Attention and individual behavioural variation in small-brained animals – using bumblebees and zebrafish as model systems

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Thesis submitted for the degree of Doctor of Philosophy

Queen Mary, University of London

2013

Abstract

A vital ability for an animal is to filter the constant flow of sensory input from the environment to focus on the most important information. Attention is used to prioritize sensory input for adaptive responses. The role of attention in visual search has been studied extensively in human and non-human primates, but is much less studied in other animals. We looked at attentional mechanisms, especially selective and divided attention where animals focus on multiple cues at the same time, using a visual search paradigm. We targeted bumblebee and zebrafish as model species because they are widely used as tractable models of information processing in comparatively small brains. Bees were required to forage from target and distractor flowers in the presence of predators. We found that bees could selectively attend to certain dimension of the stimuli, and divide their attention to both visual foraging search and predator avoidance tasks simultaneously. Furthermore, bees showed consistent individual differences in foraging strategy; 'careful' and 'impulsive' strategies exist in individuals of the same colony. From the calculation of foraging rate, it is shown that the best strategy may depend on environmental conditions. We applied a similar behavioural paradigm to zebrafish and found speed-accuracy tradeoffs and consistent individual behavioural differences. We therefore continued to test how individuality influences group choices. In pairs of careful and impulsive fish, the consensus decision is close to the strategy of the careful individual. The present thesis provides implications for the study of animal attention, individuality differences based on attentional strategies, the influence of individuality on animal group choices and an exploration of the evolutionary pressures that favour stable individual differences.

Acknowledgements

I would like to thank my supervisor Prof. Lars Chittka, and Dr. Thomas C Ings and Dr. Michael J Proulx for help with experimental design and manuscript preparation. I'd like to thank all those in the bee lab who always provided interesting ideas and discussions, including: Dr. Vivek Nityananda, Dr. Helene Muller, Dr. Sarah EJ Arnold, Dr. Aurore Avargues-Weber, Fei Peng, Erika Dawson, Kate Hunt and Oscar Ramos-Rodriguez. Special thank to my project students Krishnna Rajaratnam and Samara Polwatta who helped with my experiments.

I would like to extend my thanks to all those I've met in Queen Mary, who gave me helpful advice at all times: Tzu-Chun Chen, Dr. Elodie Briefer, Dr. Hao-Chih Kuo, Vera Sarkol, Dr. Mathieu Lihoreau and Dr. Shuichiro Taya, and also my friends supporting my life in London: Yvonne Chi, Yu-Ju Chen, Tzu-Ching Horng and Pei-Chien Tsai.

I am very grateful to have received funding from Overseas Research Student Awards Scheme, the Ministry of Education and National Science Council Taiwan Studying Abroad Scholarship, and the writing-up scholarship from SBCS.

To my parents, Jian-Ming Wang and Li-Ching Kao, I dedicate this thesis.

Summary of input and publications arising from the thesis

I am singly responsible for the content of this dissertation and the experiments reported therein, with the exception of chapters 3, 4 and 7, where some contributions were made by other persons. These contributions are as follows:

Chapter 3: Thomas C Ings and Lars Chittka designed and wrote the results for Experiment 1. The data for Experiment 1 were collected by Oscar Ramos-Rodriguez and analysed by Thomas C Ings. I performed and analysed Experiment 2 and participated in manuscript preparation.

Chapter 4: I was in charge of the experiment, data analysis, and manuscript writing. Lars Chittka and Thomas C Ings participated in the design of the experiments. Lars Chittka, Thomas C Ings and Michael J Proulx participated in manuscript preparation.

Chapter 7: I was in charge of the experiment, data analysis, and manuscript writing. Part of the data collection in Experiment 3 was performed by Krishnna Rajaratnam under my supervision. Krishnna Rajaratnam and Samara Polwatta participated in part of the video analysis.

Published chapters:

Chapter 3

INGS, T.*, WANG, M.-Y.* & CHITTKA, L. 2012. Colour-independent shape recognition of cryptic predators by bumblebees. *Behavioral Ecology and Sociobiology,* 66**,** 487-496.

* equal contribution

Chapter 4 & 5

WANG, M.-Y., INGS, T. C., PROULX, M. J. & CHITTKA, L. 2013. Can bees simultaneously engage in adaptive foraging behaviour and attend to cryptic predators? *Animal Behaviour*, 86, 859-866.

Declaration

I declare that the work in this thesis is all my own, with the exception of the contributions from others mentioned in the 'Summary of input' section above.

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April 2013

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Chapter 1 Introduction

1.1 Overview

The vital ability for animals to survive and reproduce is to access and analyse the information available in the environment, and respond appropriately to it. Animals are often faced with overwhelming amounts of environmental information which exceed the handling capacity of central processing in the brain (Marois and Ivanoff, 2005), however animals typically are able to focus on relevant stimuli without difficulty. Attentional mechanisms are the key processes to enable the brain to filter out irrelevant signals and focus on the most important information. Such mechanisms make it possible for animals to prioritize sensory input for adaptive responses and ignoring secondary cues. The experimental development of attention in humans has become fashionable since the 1950s and remained so ever since (Pashler, 1999). The work has largely relied on laboratory tests to decipher the mechanisms underpinning attentional processes in human subjects. However, attention is also of great importance in animals' everyday life when they focus on and select external stimuli and respond to them.

In the present study we aim to investigate (1) how animals prioritize visual stimuli and (2) their ability to attend to two visual stimuli at the same time, (3) consistent individual differences in visual attention tasks and (4) how these individual differences influence animal consensus decisions. We select bumblebees and zebrafish as model species because of their excellent visual systems and their wide usage in behavioural studies. Chapter 3 investigates selective attention in bees, where certain stimuli stand out from distractors but not others. Chapters 4 and 5 concern whether bees are able to divide their attention toward both foraging and predator avoidance tasks simultaneously. Chapter 6 discusses the constant individual behavioural differences in visual attention tasks, where animals prioritize decision accuracy or speed. Chapter 7 further looks into how such attentional individual differences influence animal consensus decision-making, and is followed by a general discussion in Chapter 8.

1.2 Selective attention

What we perceive is not only what enters into our sensory systems, but by information that makes it through our attentional filters. For example, when in a noisy room full of people when many sounds enter our ears at once, how do we choose to listen to what we really want to hear? This is called the 'cocktail party problem' and has been of interest to scientists for a long time (Cherry, 1953, Broadbent, 1958). Classic experiments to study this problem used selective shadowing tasks, in which two messages were played to each ear of the listener simultaneously. Participants were instructed to repeat one message but not the other. Researchers found that clear differences such as distant positions or diverse tones are important for sufficient shadowing. Interestingly, participants show very low recipient ability towards the shadowed message, even when it is as loud and clear as the target voice (Broadbent, 1958, Moray, 1959). Another example known as 'inattentional blindness', best known from Simons and Chabris's (1999) study, in which observers attended to a ball-passing game with players wearing white or black shirts. Subjects were instructed to count the number of passes from the white players. However, while subjects attended keenly to the task as instructed, they failed to notice the presence of a person in a gorilla suit (dark colour) walking across the centre of the field. Selective attention has been well documented in a wide variety of tasks in human subjects (Pashler, 1998, Yantis, 2000, Driver, 2001); however, there are relatively few studies that explore the biological relevance of attention in natural settings in humans as well as other animals.

Traditional research on selective attention in animals other than humans has been focused on different dimensions in discriminative stimuli which are reinforced or non-rewarded (Zentall and Riley, 2000). In a classic experiment, rats (*Rattus norvegicus*) were trained to attend to one dimension (e.g., colour or spatial location) of the stimuli but not to any other, and researchers also investigated how they weight conditioned cues of different aspects (Krechevsky, 1932). In natural conditions, animals have to focus on stimuli with limited conspicuousness, such as cryptic food resources or predators. It is known that they can focus on selected features of the target which assists in their efficient location, by using so-called search images (Tinbergen, 1960, Rausher, 1978, Pietrewicz and Kamil, 1979). For example, when facing cryptic prey, predators are known to utilize a prey-specific search image. This strategy enables the predator to focus on the cryptic prey, even in the presence of distractors. Some debates have been held over the nature of a search image, such as whether animals learn the entire compound as a target signal (Endler and Greenwood, 1988, Pietrewicz and Kamil, 1979), or if only certain elements of the stimuli can be useful for increasing searching efficiency (Dawkins, 1971). Several lines of evidence show that animals attend to selected features rather than to the total object (Reid and Shettleworth, 1992). For example, in pigeons (*Columba livia*), foraging for various types of grains, a change in colour but not shape of wheat grains reduces foraging detection, but in beans both colour and shape changes decrease accuracy (Langley et al., 1996). However, which parameter is involved in a search image may differ depending on situations, and this does not appear to be widely studied.

1.3 Divided attention

Divided attention, where participants process two sensory tasks at the same time, is widely studied in humans but highly under-researched in other animals (Zentall, 2005). However, in a natural environment, animals typically have to attend to more than one stimulus concurrently. For example, a foraging animal also has to be aware of potential predators at all times. Traditional explorations in animal divided attention mainly explore whether animals are able to process two elements of a compound stimulus, and whether the efficiency is the same as the existence of only one element. Not surprisingly, animals were better at processing single-element targets than compound samples (Maki Jr and Leuin, 1972, Maki Jr. and Leith, 1973). In more ecologically realistic designs, trade-offs between animals attending to foraging targets (cryptic food items, for example) and potential predators were studied. Dukas and Kamil (2000) found that blue jays engaged in a challenging foraging task for centrally located targets will often miss peripherally located targets (a secondary foraging task) and suggested that this would also cause blue jays to overlook predators in the periphery (not unlike similar research in humans (Joseph et al., 1997)). There may be trade-offs between decision time and the accuracy of stimuli processing and analysing, depending on task priority (Chittka et al., 2009). For example, when exposed to predation risk, animals tend to decrease foraging time and increase vigilance (Ydenberg and Houston, 1986, Metcalfe et al., 1987). When animals encounter more difficult foraging tasks, the probability of detecting predators decreases (Milinski, 1984, Lawrence, 1985, Godin and Smith, 1988). The attentional processes found in animals so far are not fundamentally different from those that occur in humans. When encountering compound stimuli, animals show limited capacity to process information and either divide their attention or switch rapidly between tasks. However, a better understanding of the processes should be obtained to give a clue on how animals solve multiple tasks simultaneously.

1.4 Speed-accuracy tradeoff in attentional tasks

Individual differences are common in animal decision-making (Dall et al., 2004, Thornton and Lukas, 2012). Here we aim to investigate animal behavioural individuality in attentional tasks. Past research on animal individuality focused on traits such as shyness-boldness, exploration-avoidance, activity, sociability and aggressiveness (Réale et al., 2007). In the present study we choose a different dimension, so called speed-accuracy tradeoffs, to investigate animal individual behavioural differences. Accurate decisions may require longer decision time, and impulsive choices can be faster but less discriminating. Speed-accuracy tradeoffs have long been of major concern in human psychology studies (Woodworth, 1899) such as impulsivity (Dickman and Meyer, 1988), extraversion and neuroticism (Sočan and Bucik, 1998), intelligence (Phillips and Rabbit, 1995) and information processing (Miller and Vernon, 1997). However, there are relatively few studies about other animals, despite the fact that animal studies allow us to investigate the biologically relevance of attention in natural settings. Such tradeoffs exist in animal decision making such as foraging (Chittka et al., 2003b), predator-prey interactions (Ings and Chittka, 2008, Burns and Rodd, 2008) and nest site selection (Franks et al., 2003). In many cases behavioural plasticity exists in speed-accuracy tradeoff decisions. For example when the task becomes difficult, subjects may be slower and make less accurate performance (Palmer et al., 2005), or when punishment exists they may trade speed for accuracy (Chittka et al., 2003). We focused on between group and within group speed-accuracy tradeoffs in animal colour discrimination tasks, using bumblebees and zebrafish as model species.

1.5 Consistent individual differences in speed-accuracy tradeoffs

In human psychology studies, it has long been shown that different individuals under the same conditions can behave differently, and such differences can be constant over time. Individual personality differences can influence behaviour during attentional tasks (MacLean and Arnell, 2010), however despite the burgeoning interest in human personality studies, there are few studies on individual consistency of attentional performance in other animals. In Chapter 6 we looked into the consistent individual behavioural differences when bees solving attentional tasks. For non-human animals, inter-individual differences are often treated as noise data and averaged into group behaviour. Not until recently have researchers begun to estimate the impact of individuality in animal behaviour. The terminology in both human and animal individual behavioural differences studies remains unclear. The term 'personality' has been used in humans and several other animals; however there is no unified definition (Nettle, 2006). Other phrases such as 'behaviour syndrome' (Sih et al., 2004a), 'animal temperament' (Réale et al., 2007) or 'coping styles' (Koolhaas et al., 1999) are also used in the literature (Gosling, 2001, Sih et al., 2004a). Since behavioural individuality is repeatable and predictable across a wide variety of animal groups, it appears to have ecological and evolutionary consequences and is hence favoured by selection. Within-colony diversity in social insects is shown to increase colony fitness (Mattila and Seeley, 2007) and is useful to cope with the rapidly changing environment (Burns and Dyer, 2008). The use of the term 'personality' implies, firstly, that variation between individuals is greater than within individuals. The same individual does not exhibit the whole spectrum of behaviours in the population. Secondly, the same type of behaviour is constant through time and context. Such consistency is not compared with a standard measurement but between the ranks in the population. Plasticity may also occur in individual animals where behaviour changes with the situation encountered. However the rank of each individual within the population should remain stable (Lessells and Boag, 1987, Bell et al., 2009).

Why do animals remain behaviourally consistency rather than change their strategies depending on situations? The answer is still largely unclear, however theoretical models indicate that variations in behaviour may enhance the evolution of cooperation when collaborating with stable partners (McNamara et al., 2004). Obtaining cutting-edge environmental information may be costly (McElreath and Strimling, 2006, Briffa et al., 2008) and can quickly be out of date when the environment changes rapidly. Physiological constraints such as metabolism and hormone regulation can also limit behavioural plasticity in animals (Cavigelli, 2005, Biro and Stamps, 2010). In social animals, predictable behaviour can reduce conflicts between group members and help towards establishing a stable hierarchy, thus enhancing collaboration (Dall et al., 2004, Bergmüller and Taborsky, 2010). When individuals have to constantly interact with other group members, maintaining the same strategy may help others to predict one's behaviour and thus choose to participate. An untrustworthy individual may pay higher costs than trustworthy individual when the environment is stable (McNamara and Houston, 2002). However, individuality in animal groups may be the subject of other debates, which will be discussed in the following section.

1.6 Constant individual behavioural differences and consensus decision-making

Since individuality can benefit social groups, how animals compromise with each other and make consensus decisions has become an interesting topic. Living in social groups is a widespread phenomenon and can provide many ecological benefits for group members. Grouping may enhance anti-predator protection such as the traditional 'many-eyes' theory which allows the group to spot predators more easily

(Powell, 1974, Godin and Morgan, 1985). Large group members can also reduce the opportunity for predation or can confuse predators during predation (Hamilton, 1971, Krakauer, 1995). From both predator and prey's point of view, grouping can provide benefits for both of them in foraging for food such as attacking in a team (Hector, 1986, Packer and Ruttan, 1988), information sharing (Valone, 1989, Chittka and Leadbeater, 2005) and reducing plant defences (Denno and Benrey, 1997).

Traditional investigations usually assume animals retain the same personality whether they operate singly or in a group (Camazine et al., 2003), however individual variations in traits and conditions appear in group members and can influence group structure. For example, hungry individuals are more likely to be located at the edge of the group (Romey, 1995), and in some species the individuals with higher position in the hierarchy often appear in central positions (Hemelrijk, 2000). A small difference in speed can influence the movement of the group (Gueron et al., 1996). Behavioural trait differences may be important factors shaping consensus decisions, and certain individuals are more dominant than others in the collective decision-making (Couzin et al., 2005, King et al., 2008, Stueckle and Zinner, 2008, Conradt et al., 2009). For example, bold individuals are more likely to take the lead in a group and have a greater effect on making choices (Harcourt et al., 2009, Kurvers et al., 2009). However, other behaviour traits are rarely considered and the effect on group decisions remains largely unknown. In Chapter 7 we aim to look at how individuals with different behavioural traits make consensus decisions.

1.7 Structure of thesis

Chapter 3 – Selective attention in bees: shape recognition of cryptic predators by bumblebees

This chapter investigates the selective attention in bumblebees. We exposed bumblebees to yellow flowers concealing cryptic robotic spiders, and then introduced them to a new patch of white flowers with white spiders or yellow circles on some of the flowers. Bees were able to avoid white spiders but not yellow circles. Bees disentangle shape from colour cues and thus can form a generalised search image for spider shapes, independent of colour. The innate selective attention in bees shows the focus toward the shape but not colour of the predators, which may have ecological benefit to colour-changing predators such as crab spiders.

Chapter 4 – Divided attention in bees: can bees simultaneously engage in adaptive foraging behaviour and attend to cryptic predators?

In this chapter, we ask whether bees are able to maintain efficient foraging in the presence of cryptic predators. Bees were tested with artificial flowers of similar appearance and different rewards. Predation risk was simulated by robotic spiders. We found that bees had no difficulty avoiding conspicuous spiders and still foraging adaptively, but they prioritized predator avoidance at the expense of efficient foraging when faced with detecting cryptic predators and a difficult colour discrimination task.

Chapter 5 – Divided attention in bees: is the priority in predator avoidance due to attentional limitation?

Following the previous chapter, we forced bees to attend to both flower choice and predation. Instead of rewarded in different levels, bees were required to forage from target and distractor flowers distinguished by similar colors but contain either sucrose reward or mild punishment via distasteful quinine solution to encourage color discrimination. Surprisingly, punishment with distasteful quinine motivated the bees to simultaneously avoid predation and make correct foraging choices. By demonstrating the ability to divide attention between two challenging visual tasks, these results demonstrate a high level of cognitive sophistication in bumblebees.

Chapter 6 –Individual consistency in bumblebee speed-accuracy tradeoff decisions when foraging under predation threat

We tested individual consistency in bumblebee speed-accuracy tradeoffs faced with predation risks. Bumblebees showed individually repeatable strategies in foraging decisions when conspicuous predators were introduced in the meadow. We also calculated the foraging rate for different experimental design, and found that optimal strategy changes between experiments.

Chapter 7 – Speed accuracy tradeoffs and decision making by individuals and dyads of zebrafish in colour discrimination tasks

Here we investigated whether zebrafish show individual consistency in speed accuracy tradeoffs in colour discrimination tasks, and how pairs of fish with distinct ‘personalities’ make consensus decisions. We found that zebrafish exhibit consistent between-individual differences in speed-accuracy tradeoffs. Some fish made ‘careful’ slow but accurate decisions, where others were more ‘impulsive’ and made swift but less accurate decisions. When tested in pairs of ‘careful’ and ‘impulsive’ individuals, the combined decisions were closer to those of careful individuals.

Chapter 2 Model systems and general methods

2.1 Background of model systems

Bumblebees and zebrafish make ideal models to investigate attention and behavioural individuality in vertebrate and invertebrate animals. Worker bees are proficient learners especially in the context of flower foraging, and have therefore been a popular model to study both the ecological implications of learning and memory, as well as their neural underpinnings (Menzel 1985, Menzel and Muller, 1996, Chittka et al., 1999). They are able to associate food reward with colours, shapes, patterns, odours and landmarks (Chittka and Thomson, 2001), and will also learn to avoid stimuli that are associated with penalties (Ings and Chittka, 2008).

Zebrafish have been one of the most popular model species in developmental biology for decades (Grunwald and Eisen, 2002, Sison et al., 2006), but more recent studies increasingly explore the molecular-genetic underpinnings of behaviour variation in this species. This concerns, for example, the genes associated with drug addiction (Darland and Dowling, 2001) or visual psychophysical measurements (Page-McCaw et al., 2004, Muto et al., 2005). However, many aspects of zebrafish behaviour remains largely unknown, especially insofar as the relevance of learning and memory in more naturalistic settings is concerned (Sison and Gerlai, 2010). In addition, zebrafish are shoaling fish species that swim in groups whether in their natural habitat or in artificial captivity, which makes them an excellent model for studying animal consensus decisions (Engeszer et al., 2007, Miller and Gerlai, 2007).

The visual system and learning behaviour of bumblebees

Like most hymenopterans, bumblebees show trichromatic colour vision with three types of photoreceptors with sensitivity peaks in the ultraviolet, blue and green region

(Peitsch et al., 1992). The compound eyes of bees consist of thousands of ommatidia each containing 6 green receptor cells with addition of one or two blue or UV receptors (Spaethe and Briscoe, 2005, Wakakuwa et al., 2005, Dyer et al., 2008). Behavioural tests also demonstrate that bees are able to identify colour out of different shades of greys, and are able to associate food rewards with colours even with the existence of similar colour distractors (Frisch, 1914, Daumer, 1956). With both pseudopupil and behavioural approaches, researchers find that larger worker bees exhibit better overall visual sensitivity and image resolution (Spaethe and Chittka, 2003). For smaller workers, stimulation of seven or more ommatidia is needed for detecting a target, while larger bees only require one ommatidium for the same target (Spaethe and Chittka, 2003). In this case, visual learning paradigms are suitable for addressing the attention mechanisms and the individual behavioural differences between bees.

