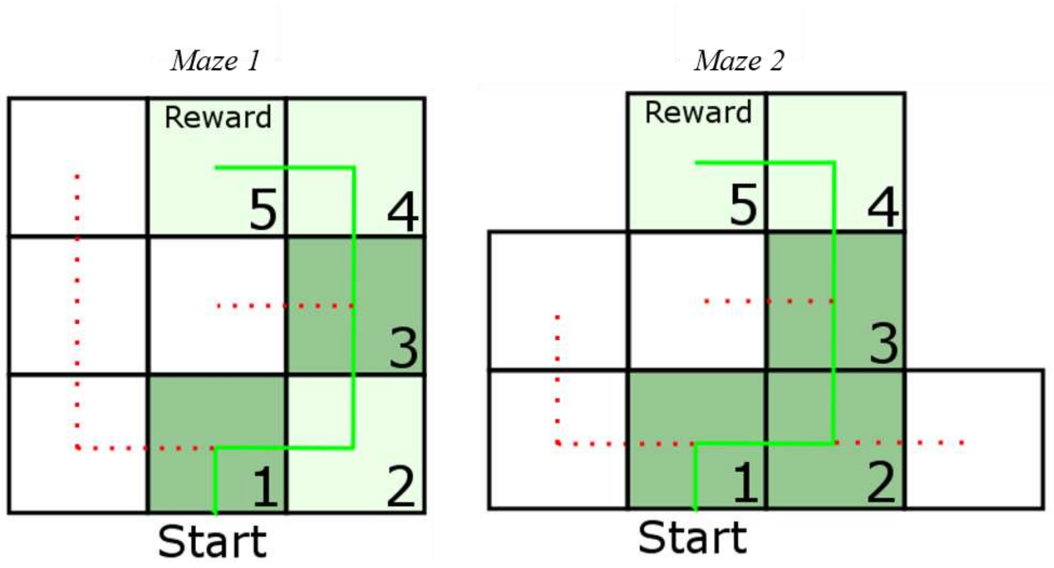

Figure A1: The two maze configurations used in Experiment 1, with the dark green boxes representing decision boxes and light green and white boxes representing non-decision boxes. The green line represents the path through the maze which led to the reward and the red line represents the paths that lead to dead ends. Maze 1 had two decision boxes while Maze 2 had three decision boxes.

Table A3.1. Post-hoc comparisons of all cognitive traits among the four honeybee species with mean standard differences (MSD) and 95% confidence intervals in parentheses, significant differences are given in bold letters.

	Associative Learning	**Sampling Frequency**	**Preference for Novelty**	**Preference for Risk**
A. dorsata* X *A. cerana	***P* < 0.001** MSD = 1.81 (0.81, 2.81)	***P* = 0.01** MSD = 2.57 (-4.78, -0.37)	*P* = 0.12 MSD = 0.31 (-0.05, 0.68)	*P* = 0.63 MSD = 0.10 (-0.34, 0.12)
A. florea* X *A. cerana	*P* = 0.63 MSD = 0.46 (- 0.54, 1.47)	*P* = 0.96 MSD = 1.41 (-1.82, 2.66)	*P* = 0.67 MSD = 0.16 (-0.21, 0.54)	*P* = 0.95 MSD = 0.04 (-0.19, 0.28)
A. mellifera* X *A. cerana	***P* = 0.01** MSD = 1.16 (0.15, 2.18)	*P* = 0.47 MSD = 1.25 (-3.50, 1.00)	*P* = 0.28 MSD = 0.26 (-0.11, 0.64)	*P* = 0.90 MSD = 0.06 (-0.30, 0.17)
A. dorsata* X *A. florea	***P* < 0.001** MSD = 1.35 (-2.13, -0.56)	***P* < 0.001** MSD = 2.99 (1.25, 4.73)	*P* = 0.54 MSD = -0.15 (-0.44, 0.14)	*P* = 0.12 MSD = 0.15 (-0.02, 0.34)
A. mellifera* X *A. dorsata	*P* = 0.14 MSD = 0.64 (-1.43, 0.14)	*P* = 0.20 MSD = 1.32 (-0.42, 3.07)	*P* = 0.96 MSD = -0.05 (-0.34, 0.24)	*P* = 0.91 MSD = 0.04 (-0.14, 0.23)
A. mellifera* X *A. florea	*P* = 0.11 MSD = 0.71 (-0.10, 1.51)	*P* = 0.07 MSD = -1.66 (-3.46, 0.12)	*P* = 0.84 MSD = 0.09 (-0.20, 0.39)	*P* = 0.43 MSD = -0.11 (-0.30, 0.08)