The visual system and learning behaviour of zebrafish

Zebrafish are diurnal fish originally from the Indian sub-continent, where they are typically in habitants in rich-vegetated silt-bottomed pools and rice paddy shallow water (Engeszer et al., 2007). Their fine visual ability may be driven by the slow-moving water and the complexity of the environment, and has become an important model for studying visual systems in animals (Bilotta and Saszik, 2001). Their retina contains four different morphological types of photoreceptors, including short-single cones (SSC), long-single cones (LSC), double cones (DC) and a rod (Risner et al., 2006). Electroretinogram data shows spectral sensitivity ranges from 320 to 640 nm (Hughes et al. 1998), and the peak sensitivity for different opsins on single cones were measured (UV_{max} = 360 - 361 nm, S_{max} = 407 - 417 nm, M_{max} = 473 - 480 nm, L_{max} = 556 - 564 nm) (Nawrocki et al., 1985, Robinson et al., 1993, Cameron, 2002, Allison et al., 2004). Behavioural spectral sensitivity experiments with zebrafish provide evidence for colour discrimination, but the result cannot be predicted from photoreceptor spectral sensitivity in a straightforward manner, suggesting that visual processing may occur at a different level (Risner et al., 2006). An innate preference in blue is reported in zebrafish (Avdesh et al., 2012). Considering the complexity of manipulating odour and vibration cues underwater, visual discrimination has become the most popular behavioural paradigm in zebrafish (Colwill, 2005). Here we used two colour signals, one conditioned with food reward and another with punishment, to test individual and dyad decisions in zebrafish.

2.2 General Methods

Keeping of animals

Bumblebees: bees (*Bombus terrestris*) were obtained from Syngenta Bioline Bees, The Netherlands. All bees were reared in a 23°C dark environment devoid of visual cues (colour and shape) prior to commencement of the experiments. Bee hive was put in a wooden box with a divider in the middle. There was a small hole on the divider so the worker bees can enter the other room but not the queen. Unlimited 50% (v/v) sucrose solution was provided daily and 10g of pollen (Syngenta Bioline Bees) was given twice per week. If fewer than three honey pots in the bee colony were full, 2 ml of 50% sucrose solution was added. After the experiment, individual bees were frozen in a -4°C freezer for one hour, and the body length, thorax width and weight were measured.

Zebrafish: wild type zebrafish (*Danio rerio*) were used in all the fish experiments. Fishes were bred and kept in the breeding room under UK Home Office regulation. The breeding room was air conditioned at 26°C with a 14h:10h light:dark cycle, with lights going on at 8am. The fishes were fed with flake food and brine shrimp three times per day. Two weeks before the experiment, the fish were moved to the experimental room and pair housed separately in 20 cm (l) × 11 cm (w) × 10 cm (h) tanks with non-transparent dividers in the middle. The temperature and light cycle for the experimental room remained the same as the breeding room. All tanks were connected to air-lines, and aquarium water (de-ionized water with added salts) was changed twice weekly. The body length and the weight of each fish were measured after the experiment.

Experimental setup

Bumblebees: In order to test how bees visually attend to colour signals and the individuality of the processes, we used an experimental setup in the lab with ecologically relevant tasks for bees to solve. In nature, foraging bees have to discriminate similar flowers with different rewards (or even no rewards), and simultaneously avoid cryptic predators on the flowers. Crab spiders are one of the most common sit-and wait predators on the flowers, thus we used ‘robotic’ spiders to mimic predation risk when foraging. All the bumblebee experiments were conducted in a wooden flight arena (l = 1 m, w = 0.72 m and h = 0.73 m) with a UV-transmitting Plexiglas lid. Two twin lamps (TMS 24 F with HF-B 236 TLD [4.3 kHz] ballasts, Philips, The Netherlands) fitted with Activa daylight fluorescent tubes (Osram, Germany) were suspended above the flight arena to provide controlled illumination. Artificial flowers (7×7 cm acrylic, 1 mm thick) were arranged in a four by four vertical grid on one of the walls of the arena on a grey background (Fig 1). The opposite wall contained an entrance hole through which the bees could enter the arena from the colony. Bees were able to access rewards (sucrose solution) through a hole which was 10 mm above a wooden landing platform (40×60 mm). Rewards were supplied to each flower and dispensed from the tips of 26G syringe needles (BD

Microlance Drogheda, Ireland; 0.45 × 13 mm) by syringe pumps (KD Scientific, KD200, Holliston, USA). A maximum droplet volume of 4.70 + 0.3 µl could be reached before it fell into a ‘waste pot’ which was not accessible to bees (thus mimicking a flower that had been emptied by a bee). Robotic ‘spider arms’ (custom-built by Liversidge & Atkinson, Romford, UK) covered with sponges were set up at the base of the flowers to simulate predation attempts. The trapping mechanism enabled us to capture bees without causing physical damage. ‘Dangerous flowers’ were fitted with life-sized crab spider (*Misumena vatia*) models (l = 12mm, made from Gedeo Crystal resin) placed on the flowers above the feeding hole.

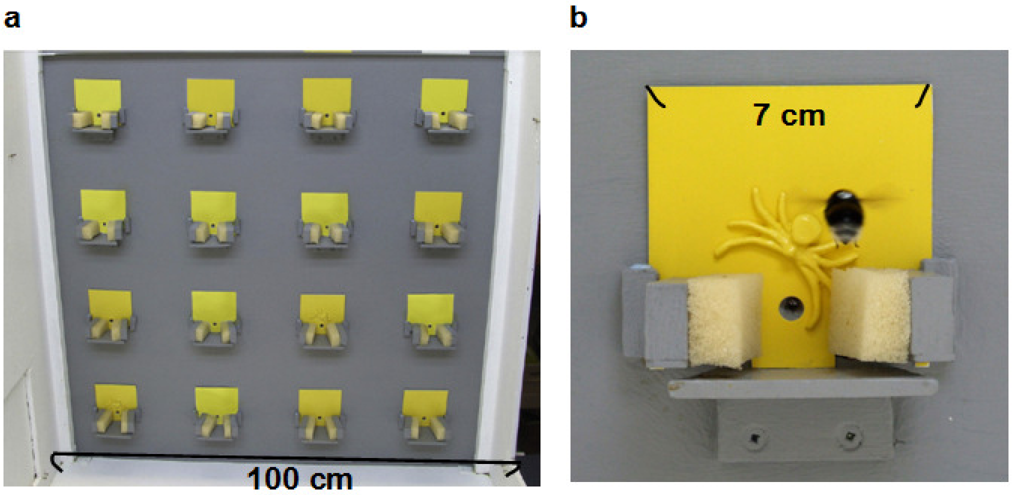


Fig 1 Experimental setup demonstrating the artificial meadow (a) containing artificial flowers (b). The positions of the flowers and spiders were randomly shuffled for each foraging bout.

Zebrafish: We used a 40 cm (l) × 25 cm (w) × 15 cm (h) tank filled with 7 cm deep water which was identical to the raising water (Fig 2). Water temperature was kept at 26°C and measured in the beginning of each experiment. Each fish was first allowed to explore and get used to the holding area of the tank for three minutes. After habituation, the experiment started by lifting up the barrier to let the fish swim freely in the tank. If a fish crossed the hole to the signal area, this was considered as decision. If the fish made a correct decision, the barrier between signal area and investigating zone was closed and the fish was given a small amount of brine shrimp (~0.5 ml, in average 140 individuals). After a fish had consumed the food, it was gradually moved back to the holding area, and the barrier was closed again. When an incorrect decision was made, we stirred the water for three seconds as punishment, as a further incentive for discrimination in difficult colour discrimination tasks (see below). When testing easily distinguishing colours, only food reward was given when fish chose the rewarding colour, and no punishment was given for incorrect choices.

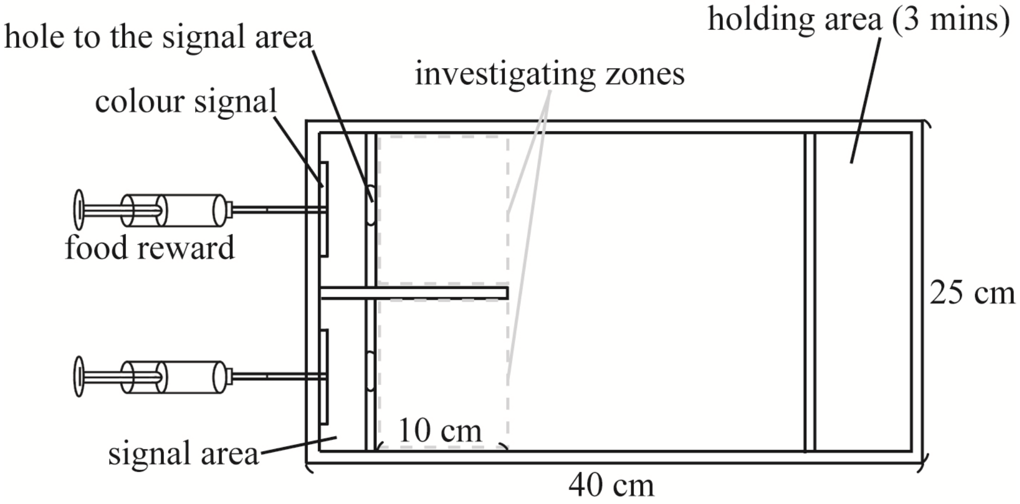


Fig 2 Experimental setup for all the zebrafish experiments. The fish was habituated in the holding area for three minutes and left inspecting the tank. The colour signals were set on the wall of the signal area and can be seen from the tank. Once the fish crossed the hole to the signal area, it was considered as a choice. We gave 0.5ml of brine shrimp solution as reward when the fish made a correct choice, and stirred the water for three seconds as punishment. The fish was gradually moved back to the holding area after that. We tested the fish continuously for 20 trials in total. The time fish spent in the investigating zone were recorded.

Tracking system

The movements and positions of animals were recorded in real time during the experiment with three-dimensional coordinates of animal positions being calculated 50 times per second using two video cameras connected to a computer running Trackit 3D software (BIOBSERVE GmbH, Bonn, Germany). Before releasing the animal, the background of the experimental setup was saved as reference, thus the 3D coordinates of anything different from the background after starting the software was recorded. The 3D position data was further analysed using R (v. 2.15.1) and Excel

2003. For experiments with dyads of fish, the videos were also analysed by Move-tr/2D (Library Co. Ltd., Tokyo). The software can break the videos into image frames and automatically track the differences in colour, luminance and pattern. In this case, we were able to calculate the two-dimensional position, speed, moving angle and the coordinate distance between two fish.

2.3 Summary of experiments

For testing selective attention in bumblebees, we design the experimental paradigm as follows which reported in Chapter 3 (**Table 1**):

Table 1 Summary of experimental design, where Y = yellow, W = white, F= flowers, S = Spiders and C = Circles. The first experimental group tested the effect of the spiders of different colour, where the second group acted as a control group to test the effect of a novel object (circle) appeared on the flowers.

Experimental group	**Number of bees**	**Training (200 trials)**	**Avoidance assay (30 trials)**
1: Naive	12	16 YF, no spider	16 WF, 4 with WS
1: Experienced	12	16 YF, 4 with YS	16 WF, 4 with WS
2: White spiders	12	16 YF, 4 with YS	16 WF, 4 with WS, 4 with WC
2: White circles	12	16 YF, 4 with YC	16 WF, 4 with WC

To make sure the task in the ‘divided attention experiment’ is difficult enough for bumblebees, we firstly determined which colour pair is difficult, but possible, for bees to discriminate. The design of the experiment is as follows (**Table 2**) and is provided in Chapter 4.

Table 2 Summary of experimental design for colour discrimination pilot study. We aim to find a colour pair which was difficult, but possible for bee to distinguish. In this case we used both sucrose reward and quinine punishment to motivate the colour discrimination.

Experimental group	**Number of bees**	**Colour signals (200 trials)**
Distinct colours	5	8 yellow flowers (50 % sucrose), 8 white flowers (0.12 % quinine)
Similar colours	5	8 dark yellow flowers (50 % sucrose), 8 light yellow flowers (0.12 % quinine)

To explore divided attention in bumblebees, we set up three experimental groups which are discussed in Chapter 4, 5 and 6 (Table 3). For Chapter 4, we described the first two experiments, which whether bees were able to solve two difficult tasks simultaneously. In Chapter 5, we added the third group to test the attentional limitation in bees. In Chapter 4 and 5, we examine the data at the group level, while in Chapter 6 we quantitatively evaluate individual differences between bees.

Table 3 Summary of experimental design in Chapter 4, 5 and 6. We aimed to test how bees focused on two difficult tasks (colour discrimination and predator avoidance) at the same time. In the first two experiments, we firstly trained the bees to two similar colour flowers with different rewarding levels, and added spiders of different detectability. In the last experiment, we would like to make sure the bees foraging in high effort thus exchange low reward to quinine punishment.

Experimental group	**Number of bees**	**Training (200 trials)**	**Testing (200 trials)**	**Chapter**
Rewarding flowers with conspicuous spiders	17 (2 stopped to forage)	8 dark yellow flowers (50 % sucrose), 8 light yellow flowers (20 % sucrose)	8 dark yellow flowers while 2 of them contained white spiders (50 % sucrose), 8 light yellow flowers (20 % sucrose)	4, 5 and 6
Rewarding flowers with cryptic spiders	17 (2 stopped to forage)	8 dark yellow flowers (50 % sucrose), 8 light yellow flowers (20 % sucrose)	8 dark yellow flowers while 2 of them contained dark yellow spiders (50 % sucrose), 8 light yellow flowers (20 % sucrose)	4, 5 and 6

Rewarding and distasteful flowers with cryptic spiders	10	8 dark yellow flowers (50 % sucrose), 8 light yellow flowers (0.12 % quinine solution)	8 dark yellow flowers while 2 of them contained dark yellow spiders (50 % sucrose), 8 light yellow flowers (0.12 % quinine solution)	5 and 6

Experiments using zebrafish (Chapter 7) are summarized in Table 4 and 5. In a pilot study we tested whether fish can learn a simple colour discrimination task (easily discriminating colours) with only food reward, or whether both reward and punishment were required. When only food reward was present, the fish chose two colours at random; thus in the following studies both reward and punishment were used.

Table 4 Pilot study in Chapter 7. We tested whether only conditioned colour signal with reward can motivate colour discrimination, or both reward and punishment were required.

Experiment	**Number of fishes**	**Colour signal**	**Trials**
Condition colour with food reward	5	Green (rewarded) and brown (no punishment)	20
Condition colour with food reward and punishment	5	Green (rewarded) and brown (punishment)	20

In the zebrafish experiments, we aimed to answer three questions: (1) Do zebrafish show speed-accuracy tradeoffs between individuals, so that some individual have a slow and accurate strategy whereas others have a fast and ‘sloppy’ strategy? (2) If they show speed-accuracy tradeoffs among individuals, is individual behaviour consistent over time? (3) How do fish with different strategies make consensus decisions in dyads? To answer these questions, we designed three sets of experiments as follows. Results are described in detail in Chapter 7. To test whether fish showed speed-accuracy tradeoffs between individuals, we used colour discrimination test conditioned with both reward and punishment. We hypothesized there would be a correlation between decision time and accuracy, which some fish would spend longer time making decisions and achieve higher accuracy, while others would be fast but more inaccurate. For the second set of the experiment, we continued using colour discrimination test and repeated three times with a 24 hours interval. We aimed to test whether the strategy of the fish was stable over time. For example, the careful fish was constantly careful on the second and the third day, and so as the impulsive fish. In the last set of the experiment, we tested how zebrafish make decision in dyads.

Table 5 Summary of design for zebrafish experiments which described in Chapter 7. In the first set of the experiment, we tested whether fish showed between individual speed-accuracy tradeoffs in a colour discrimination test. Since the fish presented speed-accuracy tradeoffs, we design the second sets of the experiment to test whether this tradeoff was consistent over time. We tested the fish with the same colour discrimination task after 24 and 48 hours. The fish showed stable strategy over time thus we were able to test how they made decisions in dyads. In the last sets of the experiment, fish were tested individually in the first day in order to gain their reference behaviour, and paired in the second day for the colour discrimination experiment.

Experiment	**Number of fishes**	**Colour signal**	**Trials**
Speed-accuracy tradeoffs in colour discrimination test	30	Green (rewarded) and turquoise (punishment)	20
Repeatability test	15	Green (rewarded) and turquoise (punishment)	20 trials for the first day, and another 20 trials 24 & 48 hours after
Consensus decision test	30	Green (rewarded) and turquoise (punishment)	Fishes were tested individually for 20 trials for the first day, and tested in pairs after 24 hours

Chapter 3 Selective attention in bees: shape recognition of cryptic predators by bumblebees

3.1 Abstract

Predators hunting for cryptic prey use search images, but how do prey search for cryptic predators? We address this question using the interaction between bumblebees and the colour-changing crab spider *Misumena vatia* which can camouflage itself on some flowers. In laboratory experiments, we exposed bumblebees to an array of flowers concealing robotic predators (a trapping mechanism combined with a 3D life-sized model of a crab spider or a circle). Groups of bees were trained to avoid either cryptic yellow spiders or yellow circles (equal area to the spiders) or remained predator naive. The bees were then exposed to a new patch of white flowers containing some cryptic predators (either white spiders, white circles or a mixture of both). We monitored individual foraging choices and used a 3D video tracking system to quantify the bees' flight behaviour. The bees trained to avoid cryptic spiders, chose 40% fewer spider harbouring flowers than expected by chance, but were indifferent to cryptic circles. They also aborted a higher proportion of landings on flowers harbouring spiders, ultimately feeding from half as many 'dangerous' flowers as naive bees. Previous encounters with cryptic spiders also influenced the flight behaviour of bees in the new flower patch. Experienced bees spent longer time inspecting the flowers they chose to reject (both with and without concealed spiders) and scanned from side to side more in front of the flowers to facilitate predator detection. We conclude that bees disentangle shape from colour cues and thus can form a generalised search image for spider shapes, independent of colour.

3.2 Introduction

Animals are exposed to a constant flow of complex sensory input. A necessary ability for them to survive is to selectively process the target stimuli and ignore others. At the same time, foraging animals must balance predator vigilance with foraging efficiency (Lima, 1985). Thus, mechanisms which enhance predator detection should benefit foraging animals (Lima and Dill, 1990). Indeed many animals possess predator avoidance responses that can either be innate (Veen et al., 2000, Berejikian et al., 2003, Turner et al., 2006) or learnt (Ings and Chittka, 2008, Brown, 2003, Kelley and Magurran, 2003). Both innate and learned avoidance responses require an animal to recognise cues that indicate the presence of their predators. In many cases the cues that indicate predator presence are salient, e.g. passing shadows (Cooper, 2009) or chemical cues such as fish kairomones (reviewed in Wisenden, 2000). However, in the case of cryptic predators, especially ambush or sit-and-wait predators, such cues are likely to be much less salient to prey (Troscianko et al., 2009).

When faced with cryptic prey, predators are known to utilise a prey specific search image (Tinbergen, 1960), defined as: "*a transitory enhancement of detection ability for particular cryptic prey types or characteristics*" (Ruxton et al., 2004). This strategy enables the predator to focus on the cryptic prey, even in the presence of distractors. But what strategies do prey use to recognise, and thus avoid, cryptic predators? Surprisingly, little attention has been given to this question. However, in a previous study (Ings and Chittka, 2008) researchers showed that bumblebees are able to learn to reliably detect cryptic predators. As the bees were unable to detect any colour contrast between spider models and their background researchers suggested that the bees must have been relying on shape-from-shading cues (Ramachandran, 1988, Hershberger, 1970). We were particularly intrigued by the report that bees were able to rapidly learn to avoid spider models and that the avoidance response was maintained for at least 24 hours. This led us to consider whether bees are developing a specific search image to enhance their detection of cryptic predators.

While it is widely known that bees possess advanced cognitive capabilities and can be trained to recognise and associate complex patterns with rewards or punishments (Chittka and Niven, 2009, Stach et al., 2004, Roussel et al., 2009) in both appetitive (rewarding or distasteful food: Menzel, 1985) and predator avoidance contexts (Ings and Chittka, 2008, Ings and Chittka, 2009), little is known about their use of search images. By inference, it appears that honeybees can use search images as they are able to distinguish camouflaged shapes after training (Zhang and Srinivasan, 1994). Field observations also indicate that pollinators are able to recognise specific elements of a spider shape (the raptorial forelegs: Gonçalves-Souza et al., 2008). However, the spider models used in that study were not cryptic on the flowers and only wild pollinators were tested, so it was not possible to determine if avoidance was an innate or learned response.

Therefore, in this study we tested whether bees can form a generalised search image for cryptic predators, independent of colour. We utilised the interaction between bumblebees and the predatory crab spider *Misumena vatia,* which is able to reversibly change its colour between white and yellow and thus camouflage itself on white or yellow flowers respectively (Morse, 2007, Insausti and Casas, 2008). One hypothesis is that bees only learn to avoid predators of the colour they have been exposed to, or they are only vigilant for predators when they encounter flowers of the same colour as those where they have experienced predation threat. Alternatively, bees might form a generalised, colour independent search image and will thus avoid spider shapes irrespectively of spider or flower colour.

3.3 Materials and methods

Two colonies of bumblebees (*Bombus terrestris*) were obtained from Syngenta Bioline Bees, The Netherlands. All bees were reared in a dark environment devoid of visual cues (colour and shape) prior to commencement of the experiments. The experiments were conducted in a wooden flight arena (l = 1 m, w = 0.72 m and h = 0.73 m) with a UV-transmittent Plexiglas® lid and lit by two twin-lamps (TMS 24F with HF-B 236 TLD (4.3 KHz) ballasts, Philips, The Netherlands) fitted with Activa daylight fluorescent tubes (Osram, Germany). The arena contained an artificial 'meadow' of 16 'flowers' arranged in four evenly spaced rows on the end wall. Each flower consisted of a landing platform, where bees could land and extend their proboscises through a hole in the wall to feed on sucrose droplets (50% v/v) being formed at the end of syringe needles (BD Microlance™ Drogheda, Ireland, 3 26G 0.45 x 13 mm, delivered at a rate 1µl min^{-1} by syringe pumps: KD Scientific, KD200, Holliston, USA), and a removable square (7x7cm) floral colour signal (for further details see Chapter 2).

Experimental design

To determine whether bees are able to form generalised, colour independent, search images of predators we carried out two sets of experiments (Table 6). Our initial focus (Experiment 1) was to determine whether bees that had learnt to avoid cryptic predators in one colour context (yellow spiders on yellow flowers) would also be able to avoid cryptic predators in a different colour context (white spiders on yellow flowers). In the second experiment (Experiment 2), we used another colony of bees to test whether the transference of avoidance responses between colour contexts represents the use of shape specific search images.

Table 6 Summary of experimental design, where Y = yellow, W = white, F= flowers, S = Spiders and C = Circles

Colony	**Experimental group**	**Training**	**Avoidance assay**
A	1: Naive	16 YF, no spider	16 WF, 4 with WS
A	1: Experienced	16 YF, 4 with YS	16 WF, 4 with WS
B	2: White spiders	16 YF, 4 with YS	16 WF, 4 with WS, 4 with WC
B	2: White circles	16 YF, 4 with YC	16 WF, 4 with WC

Experiment 1

Pre-training

Prior to training, individually marked bees foraged freely in the arena. Once motivated foragers were identified (i.e. they filled their crops and returned to the nest repeatedly) they were individually pre-trained on yellow flowers (a 7x7cm flat yellow floral signal was placed flush with the wall at each feeding position). Pre-training lasted for a minimum of 100 flower visits (134.4 ± 4.4), to ensure bees had learned to associate yellow flowers with a sucrose reward (Ings and Chittka, 2008). Subsequently, bees were allocated into two treatment groups for training (Naïve and Experienced).

Training

During training both groups of bees foraged individually within the experimental meadow of yellow flowers. Their foraging behaviour was observed and scored into four categories: 1) *Choices* - where bees chose to land on flowers; 2) *Acceptances* - where the bees remained and extended their proboscises to feed; 3) *Aborts* - where bees landed but rapidly left without attempting to feed; and 4) *Rejections* - where bees inspected flowers (by entering a defined zone [h = 9cm, w = 9 cm, d = 7 cm] in front of the floral display; Fig. 3a) but rejected them without landing. In addition, the flight paths of all bees were recorded using a 3D tracking system (Trackit, Biobserve, Germany; for further information please see Chapter 2). Before training ceased, bees were required to make a minimum of 200 flower choices after which they were allowed to continue foraging and return to the nest under their own volition (thus the total number of choices varied among bees: Naïve = 240.4 ± 7.9, Experienced = 227.0 ± 8.0). This ensured that Experienced bees received sufficient (5.9 ± 1.0) simulated predation attempts to learn about predation risk from camouflaged spiders (Ings and Chittka, 2008).

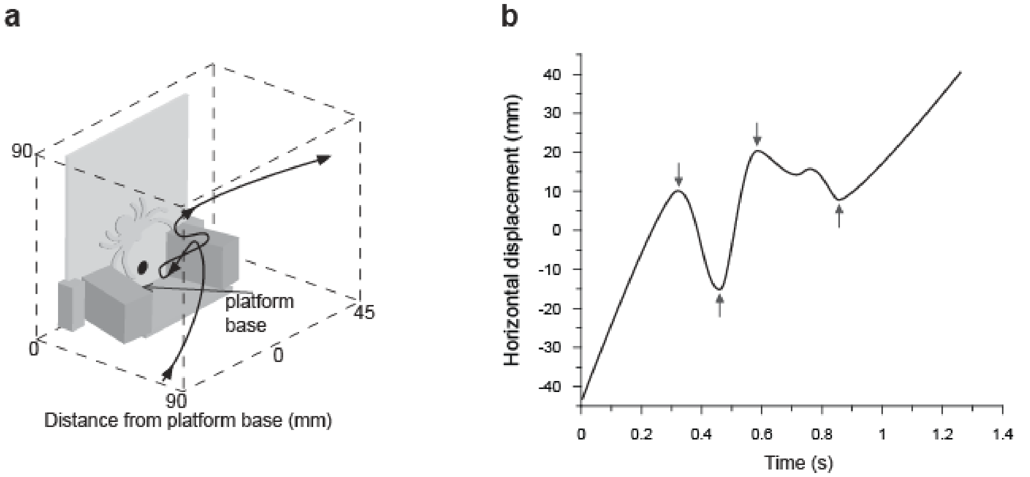


Fig. 3 Analysis of turning points. Panel a shows a dangerous flowers and the flower inspection zone (dashed cube) surrounding the flower. The bold line shows the 3D flight path of the bee as it inspects the flower. In panel b this path is translated to show horizontal (y) displacement relative to the platform against time to detect turning points (changes of direction > 5mm) which are indicated by the arrows.

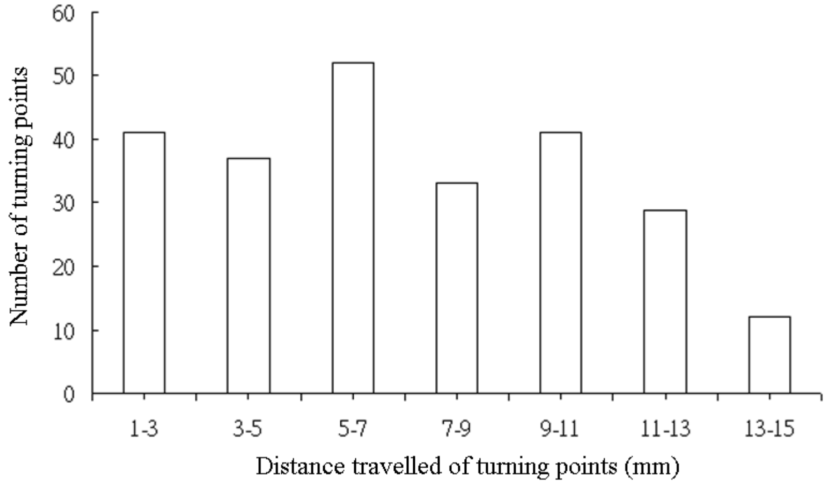


Fig. 4 Sample of numbers and the distances travelled of turning points in 100 flower choices. We selected 5mm as cutting point because it contains over 68% of the turning points, and also it contains 1.4 times more turning points than previous category. Turning points which moved small distances may be noise data which bees zigzag during their flight.

For the Naïve bees group (n=12), the artificial meadow remained the same as the pre-training phase and was free from predation risk. Bees in the Experienced group (n=12) were exposed to the same meadow of yellow flowers as Naïve bees, but there was a 25% risk of being attacked by a predator. Four randomly selected 'dangerous' flowers (out of 16) harboured cryptic 'predators'. Bees received a simulated predation attempt by a 'crab spider' whenever they landed on one of these flowers (see Chapter 2 for further information). Predators consisted of a 3D life-size model of the crab spider *Misumena vatia* (placed above the feeding hole) and a trapping mechanism that grasped bees between two foam coated pincers for 2 seconds. The pincers, which projected from the arena wall either side of the landing platform, were operated by a remotely controlled solenoid. During training, yellow spider models (painted the same yellow as the floral display) that were cryptic to bumblebees were used. To avoid bees learning the location of the spiders, their positions were randomly redistributed between foraging bouts when bees returned to the hive.

Avoidance assays

Directly after training, bees from both groups were tested in a new ‘meadow’ containing 16 white flowers. Four randomly chosen flowers harboured cryptic spiders (painted the same white as the floral displays), although bees landing on these flowers were not attacked. The behaviour and flight paths of bees in this new meadow were monitored until a minimum of 30 flower choices had been made. The majority of bees (22 out of 24) reached this criterion within their first foraging bout, only two bees, both in the Naïve group, required two foraging bouts.

Experiment 2

All 24 bees were pre-trained using the same procedure as Experiment 1 and subdivided into two treatment groups. The first group of bees were trained to avoid cryptic yellow spiders in the same was as the Experienced bees in Experiment 1. However, in the avoidance assay they were exposed to a meadow containing 16 white flowers where four randomly positioned flowers harboured cryptic white spiders and an additional four flowers harboured white circles. These circles were of similar area (323.7 mm^2) to the spiders (322.6 ± 6.5 mm^2) and protruded from the flower surface (they were made from 1mm thick plastic). Thus, the general appearance of the dangerous flowers (flat white with a 3D shape by the feeding hole) remained the same as those bearing spiders. As before, the behaviour of bees was monitored until they had made a minimum of 30 choices. A second group of bees from the same colony acted as a control group to ensure that bees are able to detect and learn to avoid circles. Therefore, the training and avoidance assays were carried out in the same manner as for the Experienced group in Experiment 1 with the exception that the four spiders were replaced with four circles in both training and avoidance assay phases (Table 6).

Statistical analysis

As we were interested in how bees' past experience of spiders, not current risk (i.e. spiders were present but the trapping mechanisms were inactive during tests), influenced their responses to the presence of cryptic spiders during the avoidance assay, only the behaviour and flight paths associated with first 30 flower *choices* were included in the analyses. One-sample t-tests were used to determine if the number of 'dangerous' flowers chosen during the avoidance assays differed from chance levels (7.5 flower visits). Where necessary, appropriate transformations were carried out to meet the assumptions of the statistical tests: for rejections of safe flowers and aborts of dangerous flowers the log(x+1) transformation was used. It was not possible to normalise the proportion of dangerous flowers accepted by transformations so these data were analysed using a Generalised Linear Model (GLM) using a binomial error distribution.

For the analysis of the flight paths the duration, distance travelled, the number of turning points and the average speed of inspection flights within the zone in front of the floral displays (Fig. 3a) were compared between treatment groups using t-tests. In the case of *acceptances* and *aborts,* the inspection flights were taken as the approach flight prior to a bee landing to feed. However, for *rejections*, the inspection flight was taken as the total flight path in front of the flower. All inspection flights of less that 0.1s duration were excluded from the analyses to prevent the inclusion of instances where bees passed through the inspection zone on their way to another flower. To quantify scanning behaviour when bees inspected and rejected flowers harbouring spiders, we plotted their flight paths in the horizontal x-y plane (Fig. 3b).

A turning point was counted when the bees' trajectory changed direction along the y axis (parallel to the flower display), and involved a displacement of at least 5mm (Fig. 1b). Statistical analyses were carried out in SPSS for Windows 11.5 and using the R statistical platform (R Development Core Team, 2004). All tests were two tailed with significance level set at 0.05.

3.4 Results

Experiment 1

By the end of training, experienced bees had learnt to avoid flowers harbouring cryptic yellow spiders: visitation rates (0.03 ± 0.01 visits per choice) to dangerous flowers during the last 30 choices were significantly below that expected (0.25) if the bees were choosing flowers at random (one sample t-test: $t_{(11)} = 16.455$, $p < 0.001$). During the avoidance assay, all bees (n=24) foraged successfully in the new patch of white flowers. There was no significant difference between groups of bees in the number of flowers chosen during their first foraging bouts (Experienced, $n = 55.5 \pm 4.9$; Naïve = 56.1 ± 5.7; $t_{(22)} = -0.078$, $p = 0.939$). Although the total number of flowers rejected during the first 30 choices (Experienced = 9.1 ± 1.7; Naïve = 17.0 ± 5.7; $t_{(22)} = -1.340$, $p = 0.197$) did not differ significantly between experienced and naïve bees, naïve bees did reject more '*safe'* (no spiders) flowers (Experienced = 2.6 ± 1.0; Naïve = 11.8 ± 4.8; [log transformed] $t_{(22)} = 3.183$, $p=0.004$), but not '*dangerous'* flowers (Experienced = 4.3 ± 0.9; Naïve = 4.6 ± 1.2; $t_{(22)} = -0.168$, $p = 0.868$) than experienced bees during this period. The latency to forage was variable among bees and, although experienced bees showed a non-significant trend to start foraging sooner (Experienced = 13.8 ± 3.2s and Naïve = 40.8 ± 12.5s) than naïve bees, ([log transformed] $t_{(22)} = -2.055$, $p = 0.052$).

Bees that had experienced attacks by cryptic yellow spiders (on yellow flowers) *chose* (landed on) 40% fewer white flowers harbouring cryptic (white) spiders than expected by chance during the avoidance assay (Fig. 5a; one sample t test: $t_{(11)} = -4.413$, $p = 0.001$). This was evident from the first flowers visited and the magnitude of the effect increased gradually as bees visited more flowers (Fig. 5a). In contrast, the total number of dangerous flowers chosen by bees with no prior experience of cryptic spiders did not deviate from that expected by chance (Fig. 5a; one-sample t-test: $t_{(11)} = -1.239$, $p = 0.241$) although there is a suggestion that it fell as bees visited more flowers. Furthermore, while naïve bees accepted almost all of the dangerous flowers they chose to land on (93.1 ± 3.0 %), experienced bees aborted many landings and only accepted fewer than 2/3 (60.9 ± 12.0 %) of the dangerous flowers they chose to land on (Fig. 5b; GLM [binomial error]: $F_{(1,21)} = 9.228$, $p = 0.006$). Thus, experienced bees ultimately fed from only half (2.9 ± 0.8) as many dangerous flowers as naïve bees (5.9 ± 0.8).

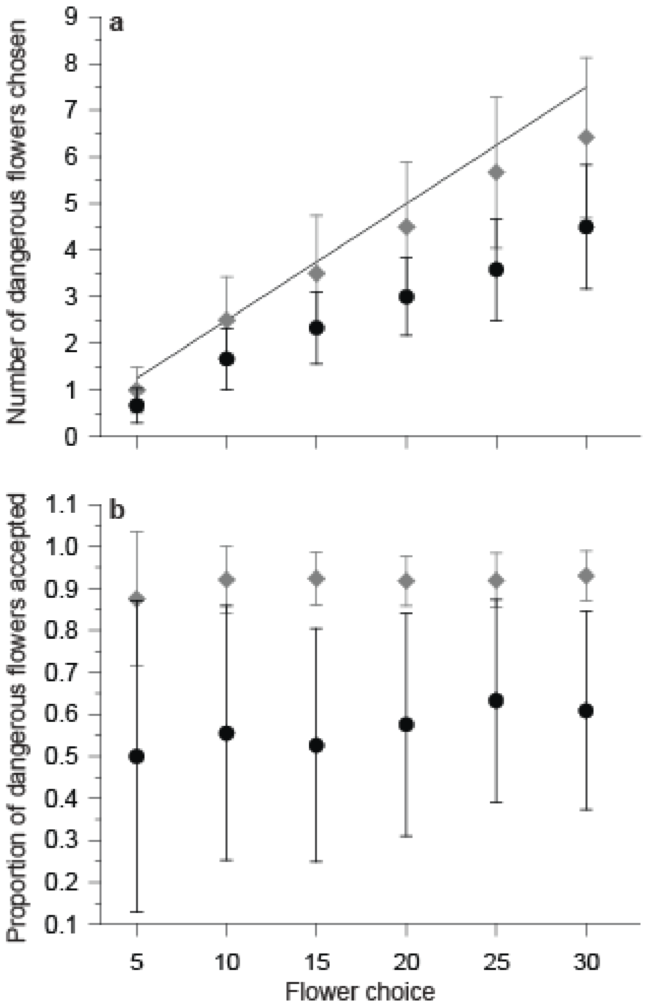


Fig. 5 Cumulative foraging choices made by bees during the first 30 flower choices of the avoidance assay. (a) The mean (±95% CI) number of dangerous flowers (harbouring cryptic white spiders) chosen by bees that had previously Experienced spiders (black circles) and spider Naïve bees (grey diamonds). The line in the panel represents the expected number of dangerous flowers chosen if bees showed no avoidance response to spiders. (b) The proportion of dangerous flowers chosen that were subsequently *accepted*, i.e. the bees continued to feed.

Prior experience of cryptic spiders also influenced bees' flight behaviour. Experienced bees spent 1.4 times longer than naïve bees inspecting safe flowers (Fig.

6a; $t_{(19)} = 3.862$, $p = 0.001$) and 1.5 times longer inspecting dangerous flowers (Fig. 6a; $t_{(20)} = 2.556$, $p = 0.019$) that they rejected. Furthermore, when rejecting flowers, experienced bees spent longer inspecting dangerous flowers than safe flowers (Fig. 6a, paired t-test: $t_{(7)} = -4.703$, $p = 0.002$), whereas there were no differences for naïve bees (Fig. 6a, paired t-test: $t_{(10)} = -1.706$, $p = 0.119$). Bees also altered the distance they travelled whilst inspecting flowers that they rejected (Fig. 6b). The flight paths of experienced bees were longer than those of naïve bees when they were rejecting both safe (2.9 ± 1.3 cm longer; $t_{(19)} = 2.279$, $p = 0.034$) and dangerous (5.0 ± 0.2 cm longer; $t_{(20)} = 2.258$, $p = 0.020$) flowers. Experienced bees also increased the length of their inspection flights for dangerous flowers relative to safe flowers (Fig. 6b, 4.7 ± 1.2 cm longer; paired t-test: $t_{(7)} = -4.007$, $p = 0.005$), but no change was observed for naïve bees (Fig. 6b; paired t-test: $t_{(10)} = -1.201$, $p = 0.258$). There were no differences in the length of inspection flights between treatment groups or flower types for flowers that were accepted (Fig. 6b).

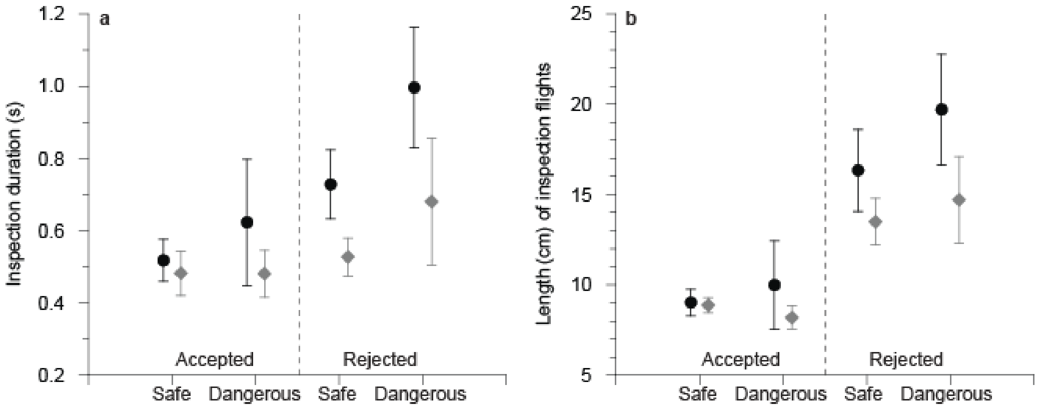


Fig. 6 Characteristics of inspection flights during the avoidance assay. (a) The mean (±95% CI) duration (seconds) of inspection flights and (b) the mean distance (cm) travelled in front of the flowers. Black circles represent bees that had Experienced spiders and grey diamonds represent spider Naïve bees.

The differences in the duration and length of inspection flights for rejected flowers corresponded to changes in the scanning behaviour of bees (Fig. 7). Experienced bees doubled the number of side-to-side scans (Figs 7, 8) when they inspected and rejected dangerous flowers (paired t-test: $t_{(7)} = -7.029$, $p < 0.001$), whereas the number of scans by naïve bees showed a slight, but non-significant, increase when they rejected dangerous flowers (paired t-test: $t_{(10)} = -1.884$, $p = 0.089$).

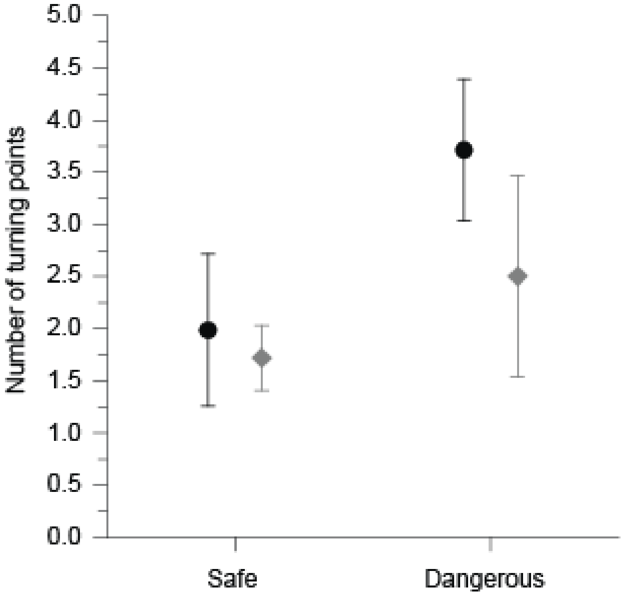


Fig. 7 The mean (±95% CI) number of turning points during left-right scanning of safe and dangerous flowers that were subsequently rejected. Black circles represent bees that had Experienced spiders and grey diamonds represent spider Naïve bees.

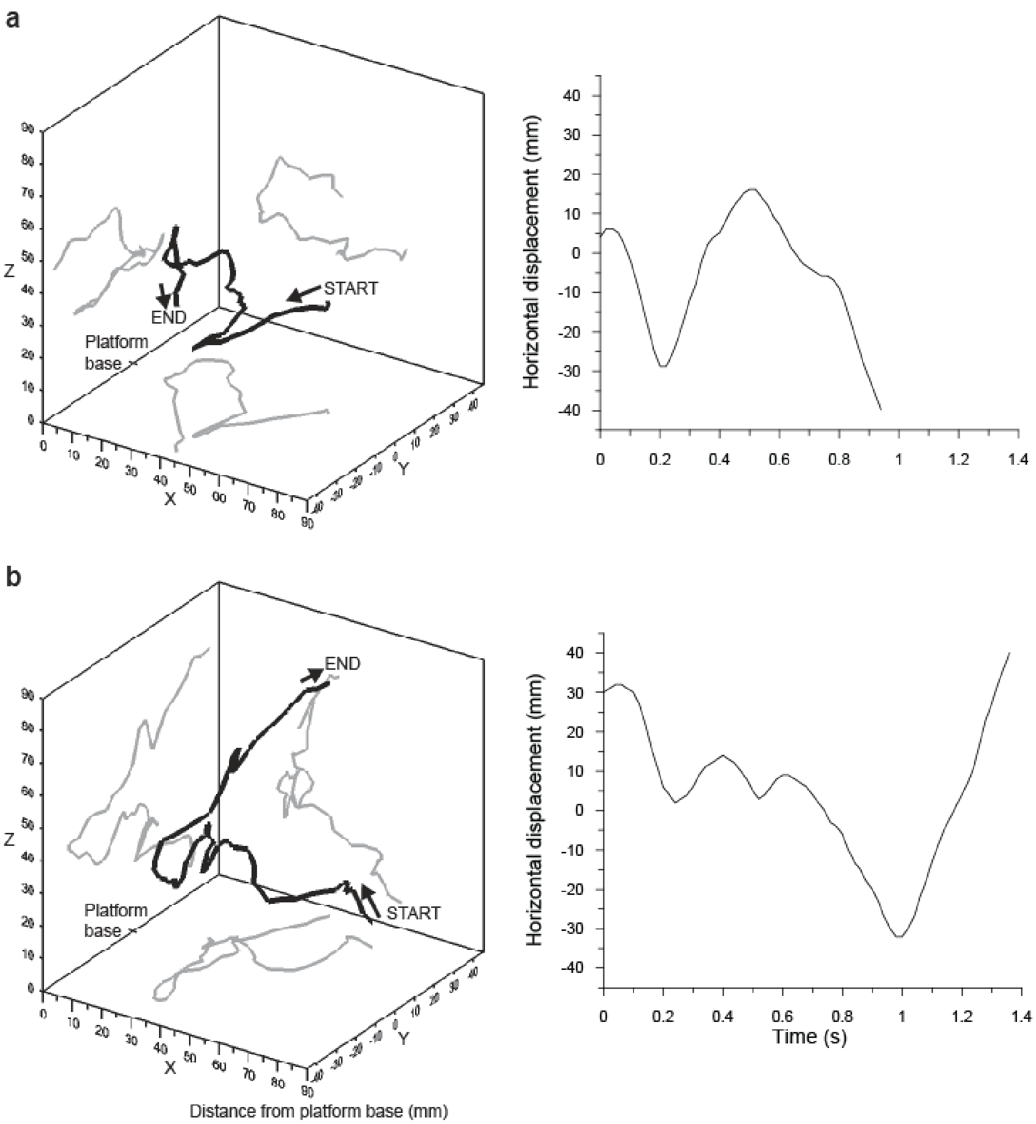


Fig. 8 An example of side-to-side scanning behaviour in front of a flower for a typical bee from the experienced treatment group: a) inspecting and rejecting a safe flower and b) a dangerous flower. Panels to the left show the 3D flight path within the flower zone relative to the base of the landing platforms (0,0,0). The bold lines are the actual trajectories and the grey lines are projections of the trajectories onto the three horizontal and vertical planes. Panels to the right show the turning points (direction changes of >5mm) of the horizontal component of the bees' inspection flight for safe (a) and dangerous (b) flowers.

Experiment 2

By the end of training, bees exposed to dangerous flowers harbouring yellow cryptic spiders (expected probability = 0.25, observed = 0.14 ± 0.03 , one sampled t-test: $t_{(11)} = -4.238$, $p = 0.001$), or yellow cryptic circles (expected probability = 0.25, observed = 0.12 ± 0.02 , one sampled t-test: $t_{(11)} = -6.434$, $p < 0.001$), chose significantly fewer dangerous flowers than expected by chance, i.e. they had learnt to avoid cryptic yellow spiders and circles. When the colour context changed (to white flowers and white spiders/circles), bees in the spider group chose less than half the number of spider harbouring flowers than expected if they were unable to recognise danger (Fig. 9: one sampled t-test: $t_{(11)} = -12.113$, $p < 0.001$). However, the same bees were indifferent to the presence of cryptic white circles on flowers: visitation rates to these flowers did not differ from chance levels. (Fig. 9: one sampled t-test: $t_{(11)} = -0.238$, $p = 0.816$). Even so, bees in the circle group (that had been trained to avoid cryptic yellow circles) also chose fewer flowers bearing cryptic white circles in the avoidance assay than would have been expected if they were unable to recognise danger (white circles: expected probability = 0.25, observed = 0.14 ± 0.02, one sampled t-test: $t_{(11)} = -6.240$, $p < 0.001$).

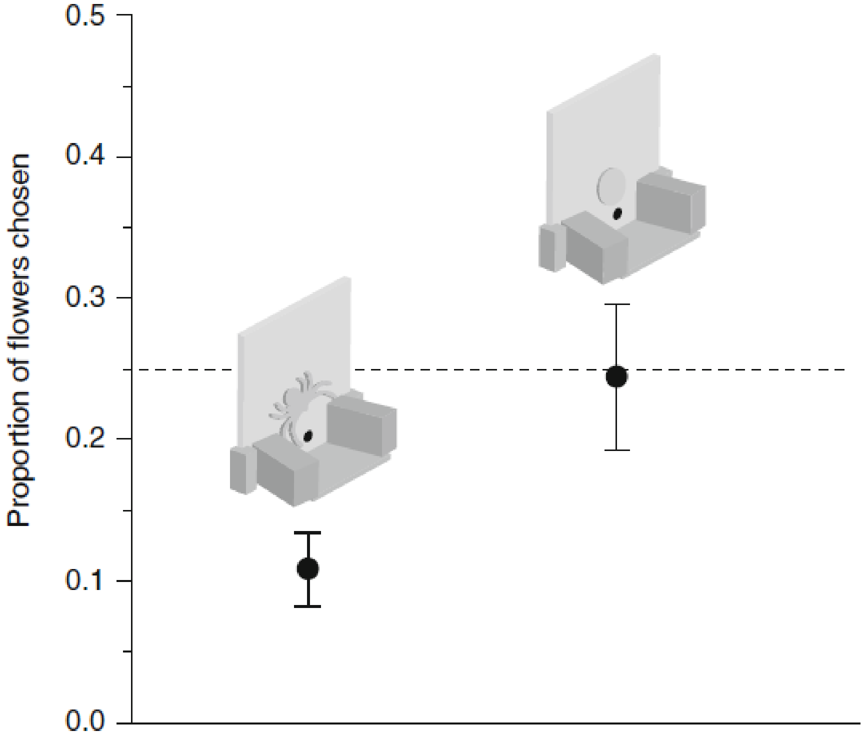


Fig. 9 The mean (± 95% CI) proportion of flowers harbouring either spiders (left circle) or circles (right circle) chosen by bees in the *spider* group during their first 30 choices during the avoidance assay. The dashed line represents the expected proportion of spider/circle flowers bees would choose if they showed no avoidance response to the shapes. Note that the shades used for the spider and circle flowers depicted were chosen for clarity and do not accurately represent the colours used in the experiments.

3.5 Discussion

We found the innate selective attention toward spider shape but not colour in bumblebees. Bees formed a colour-independent search image of cryptic predators which subsequently influenced their foraging behaviour when they were exposed to a new patch of flowers containing differently coloured cryptic predators. This ability is particularly important in the context of bumblebee-crab spider interactions where some species of spider, such as *M. vatia,* are able to reversibly change their colour (Morse, 2007, Insausti and Casas, 2008). Thus, rather than learning to detect just the cryptic yellow forms of the spiders, the bees appear to be learning complex shape cues that can be generalised (Stach et al., 2004) to other colour forms of the spider.

Previous work has shown that honeybees are able to use prior experience to enhance their ability to detect camouflaged shapes. Zhang and Srinivasan (1994) found that whilst naïve bees were unable to detect camouflaged shapes, detection was possible if they had previously been trained to discriminate the shapes in a simpler context – i.e. they had developed a search image for the shapes. We have now tested whether bees' search image of a predator consists of the shape memorised together with its colour, or whether bees are able to recognise the predator's shape irrespectively of its colour, requiring them to disentangle shape from colour features (Skorupski and Chittka, 2011). This is a non-trivial task because when both shape and colour cues are present, as is the case with the yellow spiders, bees tend to focus more on colour cues (Lehrer and Campan, 2004).

Many animals possess innate avoidance responses to major predators (e.g. birds, molluscs and fish: Veen et al., 2000, Turner et al., 2006, Dixson et al., 2010), but the possibility that bees possess an innate avoidance response to spiders has not been tested to date, although it is often alluded to (Dukas, 2001, Gonçalves-Souza et al., 2008, Reader et al., 2006). In the current study, naïve bees did not avoid flowers harbouring cryptic white spiders (Fig. 2a), which supports my previous observations (Ings and Chittka, 2008) that bumblebees do not appear to have a strong innate avoidance response to spider shapes. However, bees that had experienced attacks by cryptic yellow crab spiders, and learned to avoid such spiders, also avoided cryptic white crab spiders in a new patch of white flowers. They chose (landed on) 40% fewer spider harbouring flowers than expected by chance. Avoidance of flowers with spiders was evident right from the first few flower choices (Fig. 2a) and strengthened with increased exposure to more spider harbouring flowers. Furthermore, while naïve bees accepted nearly all of the dangerous flowers that they initially chose to land on, experienced bees aborted landings on many dangerous flowers without feeding (Fig. 2b). This suggests that bees only recognised ‘danger’ once they had briefly landed in front of the spider model – an effect already demonstrated for encounters with cryptic spiders (Ings and Chittka, 2008). Clearly, experiencing predation attempts by cryptic spiders influences the foraging behaviour of bees in a new patch of flowers, but are bees using colour-independent search images of predators?

One potential explanation for the apparent avoidance of flowers harbouring cryptic white spiders by experienced bees is that they were generally more ‘cautious’ as a result of being attacked during training (e.g. increased vigilance with higher predation risk: Lendrem, 1983, Hunter and Skinner, 1998, Winnie and Creel, 2007). However, evidence from my experiments rules out indiscriminate ‘cautiousness’. The appearance of both flowers and predators was different in the new patch and the predators were highly cryptic. Therefore, if experienced bees were more cautious overall than naïve bees, as a result of experiencing simulated spider attacks, we would have expected them to take longer to start foraging in the patch with new flowers and also to reject more safe flowers (Ings and Chittka, 2008). Yet, there was no clear evidence for an overall change in behaviour of experienced bees compared to naïve bees. In particular, there was no difference in the total number of flowers both groups of bees chose to land on during the avoidance assay and naïve bees rejected more safe flowers than experienced bees. Furthermore, bees that had experienced attacks from camouflaged spiders on yellow flowers, if anything, started foraging on the new white flowers sooner (though not significantly so) than bees that had no experience of spiders. This behaviour does suggest that, having been attacked on yellow flowers, experienced bees find white flowers more attractive (e.g. see Ings and Chittka, 2009) than naïve bees. However, overall, we argue that the reluctance of experienced bees to feed on flowers with cryptic white spiders is not a general response to being attacked by spiders, but is a specific response to their recognising the shape of the spiders which they associate with danger.

A potentially simpler explanation for the behaviour of experienced bees during the avoidance assay is that they were responding to the general appearance of the dangerous flowers relative to safe flowers rather than spider shapes specifically. In other words, even though the colour of the flowers changed between training and the avoidance assay, bees may have associated flowers that had a 3D object attached to the floral display with danger. However, evidence from experiment 2 rules out this possibility. If bees were indeed learning to avoid flowers that differed in general appearance to those that were safe in the training phase they should have avoided both flowers bearing spiders and those bearing circles. Although, having been trained to avoid cryptic yellow spiders they only avoided cryptic white spiders and were indifferent to white circles in the avoidance assay (Fig. 6). One can also rule out the possibility that indifference to cryptic circles occurred because bees were unable to detect them because bees in the *circle* group readily learnt to avoid cryptic yellow circles during training and also avoided cryptic white circles during the avoidance assay. Therefore, we argue that bees in experiment 1 and 2 had developed a search image for crab spider shapes.

Further support for the use of colour-independent spider search images by experienced bees in experiment 1 is provided by the analysis of their 3D flight paths. Experienced bees spent longer than naïve bees inspecting flowers (both with and without spiders) that they rejected (Fig 4a). More importantly, in the context of predator search images, experienced bees spent 30% longer inspecting the dangerous flowers that they rejected when compared to safe flowers. As neither group of bees had previously encountered white flowers before, these differences cannot be attributed to experienced bees associating white flowers with danger. A plausible explanation is that experienced bees invested more time into predator detection than naïve bees in response to their recent exposure to high predation risk (Lima and Bednekoff, 1999). However, there was no evidence for an overall increase in vigilance as experienced bees did not spend longer inspecting flowers that they chose to accept (Fig. 3a). This suggests that bees modulate vigilance, and potentially employ predator search-images, in response to a high 'perceived' predation threat over short time scales (Lima and Bednekoff, 1999) - even between flower visits. As bees moved rapidly from flower to flower in the meadow (mean interflower time was only 1.6 ± 0.3 seconds and the mean approach speed was 0.22 ± 0.01 ms^{-1}), the probability of detection errors (not perceiving a potential predator) is likely to be relatively high (Ings and Chittka, 2008). We therefore argue that experienced bees only shift their attention towards predator detection (i.e. used their predator search image) when they detected flowers whose appearance subtly differed (the presence of the 3D cryptic spider) from safe flowers, or after they had recently detected a threat on a nearby flower.

Evidence for this switch to predator detection upon perceiving a potential threat is provided by closer scrutiny of the bees' flight paths. Experienced bees also travelled further when they were inspecting dangerous flowers (Fig. 3b). More importantly, the greater distance travelled was a result of increased side to side scanning of the flowers (Figs 4 & 5). Although we were not able to track the relative position of bumblebees' heads and thoraxes, their scanning movements were similar to the peering flight manoeuvres recently described in honeybees (Boeddeker and Hemmi, 2010). These repeated side-to-side movements would improve edge detection by amplifying the weak spider shape signal through integration over time. For example, scanning may allow bees to use relative motion cues (Zhang and Srinivasan, 1994), i.e. changes in the appearance of shadows cast by the 3D spider models, to facilitate shape detection.

The results demonstrate that search images are important in the context of predator avoidance, when prey have to be vigilant for cryptic predators. More importantly, we found that bees are able to develop search images that do not tightly link colour and shape. Rather than search for ‘yellow spiders’, bees were able to search for ‘spider shapes’ but not other yellow material. This ability to respond to shape irrespectively of colour has only recently been recognised in hymenoptera (Lehrer and Campan, 2005, Lehrer and Campan, 2004). Here we have shown how this ability to disentangle shape from colour can enhance detection of colour changing, cryptic predators.

Chapter 4 Divided attention in bees: can bees simultaneously engage in adaptive foraging behaviour and attend to cryptic predators?

4.1 Abstract

Foraging animals need to balance efficient foraging against predator avoidance. Bees foraging for nectar often have to discriminate between flowers with similar appearance but different nectar rewards. At the same time they must be vigilant for ambush predators, such as crab spiders, which can camouflage themselves on flowers. Here we examined bees with a task where they had to discriminate between flowers with high target-distractor similarity that differ in reward level and concurrently detected predation risk from conspicuous or cryptic predators. Robotic spiders were used to simulate predation risk. We found that bees prioritized predator avoidance at the expense of efficient foraging when faced with detecting cryptic predators and a difficult colour discrimination task. Bees that encountered conspicuous spiders were able to discriminate between low and high reward flowers of similar appearance and avoid predators. When the task of discrimination became difficult, bees prioritized the avoidance of predation, but gave up priotising the identification of highly rewarding flowers.

4.2 Introduction

Animals are exposed to a constant flow of complex sensory input. Foragers, for example, must prioritize information relevant to important tasks, such as locating the most rewarding food items or detecting predators (Milinski, 1984, Godin and Smith, 1988, Clark and Dukas, 2003). Foraging and visual search often require a trade-off between attending to the foraging target (e.g. flowers) and focusing on potential danger in the environment (e.g. sit-and-wait predators on flowers). A foraging bee will spend most of its time choosing between visual targets (flowers) that vary in colour, shape, and pattern – and is under constant pressure to select the most rewarding flowers while minimizing predation risk and energetic costs (Chittka and Menzel, 1992). The task can be challenging and highly dynamic since there are distractor flowers, i.e. other plant species with different traits (Schaefer and Ruxton, 2009) and camouflaged predators in the field (Morse, 2007). Many plant species, such as those in the orchid family, have flowers which resemble the appearance or odour of co-occurring, rewarding species to attract pollinators (Dafni, 1984, Roy and Widmer, 1999). Moreover, predators can use the attractiveness of flowers to lure their prey. For example, crab spiders (Araneae: Thomisidae) are sit-and-wait predators that ambush pollinators, such as bees, on flowers (Chittka, 2001, Insausti and Casas, 2008). Some species of crab spiders can reversibly change their body colour to match that of the flower they are hunting on (Morse, 1986). They even preferentially hunt on high quality flowers (Morse, 1986), which are also preferred by foraging bees (Menzel et al., 1993). These upper level consumers can have huge impact on the entire food chain not only by directly consuming their prey, but also through non-consumptive effects such as the existence of predation threat (Luttbeg and Kerby, 2005, Preisser et al., 2005, Schmitz et al., 2008). The prey can alter their behaviour strategies with the presence of the predator and may proceed to influence their own prey and resources. Previous studies found that the existence of the predator which alters prey's traits has the same effect as when the predators actually consume the prey (Presser et al., 2005). We therefore tested how prey responded to the existence of predators in difficult foraging conditions in order to give a better understanding of how traits of both predators and prey influenced the dynamics of the ecosystems.

We have a good understanding of the individual problems facing foraging bees: how they choose between different flowers (Giurfa and Lehrer, 2001, Shafir et al., 2003, Chittka and Raine, 2006) and how they interact with predators (Heiling and Herberstein, 2004, Dukas, 2005, Reader et al., 2006). Bees can associate food rewards with specific floral traits, such as colour, and can successfully discriminate between even subtle differences in traits (colour) to maximise foraging efficiency (Dyer and Chittka, 2004a). Furthermore, bees are known to be able to learn to avoid both individual flowers harbouring predators and sets of flowers of a given type (colour) associated with predation risk (Ings and Chittka, 2008, Ings and Chittka, 2009, Jones and Dornhaus, 2011). However, as far as we are aware, no study has considered how bees perform when exposed to both flower colour discrimination and predator avoidance tasks simultaneously, a situation which bees must naturally face. Evidence from field studies suggests that bees may choose to avoid a patch harbouring predatory crab spiders (Dukas and Morse, 2003), and laboratory studies indicate that bees may also choose to switch to a less risky flower species (Ings and Chittka, 2009, Jones and Dornhaus, 2011). Therefore, we ask whether bees have the perceptual processing power to carry out such tasks simultaneously at all.

In this study we ask whether bumblebees are able to maximise energy gains by solving a difficult colour discrimination task whilst simultaneously exposed to predation threat from camouflaged or conspicuous predators. We expose bees to an ecologically relevant scenario where they forage in an artificial meadow with two visually similar flower types differing in reward quality. Visiting the highly rewarding flower type is risky because 25% of flowers harbour predatory crab spider models. If bees are able to simultaneously solve colour discrimination and predator avoidance tasks we predict that they will visit the highly rewarding species but avoid individual flowers that are risky. The null hypothesis is that bees are unable to attend to two difficult tasks simultaneously and that i) bees will prioritise predator detection and avoidance when predators are camouflaged and ii) they will continue to maximise energy gains when predators are highly conspicuous.

4.3 Materials and methods

Study Animals

Three colonies of bumblebees (*Bombus terrestris* Dalla Torre 1882) from a commercial supplier (Syngenta Bioline Bees, Weert, Netherlands) were used in the experiment. All the bees were individually tagged with number tags (Christian Graze KG, Weinstadt-Endersbach, Germany). Colonies were kept at room temperature (~23°C) and subjected to a 12 hr light/dark cycle (light on at 8am). Sucrose solution (50%, v/v) and pollen was provided ad libitum. A total of 44 foragers were used in the experiments, including 10 bees used in the colour targeting pilot study.

Experimental Apparatus

All experiments were conducted in a wooden flight arena (1.0 × 0.72 × 0.73 m) with a UV-transmitting Plexiglas lid. Artificial flowers (7×7 cm acrylic, 1 mm thick) were arranged in a four by four vertical grid on one end wall of the arena on a grey background. Bees were able to access rewards (sucrose solution) through a hole which was 10 mm above a wooden landing platform (40×60 mm). Rewards were supplied to each flower and dispensed from the tips of 26G syringe needles (BD Microlance Drogheda, Ireland; 0.45 × 13 mm) by syringe pumps (KD Scientific, KD200, Holliston, USA). A maximum droplet volume of 4.70 + 0.3 μl could be reached before it fell into a 'waste pot' which was not accessible to bees (thus mimicking a flower that had been emptied by a bee). Robotic 'spider arms' (custom-built by Liversidge & Atkinson, Romford, UK) covered with sponges were set up at the base of the flowers to simulate predation attempts. The trapping mechanism enabled us to capture bees without causing physical damage. ‘Dangerous flowers’ were fitted with life-sized crab spider (*Misumena vatia*) models (l = 12mm, made from Gedeo Crystal resin) placed on the flowers above the feeding hole. The flight behaviour and position of bees were recorded during the experiment with three dimensional coordinates of bee positions being calculated 50 times per second using two video cameras connected to a computer running Trackit 3D software (BIOBSERVE GmbH, Bonn, Germany). The details of the setup were described in Chapter 2.

Targeting colour for experimental use

The aim of the test was to find two colours which are possible, but difficult for bees to distinguish. We chose two different shades of yellow (dark yellow & light yellow) whose distance in the colour hexagon was 0.03 units. In a previous study, it was easy for bees to discriminate two colours with a distance of 0.152 hexagon units, and bees were unable to discriminate two colours that were 0.01 hexagon units apart (Dyer and Chittka, 2004a). Thus the two colours used here should be difficult, but possible, for bees to discriminate (see Fig 10 & 11). Another group of bees from the same colony foraged on dark yellow and white flowers as a control group (this is a simple discrimination for bees). Two groups of bumblebee foragers (five bees in each) randomly selected from a single colony were tested individually for 200 choices in this test. Bees that foraged on dark yellow flowers were rewarded with 50% sucrose solution while bees that visited the light yellow/white flowers were punished with 0.12% quinine hemisulfate salt solution.

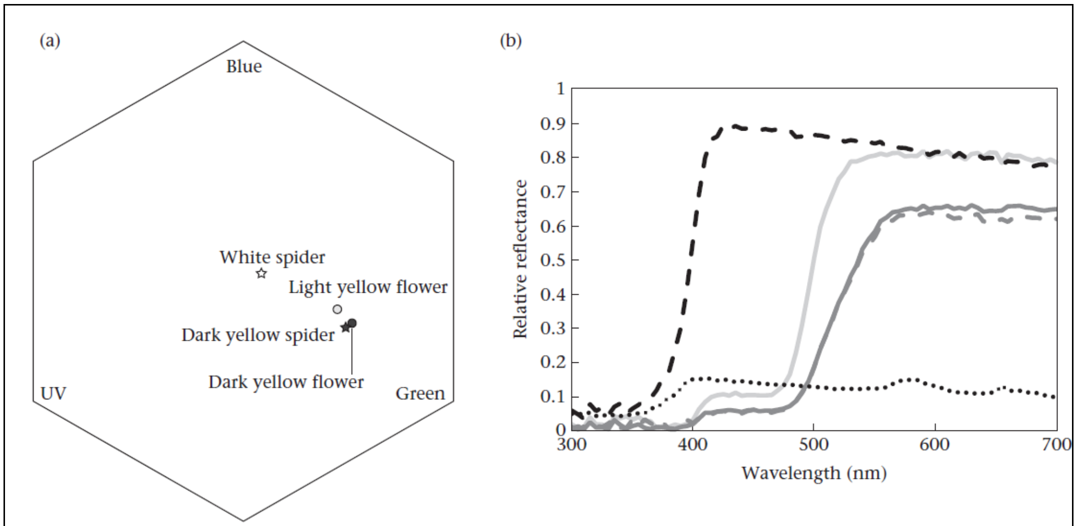


Fig 10 (a) Appearance of yellow flowers (circles: light grey for light yellow and dark grey for dark yellow) and spiders (stars: white for conspicuous spiders and dark grey for cryptic spiders) in bee colour space (calculated using *Bombus terrestris* colour receptor sensitivity functions in Skorupski et al. 2007) relative to the grey background colour (centre of the hexagon). Positions of the colour loci in the hexagon indicate excitation differences of the three bee colour receptors. The corners of the hexagon labelled UV, Blue and Green correspond to hypothetical maximum excitation of one receptor combined with zero excitation in the two others. The angular position in the hexagon (as measured from the centre) is indicative of bee subjective hue. Loci that are close together appear similar to bees and loci that are far apart appear different. (b) Spectral reflectance curves of artificial flowers, spiders and the grey background of the meadow. The dashed lines represent spiders (dark grey = dark yellow spiders and light grey = white spiders), solid lines flowers (dark grey = dark yellow flowers and light grey = light yellow flowers) and the dotted line represents the grey meadow background.

The learning curve (Fig 11) demonstrates that bees' last 30 choices seem to be stable, so we chose to compare the first and last 30 choices for the colour discrimination test. The percentage of bees choosing rewarding flowers in the average of the last 30 choices was significantly higher than the first 30 choices (first 30 choices: mean = 60.7, sd = 17.1; last 30 choices: mean = 87.3, sd = 111.4; pair t test, $t_{(4)} = 2.906$, $p = 0.020$, Cohen's $d = 1.838$). This confirmed that bees were able to learn to distinguish the two colours despite their high degree of similarity. The average percentage of correct choices during the last 30 choices was significantly higher for the easily distinguishable colours (white and dark yellow flowers) than for the more similar coloured (dark and light yellow) flowers (easily distinguishing colours: mesn = 100, sd = 0; hardly distinguishing colours: mean = 87.3, sd = 11.4; t test, $t_{(8)} = 2.484$, $p = 0.038$, Cohen's $d = 1.571$). The results showed that bees were able to discriminate the two similar shades of yellow, but with greater difficulty compared to the easy condition. This makes the highly similar flowers (dark and light yellow) suitable for the experimental conditions in which both colour discrimination and predator detection tasks were difficult enough for bees to solve, in order to discover how the bees learn to attend to the presence of the risks and rewards provided in the following experiments.

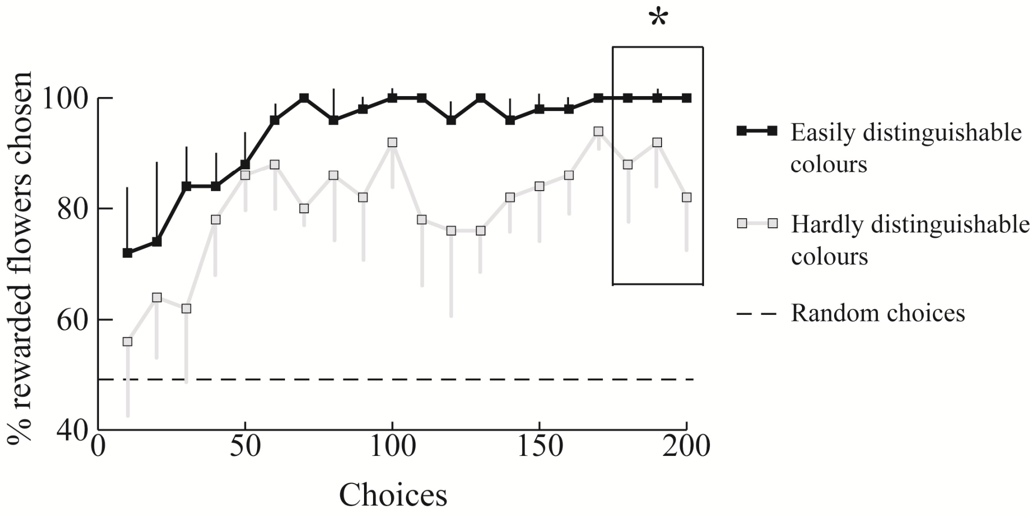


Fig 11 Discrimination test for similar and distinct colours. The black line is the average percentage of bees choosing rewarding flowers between easily distinguishable colours (white v. s. dark yellow), and the grey line is between hardly distinguishable colours (dark yellow v. s. light yellow). Each data point represents 10 choices +/- SE. Dark yellow flowers contained 50% (v/v) sucrose solution while white and light yellow flowers contained 0.12% quinine solution. Bees were able to identify the rewarding colour eventually in both colour combinations, but the average correct choices for last 30 choices between white and yellow flowers was significantly higher than dark and light yellow flowers. The two shades of yellow were distinguishable, but with more difficulty.

Pre-training

All bees were allowed to fly in the flight arena in groups without any presentation of floral signals for at least one day before the experiment. A constant flow (1.85 + 0.3 µl per minute) of 50% (v/v) sucrose solution was given as a food reward. Only bees that left the colony and fed on the flowers consistently for at least three consecutive foraging bouts were used in the experiments.

Experimental Design

In this experiment we asked whether bees exposed to an ecologically relevant scenario were able to simultaneously solve a colour discrimination task to maximise energy gains whilst avoiding conspicuous or camouflaged predators. Bees could choose between two types of flowers that were similar shades of yellow but provided food rewards of different quality - the flower colours were chosen so that bees could distinguish between them, but only with significant difficulty (see Materials and Methods section for the pilot study). The high quality flowers carried a risk of predation (25%) from either conspicuous or cryptic 'robotic spiders' (Ings & Chittka 2008). Individual bees ($N = 34$ randomly selected from 2 colonies) were initially trained to distinguish between the shades of yellow (colour discrimination training), with the darker flowers (D50) containing high quality rewards (50% v/v sucrose) and the lighter flowers (L20) providing low quality rewards (20% v/v sucrose; see Fig 12). Preference for the high quality dark flowers was assessed after bees had made 200 flower choices (if this occurred half way through a foraging bout they were left to complete the bout to avoid undue disturbance). After initial colour discrimination training (training phase), bees were randomly assigned to one of two groups exposed to predation risk on high quality flowers (25% of flowers harboured robotic spiders) by either conspicuous (white spider model on dark yellow flower) or cryptic (dark yellow spider model on dark yellow flower) spiders ($N = 17$ in each group). Predator avoidance training (testing phase) lasted for a further 200 flower choices and every time a bee landed on a high reward flower with a spider (dangerous flower) it received a simulated predation attempt whereby the bee was held by the arms of a robotic crab spider for two seconds. This emulates natural spider attacks on bumblebees, where bees are grasped by the raptorial forelegs of the spider but manage to escape, avoiding immobilization by the spider's bite.

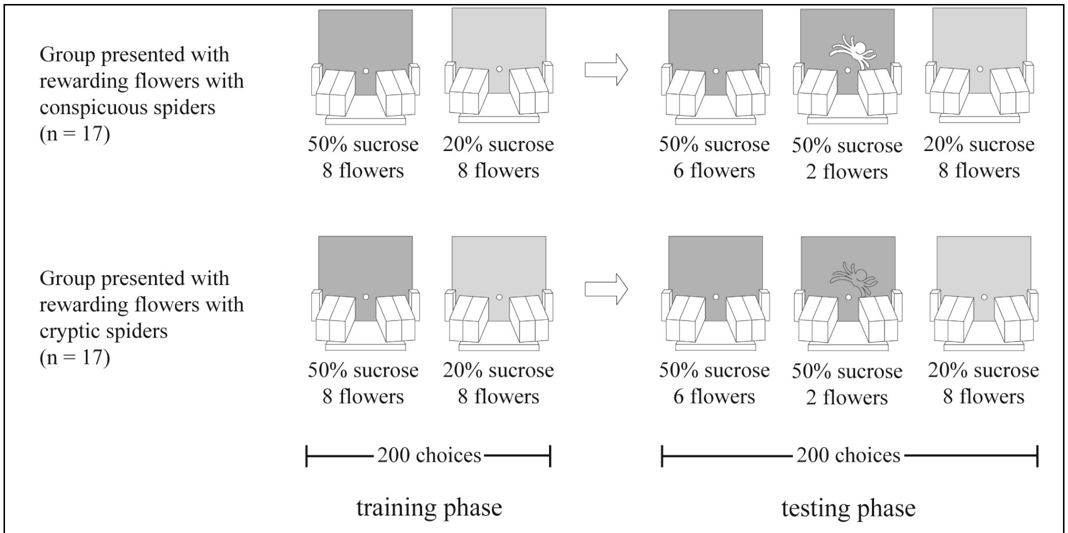


Fig 12 Experimental design for testing how bees solve difficult colour discrimination and predator avoidance tasks. In the training phase both experimental groups faced the same setup, with two similar flowers containing different reward level. In the testing phase 25 % of the highly rewarding flowers contained predators (either conspicuous or cryptic). When the bees chose the dangerous flowers they were captured for two seconds to simulate the predation threat. Two bees in each group stopped foraging (not moving for more than two hours) and were excluded from the analysis.

Data Analysis

Individual bees' preferences for dark yellow (D50) flowers were separated into four (first 100 foraging trials and last 100 trials in both training and testing phase) and compared with a one-way ANOVA test in both experiments. The effect size (Eta-square) was calculated by between subjects sum of squares divided by total sum of squares. The last preferences were then used as the baseline to compare performance during the predator avoidance training phase, where bees were exposed to predation risk. For example, under the null hypothesis of no spider avoidance, a bee reaching 80% correct choices at the end of the colour discrimination training phase would have a probability of choosing safe highly rewarding flowers (6 safe flowers out of 8 highly rewarding flowers) of $0.8 \times 0.75 = 0.6$. The last 30 choices in the testing phase were compared with hypothesized choices with a paired t test. Effect size (Cohen's *d*) is provided for the paired t test. Dangerous flowers chosen by bees were also compared with predicted choices (last 30 choices in the training phase multiplied by 0.25, which was the percentage of the dangerous flowers). The data were not normally distributed; thus a Wilcoxon test was used. Effect size is given as $r = Z/\sqrt{n}$.

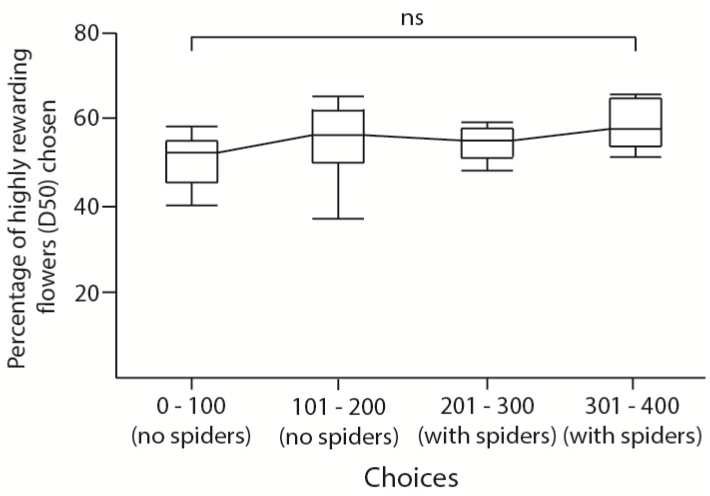
The time bees spent investigating and feeding on flowers was calculated from time and position data recorded using Trackit 3D software. Investigating zones were 7 cm (length) by 9 cm (width) by 9 cm (height) from landing platforms, and the feeding zones were 4.5 cm by 1 cm by 1 cm from the feeding hole. Investigating zones were set based on the visual angles of bumblebees (2.7 degree) where bees were able to detect both flower signals and predators using colour contrast (Spaethe et al. 2001) and feeding zones were based on observation of the position bees take whilst feeding at the flowers. Only instances when bees landed and fed on the flowers were considered as choices. Investigation duration was quantified as the time spent in the investigation zone before landing on a flower, or choosing to depart (when bees rejected the flowers without landing). The time bees spent investigating in front of the flowers was analysed with General linear models (GLM). The investigating time was set as a response variable included testing phase (with or without spiders) as covariates and the detectability of spiders as a fix factor. Interactions between covariate and fix factors were also explored. Rejection of flowers was compared by t test between conspicuous and cryptic spider group. For paired or unpaired t tests Cohen's *d* was given as effect size. Data were analysed using R (v. 2.15.1) and JMP (v. 7, SAS Institute). Four bees (two from each experiment) stopped foraging during training and were excluded from the analyses.

4.4 Results

There was no significant difference of highly rewarding (D50) flowers visited by bees before and after conspicuous spiders were added to the meadow (ANOVA, $F_{(3, 56)} = 0.843$, $p = 0.476$, $\eta^2 = 0.043$; Fig 13). However, when the spiders were cryptic, the effect of spiders was significant (ANOVA, $F_{(3, 56)} = 7.849$, $p < 0.001$, $\eta^2 = 0.296$; Fig 13). When comparing only safe highly rewarding flowers visited by bees (excluded ones with spiders), we calculated the hypothesized choices based on the last 30 choices in the training phase (170 – 200 choices, no spiders), because after spiders were added the number of safe highly rewarding flowers decreased. The last 30 choices in the testing phase (370 – 400 choices, with spiders) were significantly lower than hypothesized choices (hypothesized choices: mean = 45.7, sd = 8.7; last 30 choices: mean = 38.4, sd = 10.1; paired t test: $t_{(14)} = 3.092$, $p = 0.008$, Cohen's $d = 0.768$; Fig 14), but when spiders were conspicuous the effect was not significant (hypothesized choices: mean = 44.3, sd = 7.1; last 30 choices: mean = 54.0, sd = 17.2; paired t test: $t_{(14)} = 3.092$, $p = 0.063$, Cohen's $d = -0.737$; Fig 14).

In both groups bees were able to avoid dangerous flowers in the end of testing phase (conspicuous spider group: hypothesized mean = 14.8, sd = 2.4, last 30 visits to dangerous flowers mean = 1.6, sd = 2.8, Wilcoxon test $Z = -4.718$, $n = 15$, $p < 0.001$, effect size calculated by $r = Z / \sqrt{n} = -0.629$; cryptic spider group: hypothesized mean = 15.2, sd = 2.9, last 30 visits to dangerous flowers mean = 1.8, sd = 2.5, Wilcoxon test $Z = -4.722$, $n = 15$, $p < 0.001$, effect size $r = -0.630$, Fig 14).

(a) Conspicuous spiders



(b) Cryptic spiders

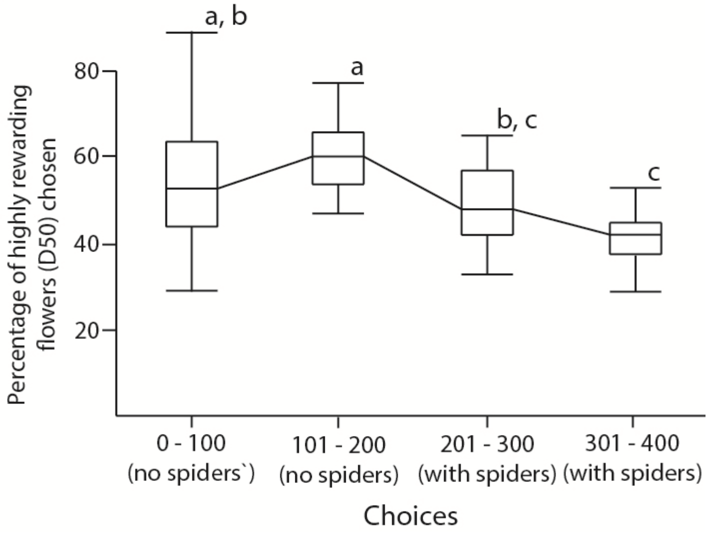
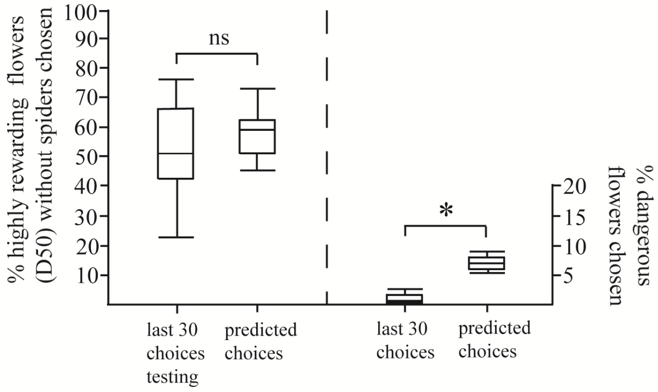


Fig 13 Percentages of rewarding flowers (D50) chosen by bees in both (a) conspicuous spiders group and (b) cryptic spiders group before the spiders were added (training phase, 0 – 200 choices) and after the spiders were added (testing phase, 201 – 400 choices). There is no significant difference before and after the spiders were added when the spiders were easy to detect (a), but when the spiders were cryptic, the percentages of correct choices significantly dropped (b). The symbols represent the groups in the Tukey's HSD post hoc test.

(a) Rewarding flowers;
conspicuous spiders



(b) Rewarding flowers;
cryptic spiders

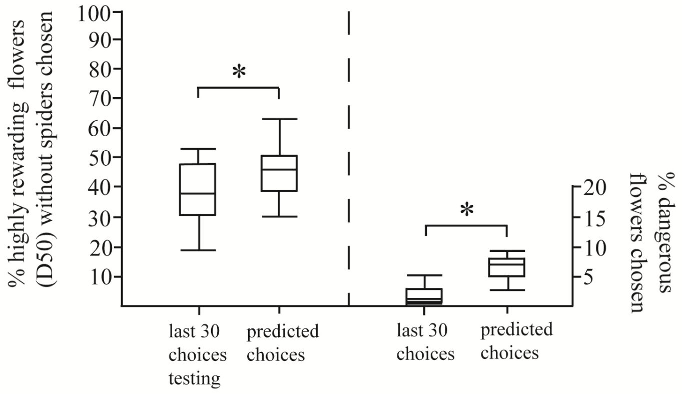


Fig 14 No matter whether the spiders were conspicuous or cryptic, bees were able to avoid the dangerous flowers (visited significantly fewer dangerous flowers than predicted value). When the spiders were easy to detect, bees maintained the choices to the safe highly rewarding flowers (no significantly difference from predicted choices) while bees encountered cryptic spiders decreased their performance (chose significantly lower safe highly rewarding flowers than predicted).

There was no significant difference in the time bees spent in front of the flowers in either before or after spiders were added or in different experimental groups (GLM,

$F_{(3,56)} = 0.516$, $p = 0.673$). Bees in the cryptic spiders group rejected (investigated but did not land) significantly more high and low rewarding flowers (highly rewarding flowers rejected: conspicuous spider group mean = 12.0, sd = 7.6, cryptic spider group mean = 31.9, sd = 28.1, $t_{(28)} = 2.601$, $p = 0.015$, Cohen's $d = -0.950$; low reward flowers rejected: conspicuous spider group mean = 8.5, sd = 6.3, cryptic spider group mean = 17.3, sd = 11.1, $t_{(28)} = 2.648$, $p = 0.013$, Cohen's $d = -0.970$). However, there was no significant difference in the number of dangerous flowers rejected (conspicuous spider group mean = 15.3, sd = 6.6, cryptic spider group mean = 18.3, sd = 7.5, $t_{(28)} = 1.168$, $p = 0.252$, Cohen's $d = -0.427$). Overall, bees were unable to simultaneously avoid predators and discriminate between highly similar flowers colours associated with different rewards.

4.5 Discussion

In this chapter we have presented bees with two ecologically relevant tasks that potentially lead to attentional competition. The first task was to maximise energy gains by using flower colour to differentiate between reward qualities. The second was to detect and avoid predators that were either conspicuous or cryptic. We found that when predator detection was difficult, that is predators were cryptic rather than conspicuous, bees prioritised predator avoidance over floral colour discrimination.

During the training phase, bees from both experimental groups learned to perform a difficult colour discrimination task in order to maximise energy gain from flowers. By the end of the training they had learnt to avoid distractor flowers containing low rewards (20% v/v sucrose solution) and focussed their foraging efforts on the most rewarding (50% v/v sucrose) flower type. While the error rate was relatively high (~40% visits to poorly rewarding flowers), it has been demonstrated in humans that increased task difficulty decreases attention to even highly conspicuous distractors.

This implies that bees employ a degree of attentional modulation depending upon the balance of risks and rewards (Giurfa, 2013). Further support for this is given by bees' responses to the introduction of predation risk from either conspicuous or cryptic spiders during the colour discrimination task.

All bees rapidly responded to the presence of predators and were able to avoid risky flowers by the end of the experiment, irrespective of the detectability of the spiders. However, avoidance of spiders affected bees' ability to discriminate between high and low rewarding flowers differently depending on the detectability of spiders. When spiders were conspicuous, bees were able to avoid dangerous flowers and still maintain their preference for the highly rewarding flowers. In contrast, when spiders were highly cryptic, bees avoided the spiders but did not discriminate between the remaining safe flowers.

It has recently been suggested (Morawetz and Spaethe, 2012) that bumblebees may carry out restricted parallel visual search – i.e. where the whole visual field is processed simultaneously and the targets "pop out" from distractors. Assuming this is true, bees in our study could be assessing the meadow every time they approach the meadow wall and focusing their attention on flowers that match their search image (i.e. dark yellow flowers = highest reward). In the case of bees exposed to conspicuous predators, flowers with spiders will sufficiently alter the appearance of the dark yellow flowers (i.e. a large central white area on the dark yellow background) so that they are processed as distractors and hence avoided. However, when spiders are cryptic, and thus do not create sufficient colour contrast against the floral display to alter the appearance of the flower relative to the bees' search image for high reward flowers, we would expect bees to initially choose mostly dark yellow flowers. This is exactly what we observed during the first few choices made by bees in the cryptic spider group. Experience of predation attempts at flowers with spiders would thus lead bees to develop a new search image for dangerous flowers (Ings et al., 2012). Maintaining two very similar search images with opposing values (i.e. dark yellow flower = high food reward whilst dark yellow flower with dark yellow spider = predation risk) is likely to be cognitively demanding. Indeed, in this experiment, bees encountering cryptic spiders did prioritise predator avoidance at the expense of discriminating floral reward quality of the remaining safe flowers.

Bees do not simultaneously attend to difficult colour discrimination and predator avoidance tasks. Alternatively, focusing on predator avoidance may be an efficient strategy employed by bees rather than evidence of sensory processing limitations. The costs of attending to both tasks simultaneously may outweigh the benefits of choosing highly rewarding over low rewarding flowers. Even in the absence of spiders, the difficulty of the colour discrimination task meant that bees only chose 10% more high rewarding flowers than they would have if choosing flowers at random. Thus, because making an error in terms of avoiding a predator is potentially more costly under natural conditions (injury or death) it was more efficient for bees to focus on predator avoidance. In this chapter we ask whether bees can maintain efficient foraging in the presence of cryptic predators, finding that bees had no difficulty avoiding conspicuous spiders while still foraging adaptively. However, they prioritised predator avoidance at the expense of maximising energy intake when faced with detecting cryptic predators and a difficult colour discrimination task.

We found that when flowers were rewarded in different levels, bees failed to solve both flower discrimination and predator avoidance tasks. The bees chose to concentrate on predator avoidance and ignoring the reward variation. It is possible that the reward disparity influenced bee’s choices. In previous studies, researchers demonstrated that when two flowers were rewarding at the same level, and one species harboured spiders, bees would switch to the flower species which did not contain spiders (Ings and Chittka, 2008, 2009). In the current study we selected 50% and 20% (v/v) sucrose reward because they are ecologically realistic and can be found frequently in nature (Tamm and Clifton, 1986). When the reward level was too low or no reward was present, bees would not have motivation to visit the flower.

In the present experiment we analyse the data at the level of bee groups exposed to different treatments. When the predator avoidance task became difficult (spiders were difficult to detect), bees' colour discrimination decreased, while they maintained the same investigating time. However, we recorded individual behavioural differences within groups of bees. There were different strategies used by different individuals, so that some foragers were 'careful' (spending a longer time and receiving higher accuracy) and others were 'impulsive' (investigating flowers more briefly and visiting them at random). We will discuss this in Chapter 6.

Chapter 5 Divided attention in bees: is the priority in predator avoidance due to attentional limitation?

5.1 Abstract

Foraging animals need to engage in efficient foraging whilst simultaneously avoiding predators. Bees foraging for nectar often have to discriminate between flowers with similar appearance but different nectar rewards. At the same time, they must be vigilant for ambush predators, such as crab spiders, which can camouflage themselves on flowers. Here we ask whether bees can maintain efficient foraging in the presence of cryptic predators. Bees were individually tested in a tightly controlled laboratory experiment using artificial flowers whose nectar supplies were administered with precision pumps. Predation risk was simulated by automated crab spider 'robots' that captured bees for a limited duration without injuring them. Bees' behaviour was monitored by a 3D video tracking system. We experimented both with cryptic and conspicuous spiders, finding that bees had no difficulty avoiding conspicuous spiders while still foraging adaptively. However, they prioritised predator avoidance at the expense of maximising energy intake when faced with detecting cryptic predators and a difficult colour discrimination task. This was not due to attentional constraints or limited sensory processing ability as bees were able to discriminate between similar flower types under predation risk and when choosing the safe flower type incurred a gustatory punishment in the form of bitter quinine solution. However, this resulted in bees incurring substantially higher costs in terms of floral inspection times. We conclude that bees have the capacity to attend to difficult flower foraging tasks while simultaneously avoiding cryptic predators, but only do so when avoidance of gustatory punishment justifies the increased costs.

5.2 Introduction

A vital ability for an animal is filtering the tremendous sensory input from the environment to focus on the most important objects and locations. In humans (and other animals) there are extensive capacity limitations in perceptual processing (Kahneman, 1973). Attention is used to prioritize sensory input for adaptive responses. The role of attention in visual search has been studied extensively in human and non-human primates using behavioral (Treisman and Gelade, 1980) and neuroscientific (Gottlieb et al., 1998) approaches. Much research, particularly in humans, has revealed the significant costs of divided attention for performing the precise discrimination of more than one stimulus dimension (Pashler, 1998). Even with the enormous processing capacity of a large-brained animal, such as a human, there are severe consequences when one must divide attention for two forms of visual input as simple as shape and orientation (Joseph et al., 1997). Such capacity limitations might be all the more important in much smaller animals with concomitant smaller neural systems, such as bumblebees, which have long been a model system for exploring the functional significance of cognition in the economy of nature.

When animals move about their environment, they generally have to divide attention between tasks such as the search for food and the detection of potential danger in the environment. When exposed to predation threat, animals usually reduce foraging time and increase vigilance (Ydenberg and Houston, 1986), but the probability of detecting predators decreases when they encounter more difficult foraging tasks (Godin and Smith, 1988). The existence of predation risk may also change their fighting (Brick, 1998), playing and courtship behavior (Taylor et al., 2005). Dukas and Kamil (2000) found that blue jays engaged in a challenging foraging task for centrally located targets will often miss peripherally located targets (a secondary foraging task) and suggested that this would also result in blue jays overlooking predators in the periphery (not unlike similar research in humans (Joseph et al., 1997)). Here we developed a dual task experiment for bees, an ideal model organism for studying visual search because they are natural visual experts for which search plays a crucial role in foraging, and thus they provide an ecologically intriguing model system to study divided attention in visual search (Spaethe et al., 2006). Recent work in bees led to the development of a realistic foraging scenario with simulated predation rather than additional targets presented as a proxy for predators (Chittka et al., 2003), and we extend that method here to the question of divided attention.

Although previous research has established that bees can attend to either discrimination of similar flower types (Dyer and Chittka, 2004a) or predation (Jones and Dornhaus, 2011, Ings et al., 2012), there has not yet been an investigation of whether bees can do both simultaneously. We tested here that whether bees can simultaneously attend to multiple, complex visual search tasks from different contexts, risks, and rewards. As discussed in the previous chapter, bees were encouraged to discriminate highly similar target and distractor colors by receiving sucrose rewards for target selection contrasted with the risk of mild punishment via distasteful quinine for distractor selection, similar to how value can influence attentional prioritization in humans (Anderson et al., 2011). After learning the task, bees were exposed to predation risk by the addition of camouflaged robotic crab spiders (see Figure 9), to examine whether bees could maintain visual search foraging strategies even in the presence of predators.

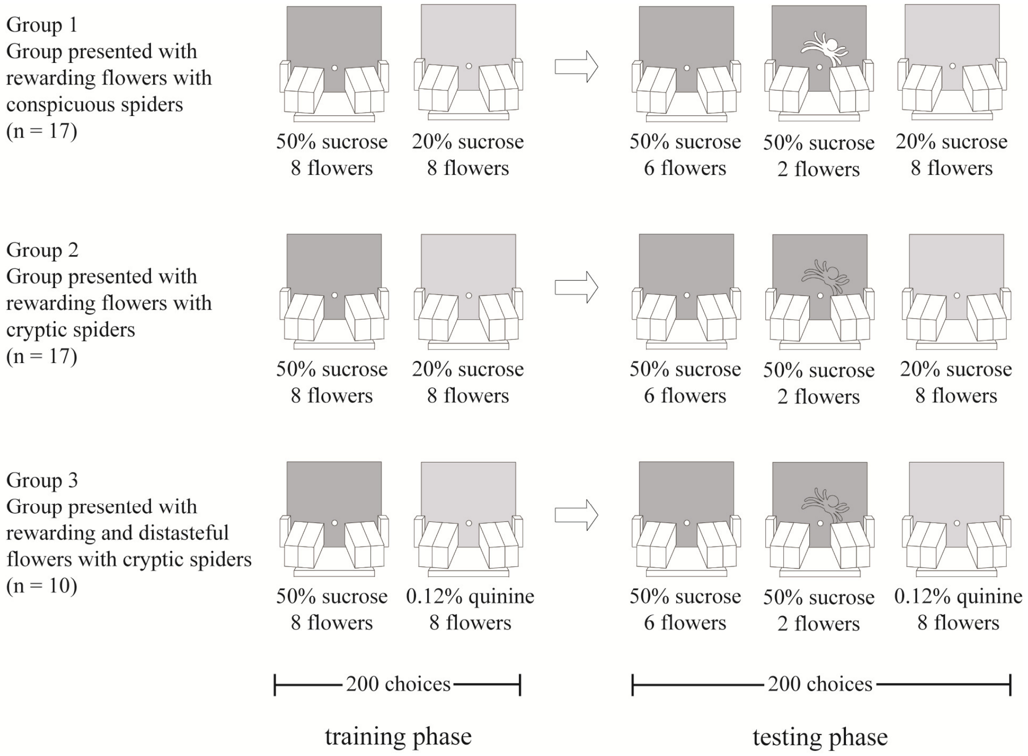


Fig 15 Protocol for the experimental design. Based on the previous chapter (Group 1 & 2), we further tested the mechanisms of attention in bees (Group 3). Bees were trained with two similar shades of yellow artificial flowers with either different quality of sucrose reward (20 vs. 50%) or reward (50% sucrose) and quinine punishment. After 200 choices, conspicuous or cryptic spiders were added to 25% of the low or highly rewarding flowers. Bees were captured by the robotic arms for two seconds when they visited dangerous flowers.

5.3 Materials and methods

Based on the previous chapter, we further tested how bees discriminate gustatory punishment and reward under predation risk. The details of the housing condition, pre-training and experimental setup were described in Chapter 2. To determine whether the apparent inability of bees to solve colour discrimination and predator avoidance tasks simultaneously was due to limitations in sensory processing or attention we conducted a second experiment where the balance of risk and reward was adjusted beyond that naturally encountered. In this experiment, a third group of bees (n = 10 from a new colony) was forced to discriminate between the shades of yellow flower by replacing the low rewards with a form a gustatory punishment, a distasteful quinine solution that bees rapidly learn to avoid (Chittka et al., 2003). Thus, bees were initially trained to distinguish between dark yellow rewarding flowers (D50) containing 50% v/v sucrose solution and light yellow distasteful flowers (LQ) containing 0.12% quinine solution. After colour discrimination training for 200 flower choices bees were then exposed to predation risk (25%) from cryptic spiders (Fig 15).

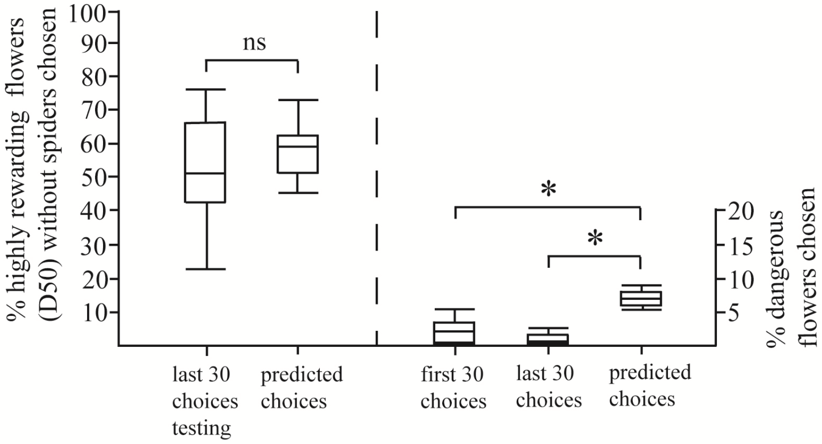
Statistical analysis

Individual bees' preferences for dark yellow (D50) flowers were separated into four (first 100 foraging trials and last 100 trials in both training and testing phase) and compared with ANOVA. The effect size (Eta-square) was calculated by between subjects sum of squares divided by total sum of squares. The last performance in the training phase was used as baseline for the hypothesized choices and compared with percentages of safe highly rewarding flowers (paired t test) or dangerous flowers chosen (Wilcoxon test, since data were not normally distributed) in the end of testing phase. For paired or unpaired t tests Cohen's *d* was provided as effect size, while for Wilcoxon test $r = Z/\sqrt{n}$ was provided as effect size. Investigating time and the number of flowers rejected were analysed with ANOVA followed with Tukey's HSD post hoc test.

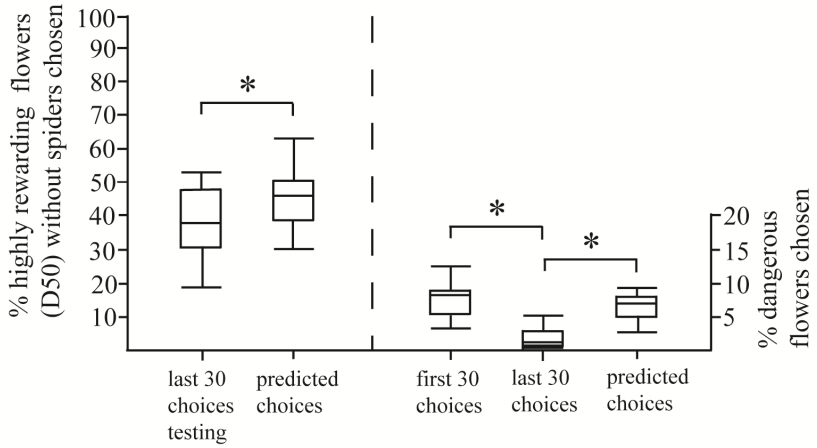
5.4 Results

When failure to choose the correct shade of yellow flower incurred a gustatory punishment (distasteful quinine), rather than a lower quality reward, bees were able to simultaneously solve the colour discrimination task and avoid cryptic predators on the rewarding flower type (Fig 16 & 17). There was significant difference between the beginning and the end of training phase (ANOVA $F_{3, 36} = 15.609$, $p < 0.001$, $\eta^2 = 1.300$, Tukey's HSD < 0.001, Fig 17), and the performance was maintained until the end of the testing phase. At the end of the testing phase, bees chose safe highly rewarding flowers no different from predicted choices (last 30 choices mean = 81.0, sd = 15.5, predicted mean = 70.8, sd = 10.8, $t_{(9)} = 1.705$, $p = 0.105$, Cohen's $d = -0.823$, Fig. 16). Although bees initially visited dangerous flowers at random (Fig. 16), they rapidly learnt to avoid cryptic spiders after experiencing simulated predation attempts (last 30 choices mean = 2.2, sd = 2.3, predicted mean = 15.8, sd = 3.2, $t_{(9)} = 15.058$, $p < 0.001$, Cohen's $d = 6.734$, Fig. 16).

(a) Group 1
Rewarding flowers;
conspicuous spiders



(b) Group 2
Rewarding flowers;
cryptic spiders



(c) Group 3
Rewarding and distasteful flowers;
cryptic spiders

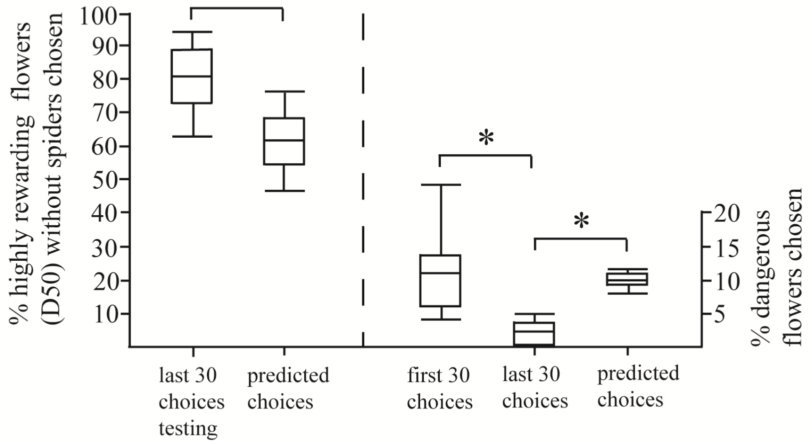


Fig 16 Percentages of safe highly rewarding flowers and dangerous flowers chosen by bees from all three experimental groups. All bees were able to avoid predation risk (chose significantly fewer dangerous flowers than hypothesized). When the spiders were easy to detect bees were able to visited more highly rewarding flowers, however when spider detection became difficult bees decreased their performance. When low rewarding flowers were replaced with distasteful solution bees increased their performance even when the spiders were cryptic.

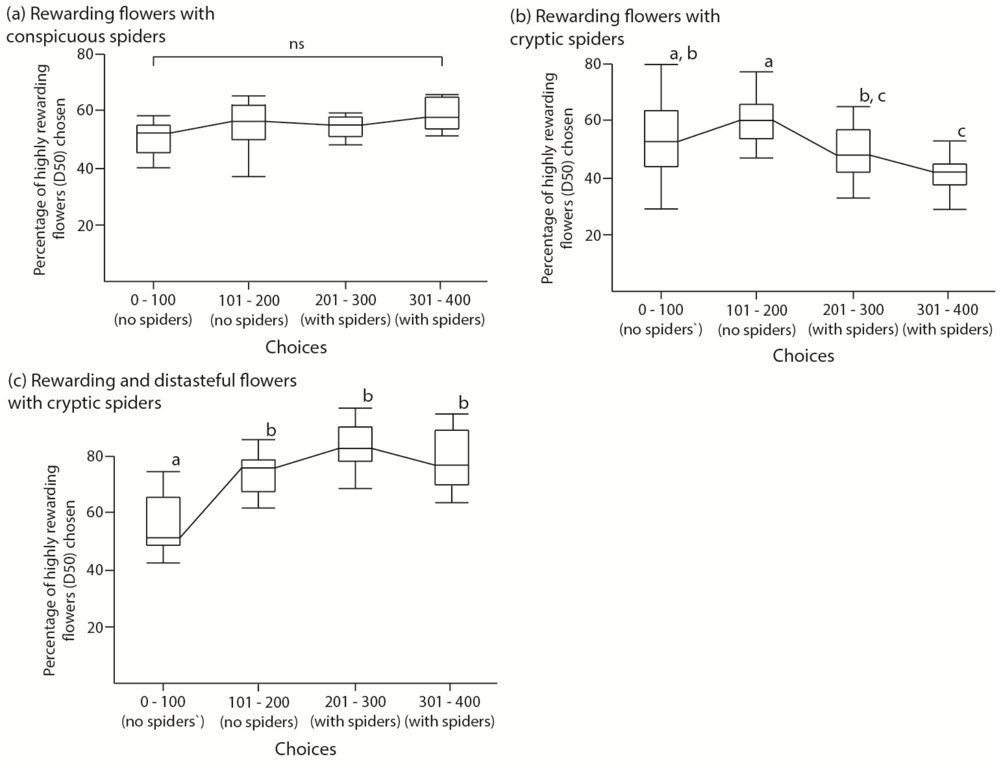


Fig 17 Learning curves of the three experimental groups. Bees maintain the performance when the spiders were conspicuous, while in cryptic spider group they gave up discriminating highly rewarding flowers and forage at random. When forcing bees to discriminate flowers with quinine punishment, bees were able to choose more high reward flowers even when the spiders were cryptic.

Once exposed to predation threat, bees spent 28% more time inspecting flowers before making their choices than they did before learning about predation risk (paired t test, $t_{(9)} = 7.442$, $p < 0.001$, Cohen's $d = 4.961$). This increase in investigation time was also significantly greater than observed for bees exposed to conspicuous spiders in the reward quality experiment (investigating time in the last 30 choices after adding spiders minus the last 30 choices during the training session with no spiders; one-way ANOVA: $F_{2, 34} = 3.395$, $p = 0.045$, effect size r = 0.029; Tukey's HSD post hoc analysis: $p = 0.013$, Fig 18). This substantial increase in temporal costs is presumably the reason why bees do not solve the difficult colour discrimination task and cryptic predator avoidance task simultaneously, unless they have to because of the introduction of quinine penalties.

When presented with rewarding and distasteful flowers, bees investigated and rejected significantly more dangerous flowers than bees in experiment 1, where only sucrose rewarded flowers were encountered (average number of rejections in dangerous flowers: Group 1: 15.2 + 5.5, Group 2: 17.9 + 5.8, Group 3: 27.4 + 12.6; one-way ANOVA, $F_{(2, 32)} = 4.47$, $p = 0.019$, effect size r = 5.730; Tukey's HSD post hoc analysis between Group 1 & 3: $p = 0.006$, between Group 2 & 3: $p = 0.031$).

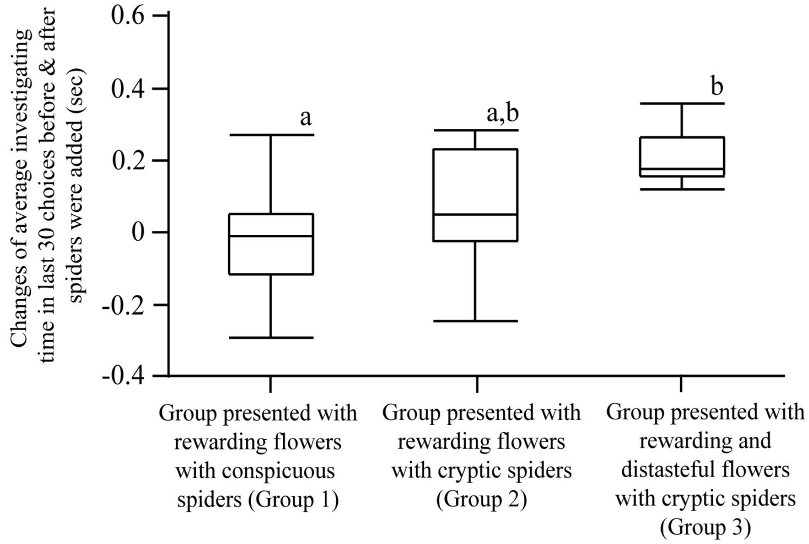


Fig 18 Difference in investigating time between training and after adding cryptic or conspicuous spiders (investigating time after adding spiders minus before adding spiders). The investigating time in the rewarding/distasteful flowers with cryptic spiders group significantly increased after adding spiders, and is significantly higher than rewarding flowers with conspicuous spiders group. There is no significant difference between either rewarding flowers or rewarding and distasteful flowers with cryptic spiders. Letters indicate the significant differences based on Tukey's post hoc test. Bees increased investigating time when forced to make color discrimination by using both sucrose reward and quinine punishment.

5.5 Discussion

In this chapter, the use of quinine punishment to aid in discrimination of the target (dark yellow) and distractor flowers (light yellow) resulted in a different performance profile to that seen in the previous chapter, where reward quality differed. When one flower colour was associated with a positive value (sucrose reward) and the other with a negative value (quinine), bees were able to maintain two value-defined categories for the task (light yellow = punishment, dark yellow = reward). As a result, discrimination between high and low reward flowers was higher (~80% visits to rewarding flowers) than observed in the previous chapter (60% visits to highly rewarding flowers). In the previous chapter, both flower types could be classified as rewarding, motivationally salient, but of high similarity in value decision space, and therefore require persistent attentional resources for discrimination.

Upon their first exposure to predation risk from cryptic spiders, bees in the quinine punishment group attended primarily to colour discrimination and thus failed to detect the predators. They maintained their strong preference for dark yellow rewarding flowers, but initially chose significantly more dangerous flowers than bees in the cryptic spider group when both flower types were rewarding. This observation supports the notion that dangerous flowers with cryptic spiders would initially be processed as target flowers, assuming that bumblebees use restricted parallel visual search (Spaethe et al., 2006, Morawetz and Spaethe, 2012).

After experiencing several attacks from spiders, bees in the quinine punishment group rapidly learnt to avoid dangerous flowers harbouring cryptic spiders whilst simultaneously maintaining high levels of accuracy in the colour discrimination task. This result is surprising given that, for foraging animals, the probability of detecting predators tends to decrease when they encounter more difficult foraging tasks (Godin and Smith, 1988). Furthermore, it implies that bees are simultaneously maintaining a search image for high reward flowers (positive value) with one for dangerous flowers (negative value). Support for this is given by the fact that bees here inspected and rejected more than twice as many light yellow flowers (distasteful) and over 1.5 times as many dangerous flowers as bees in the previous chapter, where light yellow flowers contained low quality rewards. These results lead to the conclusion that bumblebees are able to divide their attention between two distinct visual tasks.

An alternative explanation is that bees are categorising flowers into “good” or “bad”, irrespective of context. Light yellow flowers, which all contain quinine, could be classed as poor foraging options, as could dark yellow flowers harbouring cryptic spiders. Dark yellow flowers without spiders could be classed as desirable foraging options. Thus, a bee only needs to follow a simple rule – i.e. if the flower matches the search image for good then visit, otherwise avoid. Although this is perhaps more parsimonious than an explanation based on divided attention, it would still require advanced cognitive abilities with up to three search images being employed simultaneously. Such a strategy might also imply serial visual search, whereby each individual flower is assessed sequentially (Spaethe et al., 2006).

We chose the colours that were difficult to discriminate for bees (dark and light yellow) and the different reward levels (20% and 50% v/v sucrose) based on pilot experiments and previous studies (Ings and Chittka, 2008, 2009). However, it is possible that dynamic results appear if we modify the colour signals and the reward levels to different level. For example, if we increase the difference between reward levels (for example, 10 % or even no reward vs 50 % sucrose solution), bees may be more willing to discriminate between similar colours. In this study we chose ecologically realistic reward levels (Castellanos et al., 2002) which we can find commonly in nature. It is possible to have low rewarding flowers which mimicking the appearance of high rewarding ones to steal their benefit, however they should not appear too frequently, otherwise the mimetic pattern may not work anymore. In previous studies researchers use the same reward level for flowers of different colours, and the bees are able to shift to only one flower colour (Ings and Chittka 2008, 2009).

This study clearly shows that bumblebees are able to simultaneously discriminate floral rewards based upon subtle visual differences (colour) and avoid predators, but will only do so when the benefits outweigh the costs. When colour discrimination was incentivised by gustatory punishment, bees had to inspect and reject more flowers than bees that chose not to discriminate between similar flower colours whilst avoiding predators. Rejection of flowers is costly to bees because inspection of flowers is carried out in flight (Ings et al., 2012), which is an energetically demanding activity (Kacelnik et al., 1986, Hedenström et al., 2001). Therefore, although we showed that bees can attend to both colour discrimination and predator avoidance, the costs associated with such cognitively demanding tasks imply that cryptic predators can have strong trait-mediated effects on plants and may benefit mimic plant species that produce little or no floral rewards.

Chapter 6 Individual consistency in bumblebee speed-accuracy tradeoff decisions when foraging under predation threat

6.1 Abstract

Animal ‘personality’ has been described as the consistency of behaviour through time across contexts. Such constant individual differences have been investigated in many animals, but the formation and the ecological significance of the syndrome is still highly underestimated. We tested individual consistency in bumblebee speed-accuracy tradeoff faced with predation risks. Bumblebees showed individually repeatable strategies with respect to foraging decisions when conspicuous predators were introduced in the meadow. Some bees made persistently careful choices, while others had shorter decision times and achieved less accurate choices. When we increased the task difficulty by adding conspicuous spiders, careful bees tended to become more careful and impulsive bees turned even more impulsive. We calculated the foraging rate (energy intake / foraging time) for each experiment and found that the optimal strategy changed with different experimental designs. The modelling showed that when flowers were rewarding at different levels, a slow-and-careful strategy was beneficial; however, when the penalty of making erroneous choices increased, an impulsive strategy led to higher net nectar gains. Despite these predicted differences, bees maintained constant strategies instead of displaying behavioural plasticity depending on the environment.

6.2 Introduction

Many animals show repeatable individual behavioural differences over time or across situations. Such consistent differences between individuals are also variously referred to as animal personality (Gosling and John, 1999), behaviour syndromes (Sih et al., 2004a), animal temperament (Réale et al., 2007) or coping styles (Koolhaas et al., 1999), comparable with human personalities (see (Gosling, 2001, Sih et al., 2004a) for review). In eusocial animals such as bees, diversity of personalities can enhance colony fitness (Mattila and Seeley, 2007). Such differences within a colony may be helpful for colonies to cope with rapidly changing environments, where the optimal strategy shifts with conditions (Burns and Dyer, 2008).

In this chapter we explore individual consistency in speed-accuracy tradeoffs in bumblebees faced with predation risk from crab-spiders, which are sit-and-wait predators that lurk on flowers to capture pollinators. For pollinators screening flowers for such predators, accurate decisions may require longer decision time, while impulsive choices can be faster but less discriminating. Speed-accuracy tradeoffs have long been a major focus in human psychology studies (Woodworth, 1899) as has been their relationship to impulsivity (Dickman and Meyer, 1988) and information processing (Miller and Vernon, 1997). Past research found such tradeoffs exist in animal decision making such as foraging (Chittka et al., 2003), predator-prey interactions (Ings and Chittka, 2008, Burns and Rodd, 2008) and nest site selection (Franks et al., 2003). In many cases behavioural plasticity exists in speed-accuracy tradeoff decisions. For example when the task becomes difficult, subjects may be slower and display less accurate performance (Palmer et al., 2005), or when punishment exists they may trade speed for accuracy (Chittka et al., 2003). In natural conditions, situations exist where accuracy is strongly favoured over speed, such as predator detection where the fitness costs of errors can be high. Time for detecting possible predators may be substantial especially when predators are cryptic or adapt behavioural tactics to catch prey. On the other hand, animals making foraging choices may prefer shorter investigating time since the error penalties may simply be lower rewarding levels (Burns, 2005). Consistent individual differences have been found in both speed and accuracy when animals face different conditions such as varied target-distractor ratios (Burns and Dyer, 2008), however so far there is no study looks at how individuals react to both foraging and predator detection tasks.

In this chapter, we have investigated how different situations change both between and within individual behaviour in the speed-accuracy tradeoff paradigm, and the efficiency for each strategy. When animals move about their environment, they generally have to divide attention between tasks. We selected biologically realistic plant-pollinator and pollinator-predator systems as models to examine pollinator decision making and its consistency. These are important challenges for pollinators since there are flowers of different species, which differ in profitability and existence of camouflaged predators in the field. Many plant species, such as many of those in the orchid family have flowers which resemble the appearance of co-occurring, rewarding species to attract pollinators (Dafni, 1984, Roy and Widmer, 1999). Moreover, many predators use the attractiveness of flowers to lure their prey. For example, crab spiders (Araneae: Thomisidae) are sit-and-wait predators that ambush pollinators such as bees on flowers (Chittka, 2001, Insausti and Casas, 2008). Some species of crab spiders can even change their body colour to fit the flower they sit on or select the high quality flowers (Morse, 1986). How pollinators choose between flowers and how they avoid predators has been well studied, but few take both tasks into account.

We selected bumblebees as a model to address the issue of individual consistency and its relationship to speed-accuracy tradeoffs when bees must make economic flower choices while also detecting predators. The bees were trained to visit artificial flowers with ‘robotic’ spiders in different detectability and rewarding levels to investigate their choices in contrary environments. We predicted that between-group and within-group speed-accuracy tradeoffs exist in bumblebee foraging: bees may trade accuracy or speed in exchange for another when encountering flowers and spiders of different detectability. Within experimental groups, we hypothesised that diversity exists in foraging strategy, where a correlation between decision speed and accuracy can be found. Also, if consistent individual differences exist, bees would use the same strategy after predators were introduced in the meadow.

6.3 Materials and methods

Experimental design

The experimental apparatus and design are identical to Chapters 4 & 5 and were described in Chapter 2. Bees from three colonies were assigned to three experimental groups. In Group 1 bees were trained with artificial flowers of two shades of yellow containing different reward levels (dark yellow: 50 % (v/v) sucrose solution, light yellow: 20 %) for 200 choices. Then conspicuous (white) spiders were added randomly on two of the highly rewarding flowers. Bees were captured for two seconds when landed on the dangerous flowers. Group 2 was identical to Group 1 except spiders were cryptic (dark yellow). In the training phase of Group 3, low rewarding sucrose was replaced by quinine solution, and the spiders remained cryptic. Positions of the flowers and spiders were changed randomly for every foraging bout.

Data analysis

Decision speed and accuracy were analyzed using General Linear Models (GLM) compared across experimental groups and before/after spiders were added. The decision speed and accuracy of bees in all three experimental groups were tested with likelihood-ratio tests for normality. Since all the data were randomly distributed (Shapiro-Wilk test, rewarding flowers & conspicuous spider group: speed: $W = 0.926$, $p = 0.386$, accuracy: $W = 0.915$, $p = 0.202$; rewarding flowers & cryptic spider group:

speed: W = 0.980, $p = 0.290$, accuracy: W = 0.978, $p = 0.779$; rewarding and distasteful flowers & cryptic spider group: speed: W = 0.967, $p = 0.694$, accuracy: W = 0.923, $p = 0.112$), speed and accuracy before and after spiders were added were analyzed by paired t test. Cohen's *d* was given as effect size.

Repeatability (Lessells and Boag, 1987, Nakagawa and Schielzeth, 2010) of investigating time, accuracy and inverse efficiency (IE, investigating time divided by correct choices rate (Murphy and Klein, 1998)) were calculated for each individual bee before and after spiders were added. Repeatability, or intraclass correlation coefficient, details that the proportion of variance within individuals is greater than between group variance. It can be calculated from a one-way ANOVA, where repeatability $r = S_A^2 / (S^2 + S_A^2)$. S_A^2 is the among-group variance and S^2 is the within-group variance. The variances are calculated from the mean square in the ANOVA: $S^2 = MS_W$ and $S_A^2 = (MS_A - MS_W) / n$ (Lessells and Boag, 1987). Data were analysed using R (v. 2.15.1; repeatability calculated from the rptR package) and JMP (v. 7, SAS Institute).

Within the experimental group where flowers were rewarded in different levels and spiders were easy to detect (Group 1), bees showed different strategies when foraging. We classify their strategies by Two Step Cluster Analysis. Five variables were used for classification (decision time and accuracy before and after spiders were added, and to which dimension bees moved in the speed-accuracy tradeoff plot). The cluster data were further used as baseline classification in Groups 2 & 3 to target the 'careful' and 'impulsive' bees. Discriminant analysis was used to classify bees of different groups in Group 2 & 3 using SPSS 22.0.

Foraging rate

We calculated the foraging rate for each bee:

$$\text{Foraging rate} = \frac{c \times E_1 + (1 - c) \times E_2}{n \times (r \times E_3 + i \times E_3 + h \times E_4)}$$

where *c* is the percentage of correct choices (safe and highly rewarding flowers chosen by bees); E is the energy intake/output (E_1 = 4.7 µl (nectar volume per flower) of 50% v/v sucrose solution = 50 Joule; E_2 = 4.7 µl 20% v/v sucrose solution = 17 J; E_3 = 0.052 Joule, which represents the energy budget during per second flight (Heinrich, 1975, Pyke, 1980); E_3 = 0.007 Joule, which is the energetic cost for nonflight activity per second (Kammer and Heinrich, 1974, Pyke, 1980)); *n* is the number of choices (200 in training phase and 200 in testing phase); *i* is the investigating time individual bees spent in front of the flowers, and h is the handling time (7.4 seconds, the average handling time in all three groups); *r* is the interflower flight interval excluding bee investigating time. We found no significant difference in the interflower flight intervals of different experimental groups (ANOVA, $F_{(2, 32)} = 1.953$, $p = 0.160$) and between bees that chose 'careful' or 'impulsive' strategies after spiders were added ($t_{(11)} = -0.501$, $p = 0.633$), so we used the average 3.79 seconds.

We calculated the foraging rate for careful and impulsive bees classified by cluster analysis (Group 1) and discriminant analysis (Groups 2 & 3) in both the training and testing phase. The foraging rates were compared with a t test to determine which strategy was better. Effect size were given for the t test as Cohen's *d*.

6.4 Results

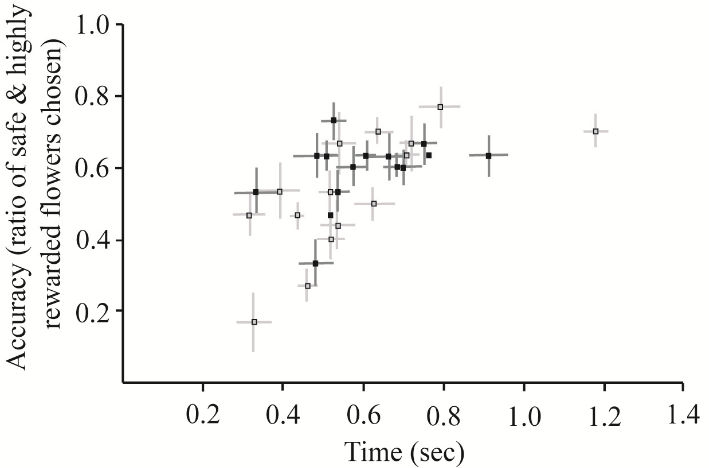
Bees showed both between group and within group speed-accuracy tradeoffs

We compared decision speed and accuracy under different experimental groups and before/after spiders were added. The decision time of bees did not differ between experimental groups (GLM: $F_{(2, 73)} = 2.653$, $p = 0.077$). The decision time of bees can be significantly explained by speed (GLM: $F_{(1, 73)} = 15.252$, $p < 0.001$, Fig 19), which suggested bees showed within group speed-accuracy tradeoffs. Whether the spiders were added or not also has a significant influence on bee decision time (GLM: $F_{(1, 73)} = 8.518$, $p = 0.005$). There was no interaction between different experimental groups and whether the spiders were added (GLM: $F_{(2, 73)} = 2.580$, $p = 0.083$).

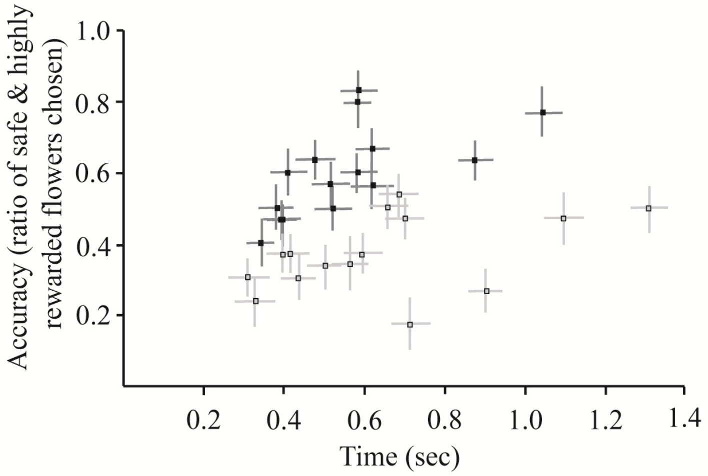
In the group presented with rewarding flowers one quarter of which harboured conspicuous spiders, there were no significant differences between time or accuracy before and after adding spiders (speed: before spiders were added mean = 0.6, sd = 0.1, after spiders were added mean = 0.6, sd = 0.2, paired t test (two tailed), $t_{(14)} = 0.520$, $p = 0.611$, Cohen's $d = 0$; accuracy: before spiders were added mean = 0.6, sd = 0.2, after spiders were added mean = 0.6, sd = 0.2, paired t test (two tailed), $t_{(14)} = 0.494$, $p = 0.061$, Cohen's $d = 0$). In the rewarding flowers with cryptic spiders group, the accuracy of bees in the testing phase (after adding cryptic spiders) was significantly lower than in the training phase (before spiders were added mean = 0.6, sd = 0.1, after spiders were added mean = 0.4, sd = 0.1, paired t test (two tailed), $t_{(14)} = 9.636$, $p < 0.001$, Cohen's $d = 2.000$), but the time bees spent investigating the flowers was not significantly different (before spiders were added mean = 0.6, sd = 0.2, after spiders were added mean = 0.6, sd = 0.3, paired t test (two tailed), $t_{(14)} = -1.47$, $p = 0.163$, Cohen's $d = 0$). In the group presented with the two types of flowers which were either rewarding or distasteful flowers, and which were also faced with cryptic spiders, the accuracy did not differ with the existence of spiders (before spiders were added mean = 0.8, sd = 0.1, after spiders were added mean = 0.8, sd =

0.2, paired t test (two tailed), $t_{(9)} = 0.899$, $p = 0.392$, Cohen's $d = 0$), while the time bees spent investigating the flowers was not significantly different (before spiders were added mean = 0.6, sd = 0.1, after spiders were added mean = 0.7, sd = 0.1, paired t test (two tailed), $t_{(9)} = -2.784$, $p = 0.021$, Cohen's $d = -1.000$). Bees traded off accuracy to maintain the same foraging speed when cryptic spiders were introduced, but traded off speed for accuracy when using both rewarding and distasteful flowers.

(a) Group 1
Rewarding flowers with conspicuous spiders



(b) Group 2
Rewarding flowers with cryptic spiders



(c) Group 3
Rewarding and distasteful flowers with cryptic spiders

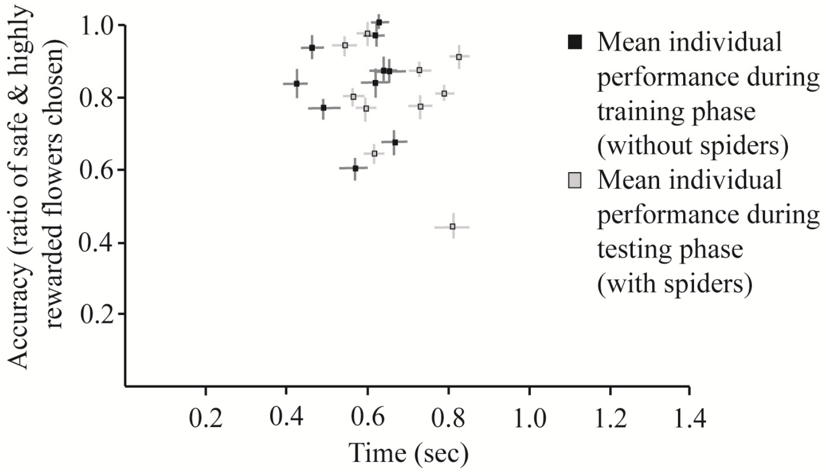


Fig 19 Speed-accuracy tradeoff plots for all three bumblebee experimental groups described in Chapter 2. Each point represents the mean decision speed and accuracy of single bee, the error bar represents ± 1 standard error mean. When the spiders were easy to detect, bees did not modify their performance. When the spiders were cryptic, bees maintained same investigating time but decreased accuracy. When flowers were either rewarding or distasteful while the spiders were cryptic, bees kept the accuracy but increased the decision time.

Bees showed consistent strategies when presented with rewarding flowers, but not when encountering distasteful flowers

When the flowers were rewarded in different levels, bees showed individually repeatable behaviours before and after conspicuous spiders were added to 25% of the rewarding flowers, no matter the detectability of the spiders. Which means, the within bee variance was smaller than between bee variance (repeatability test, investigating time: within bee variance $S^2 = 0.003$, between bee variance $S_A^2 = 0.006$, $R = 0.671$, $p = 0.002$; accuracy: $S^2 = 0.002$, $S_A^2 = 0.003$, $R = 0.635$, $p = 0.003$; IE (inverse efficiency): $S^2 = 0.008$, $S_A^2 = 0.009$, $R = 0.542$, $p = 0.013$). In the group presented with rewarding flowers with cryptic spiders, the investigating time and accuracy was significantly repeatable after spiders were added in the meadow (repeatability test with correction, investigating time: $S^2 = 0.030$, $S_A^2 = 0.037$, $R = 0.493$, $p = 0.024$; accuracy: $S^2 = 0.001$, $S_A^2 = 0.002$, $R = 0.679$, $p = 0.001$) but not the IE scores ($S^2 = 0.855$, $S_A^2 = -0.026$, $R = -0.297$, $p = 0.864$). In the experimental group which was presented with rewarding and distasteful flowers with cryptic spiders, the behaviour displayed by the bees was not repeatable in speed, accuracy or IE scores after cryptic spiders were introduced (investigating time: $S^2 = 0.015$, $S_A^2 = -0.007$, R = -0.308, p = 0.824; accuracy: $S^2 = 0.013$, $S_A^2 = 0.001$, R = 0.348, p = 0.137; IE: $S^2 = 0.079$, $S_A^2 =$ 0.002, R = 0.096, p = 0.381).

Bees performed different strategies (careful and impulsive) when foraging

From a group point of view, bees from rewarding flowers with conspicuous spiders group retained the same strategy before and after spiders were added (Fig 19, decision time and accuracy were not significantly different). But when the individual performance was evaluated, different bees used different strategies after the addition of conspicuous spiders. Cluster analysis classified the bees into two clusters with a good cluster quality (Average Silhouette = 0.6). There was no swamping variable and the importance of each variable did not dramatically differ (accuracy after spiders added = 1.0, dimension bees moved after spiders added = 0.89, speed after spiders added = 0.69, accuracy before spiders added = 0.37, speed before spiders added = 0.21). The six bees which clustered into a group chose either 'careful' strategy (increased investigating time and accuracy, five individuals) or 'super' strategy (decreased investigating time but increased accuracy, one individual). The nine bees in the other group all chose the 'impulsive' strategy (decreased investigating time and accuracy).

The accuracy of bees that chose a careful strategy before adding spiders was significantly higher than that of bees that chose an impulsive strategy ($t_{(13)} = -1.841$, p = 0.040, Cohen's d = -0.36), but the investigating time was not significantly different ($t_{(13)} = -0.973$, p = 0.183, Cohen's d = 0.334). The bees that made more accurate choices before adding spiders were more likely to choose the careful strategy after adding conspicuous spiders. There was no significant correlation between bumblebee individual accuracy and predator avoidance speed (Spearman's rank correlation: r = 0.259, n = 15, *p* = 0.351). Careful bees did not avoid spiders more efficiently (Wilcoxon test, Z = 0.429, *p* = 0.671) and did not learn faster in the colour discrimination task (t test, $t_{(13)} = 1.295$, *p* = 0.113, Cohen’s *d* = 1.300).

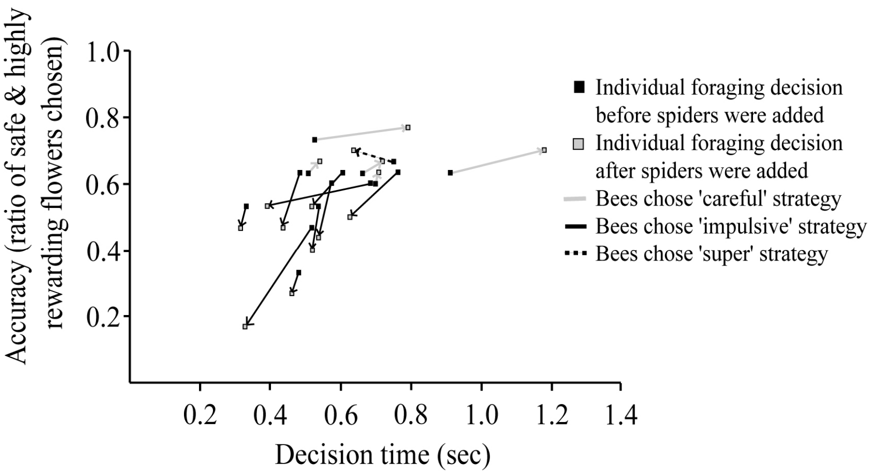


Fig 20 Individual foraging strategies of bees before and after conspicuous spiders (Group 1) were added to the ‘meadow’. Bees increased both decision time and accuracy (‘careful’ strategy) and decreased investigating time but increased accuracy (‘super’ strategy) after spiders were added were classified in the same group by cluster analysis. Bees which chose an ‘impulsive’ strategy (decreased investigating time and accuracy) were classified as another cluster. Bees which chose the careful strategy in the beginning were more likely to be ‘careful’ when the choices became difficult, so did the more ‘impulsive’ bees.

Better strategy changes with experimental groups

Since the bees displayed a constant strategy when the tasks became more difficult (spiders were added to the meadow), we calculated the foraging rate (energy intake divided by time × energetic cost) for bees choosing different strategies. We used the two groups classified by cluster analysis in Group 1 as a baseline to discriminate the bees which were ‘careful’ or ‘impulsive’ in Groups 2 & 3. 78.3% of the original group cases were correctly classified. Three of the five variables had high explanatory power. After targeting the strategies of the bees, we calculated the foraging rate of bees in different strategies from all three experimental groups. In Group 1 (rewarding flowers with conspicuous spiders), there was no better strategy before spiders were added (t test, careful bees foraging rates mean = 165.9, sd = 10.1, impulsive bees foraging rates mean = 155.4, sd = 13.2, $t_{(13)} = -1.738$, $p = 0.107$, Cohen’s $d = 0.893$). After spiders were added, the careful strategy became more efficient (t test, careful bees foraging rates mean = 137.5, sd = 6.1, impulsive bees foraging rates mean = 112.6, sd = 14.7, $t_{(13)} = -4.517$, $p < 0.001$, Cohen’s $d = 2.213$). In Group 2 (rewarding flowers with cryptic spiders) the careful strategy was better before spiders were added (t test, careful bees foraging rates mean = 139.7, sd = 11.9, impulsive bees foraging rates mean = 124.1, sd = 7.3, $t_{(13)} = -3.118$, $p = 0.009$, Cohen’s $d = 1.580$). After spiders were added there was no better strategy (t test, careful bees foraging rates mean = 103.8, sd = 14.9, impulsive bees foraging rates mean = 101.7, sd = 5.1, $t_{(13)} = -0.383$, $p = 0.711$, Cohen’s $d = 0.189$). Before spiders were added in Group 3 (rewarding and distasteful flowers with cryptic spiders) the impulsive strategy was superior (t test, careful bees foraging rates mean = 148.9, sd = 13.6, impulsive bees foraging rates mean = 169.5, sd = 8.0, $t_{(13)} = 2.924$, $p = 0.024$, Cohen’s $d = -1.846$). After spiders were added there was no better strategy (t test, careful bees foraging rates mean = 139.4, sd = 19.7, impulsive bees foraging rates mean = 162.4, sd = 10.2, $t_{(13)} = 2.311$, $p = 0.060$, Cohen’s $d = -1.466$). The better strategy was different depend on the experimental design, however, the bees maintained the same strategy instead of shift to the optimal strategy.

6.5 Discussion

The experiment presented bees with two challenges, colour discrimination and predator avoidance tasks of different difficulties, to assess the intra- and inter-task speed-accuracy tradeoffs in bumblebees. Bees showed consistent decision making strategies, where some bees made rapid but inaccurate choices while others were more careful but required longer decision time. There was a positive correlation between decision speed and accuracy when bees faced only rewarding flowers, which is consistent with the findings in Chittka et al. (2003). We also found that speed-accuracy tradeoffs occurred at the group level. When flowers were rewarded in different levels, bees traded off foraging accuracy for speed and visited safe flowers at random. As the penalties for incorrect choices became more significant, bees shifted to spend longer decision time for retaining the same accuracy. When a certain strategy is preferred, such as predator avoidance for which the cost of failure may be huge, bees changed their foraging behaviour and gave up colour discrimination. The results showed that, like in humans and other animals (Zenger and Fahle, 1997, Franks et al., 2003), bees showed dynamic decision making where foraging behaviour changed depending on the context of the tasks to minimize the possible cost.

While we showed there was behavioural plasticity in bumblebees depending on the nature and difficulty of the task, there were also constant individual differences between tasks observed in the same group. Individual bees showed consistent individual strategies when the task became difficult (spiders added in the meadow) where careful bees remain careful, and impulsive bees constantly choose the impulsive strategy. What is interesting is that when careful bees encountered a more difficult situation, they tended to spend even longer decision time to gain higher accuracy, while impulsive bees became more impulsive with the presence of spiders. Even when a ‘careful’ strategy should result in a higher nectar intake, the ‘impulsive’ individuals still maintained their strategy. After introducing quinine penalties, both speed and accuracy were not repeatable in individual bees. When bees were forced to solve both tasks at the same time, and a certain level of accuracy had to be retained, they did not show constant decisions in both speed and accuracy. When bees had to use most of their efforts to reach a certain percentage of accuracy, they all sacrificed foraging speed and the individual differences were no longer significant. Bees also showed no correlation between the speed and accuracy of learning distinguishing colours and avoiding predators, indicating that the same individual did not use the same careful-impulsive dimension in different behavioural paradigms. Individual bees which were fast in learning colour discrimination tasks did not necessarily learn rapidly to avoid predators. Some studies define animal ‘personality’ as the same personality traits appearing constantly across behavioural contexts (also called ‘behavioural syndromes’ (Johnson and Sih, 2007, Logue et al., 2009)). However the same trait may not necessarily appear in different context. For example an individual that is careful in food searching may not necessarily be careful when facing predation threat.

The foraging rates showed that the superior strategy changed depending on the experimental context. When the flowers were rewarding at different levels, being careful was the better strategy for bees. But when one flower type did not contain reward and instead contained bitter quinine penalties, an impulsive strategy was more efficient. The optimal strategy changes with different experimental setups, and there is no single strategy which is efficient in all situations. This might be a possible explanation of why stable behavioural variability is maintained in bee colonies. The individuality in foraging behaviour may decrease variation in resource acquisition from different environments at the colony level. The flowering species may shift rapidly over time, thus the environment an individual bee encounters is constantly changing. Searching for the optimal strategy may be energy and time consuming, and information may be quickly out of date when the environment changes swiftly. In this study we demonstrated consistent behavioural individuality in bumblebee speed-accuracy tradeoff decisions, and the efficiencies of different foraging strategies. We also showed that the optimal strategy changes with different experimental conditions.

Chapter 7 Speed accuracy tradeoffs and decision making by individuals and dyads of zebrafish in colour discrimination tasks

7.1 Abstract

Speed accuracy tradeoffs are well studied in human decision making, but we are only beginning to understand how such tradeoffs affect animal decision making in biologically realistic settings. Here we investigated whether zebrafish show individual consistency ('personality') in speed accuracy tradeoffs in colour discrimination tasks and how pairs of fish with distinct 'personalities' make consensus decisions. We used state of the art 3D video tracking to evaluate the fishes' individual choice strategies. We found that zebrafish exhibit consistent between-individual differences in speed-accuracy tradeoffs. Some fish made 'careful' slow but accurate decisions, while others were more 'impulsive' and made swift but less accurate decisions. When tested in pairs of 'careful' and 'impulsive' individuals, the combined decisions were closer to those of careful individuals.

7.2 Introduction

Repeatable individual behavioural differences have been found in many animal groups (Chittka and Thomson, 2001, Gosling, 2001, Sih et al., 2004b). Diverse terms such as animal personality (Gosling and John, 1999), behaviour syndromes (Sih et al., 2004a), animal temperament (Réale et al., 2007), or coping styles (Koolhaas et al., 1999) were used to describe such findings. Here, we tested the most general definition, which was whether the behavioural differences between individuals are constant over time and contexts. We tested consistency in the speed-accuracy tradeoff to address the questions of whether constant individual differences exist in zebrafish, and how they compromise with each other when foraging in pairs. When animals make decisions, tradeoffs may exist between speed and accuracy, where accurate decisions require longer manipulation time and vice versa. This has long been considered in human perceptual studies (Woodworth, 1899); however, ethological research has mainly focused on discrimination accuracy but rarely on the handling time. In this study, we used colour discrimination tasks, which have been analysed for zebrafish in previous research (Bilotta et al., 2005, Colwill et al., 2005, Spence and Smith, 2008, Sison and Gerlai, 2010), to test the between-individual and pairwise speed-accuracy tradeoffs in zebrafish.

The zebrafish has been one of the most popular model species in developmental and genetic biology for decades (Grunwald and Eisen, 2002, Sison et al., 2006). Here, we selected zebrafish to address three levels of questions: (1) do within-group and between-group speed-accuracy tradeoffs exist in zebrafish colour discrimination tasks? (2) if speed-accuracy tradeoffs exist in zebrafish foraging decisions, is it consistent over time and contexts; and (3) how do fish in pairs compromise with each other when they have different strategies? The goal of the present paper is to investigate whether constant individual differences exist in zebrafish decision making, and how the differences influence consensus decisions.

7.3 Materials and methods

Study animals and pre-training

A total of 85 adult AB wild-type zebrafish were tested in three sets of experiments. Prior to the experiment fish were housed individually at 28°C (room temperature) with a 14h:10h light:dark cycle. Fish were fed with brine shrimp twice per day or during experiment/pre-training. One day before the experiment, fish were pre-trained in an apparatus that was identical to the actual experimental setup (Fig 21) but without colour signals for 20 discrete trials. Each fish was first allowed to explore and get used to the holding area of the tank for three minutes. After habituation, the barrier of the holding area was lifted up and fish were able to make decisions. A fish crossing the hole to the signal area was considered as a decision. The barrier was closed and the fish were given a small amount of brine shrimp (~0.5 ml). When fish showed no interest in food they were gradually moved back into the holding area with a barrier. The choices and the investigating time of the fish were recorded. When a fish showed an innate preference for a certain position (visited the same chamber for more than seven trials, which would be significantly different from random choice), or did not enter the chamber for more than two hours, it was removed from the experiment. The positions of the fish were recorded with three-dimensional coordinates calculated 50 times per second using two video cameras connected to a computer running Trackit 3D software (BIOBSERVE GmbH, Bonn, Germany). The tracking software provided live time tracking which allowed us to identify the two fish. The experiments were also recorded by video camera so we were able to analyse the video as well. The videos were analysed by Move-tr/2D (Library Co. Ltd., Tokyo). The software can calculate the two-dimensional position, speed, moving angle and the coordinate distance between the two fish. There were no two fish that looked exactly the same and we were able to tell them apart by the appearances with the naked eye (Fig 22a), but with the tracking software we could discriminate the dyads of fish more easily (Fig 22b).

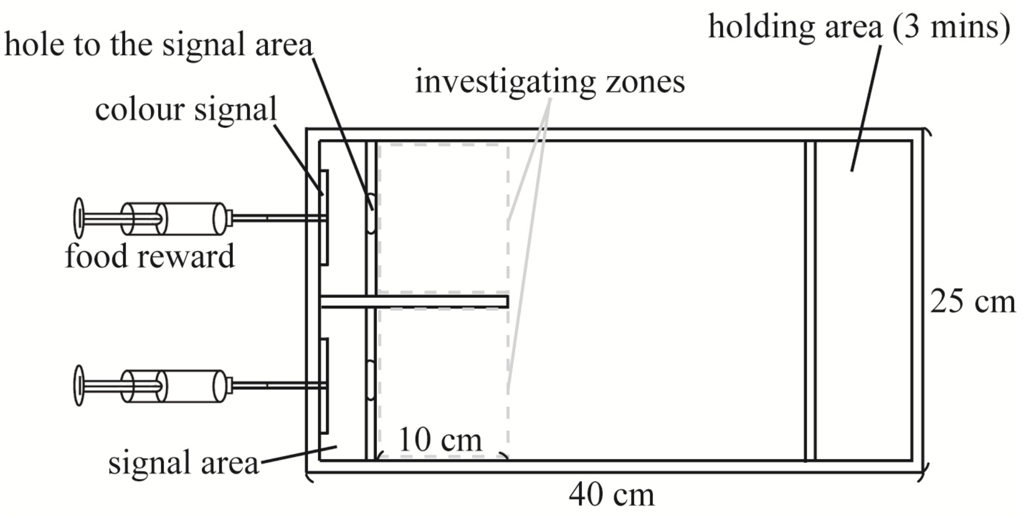


Fig 21 Experimental apparatus for all three experiments. Fish were habituated to the holding area for three minutes, and allowed to explore the tank. When the fish passed the hole to the signal area, this was considered as a decision. Fish were held in the signal area and given food reward when making correct choices, while punishment (stirring the water) was applied when fish chose the wrong colour. Investigating time was considered as the time spent by fish in the investigating zones and when fish were facing the colour signals.

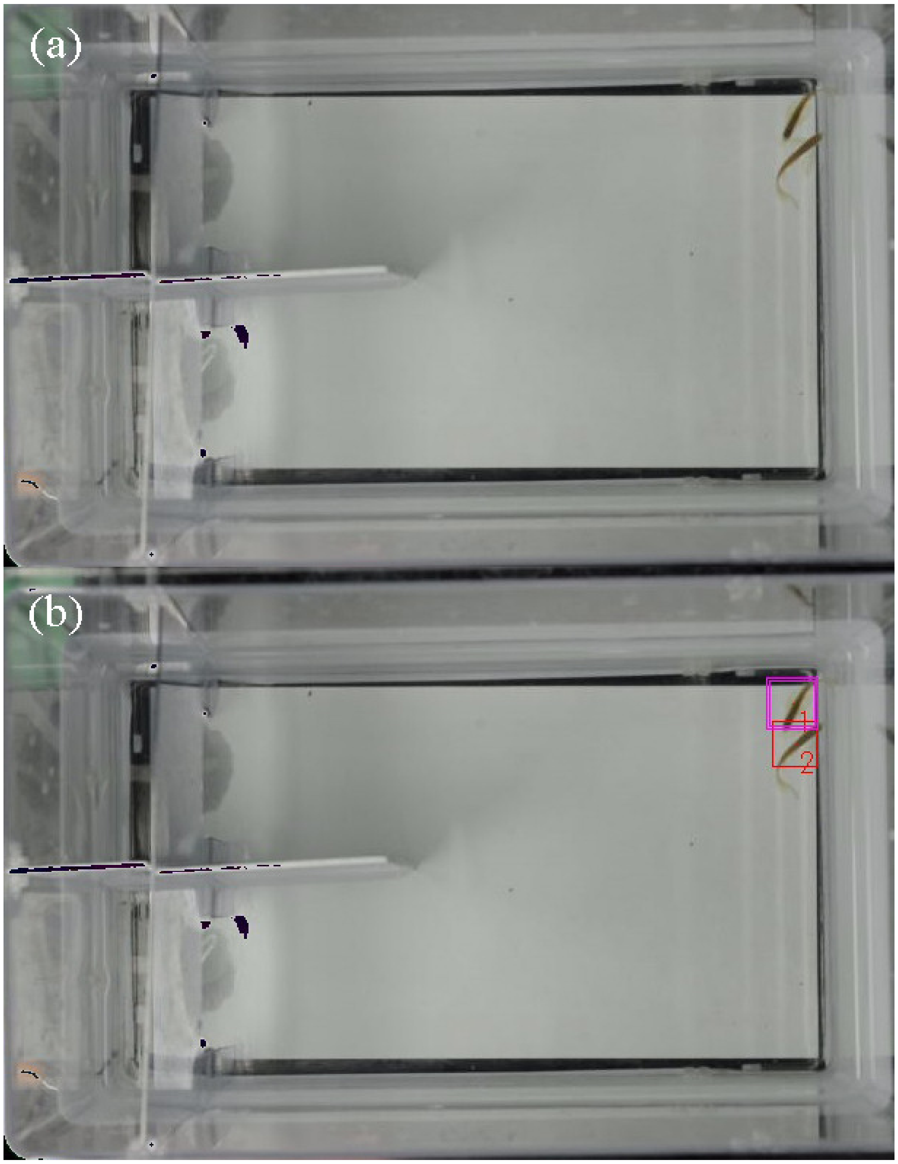


Fig 22 (a) A sample of two fish used in the pair experiment. The appearances of the fish were different and we could tell them apart by visual inspection. (b) The real-time tracking system allowed us to discriminate the fish more easily. During the experiment, we can check how tracking system track the fish on a computer screen as shown in (b). Sometimes when fish crossed over the tracking system might track the wrong fish, in this case we could manually adjust the target. We also recorded all the experiments by video camera and analysed the video by Move2D software and carefully checked every crossed over.

In a pilot experiment, we tested whether punishment was necessary for fish colour discrimination test. We used two distinct colours (green and brown) to decrease the difficulty of discrimination. When choosing one colour is rewarding and no punishment was given for incorrect choices, fish chose the colours at random (green = rewarded, one sample t test, random = 50, mean = 51.0, sd = 6.6, $t_{(4)} = 0.343$, $p = 0.749$). Only when punishment was introduced, the fish chose significantly more of the rewarded colour (green = rewarded, brown = punishment, one sample t test, random = 50, mean = 63.333, sd = 5.700, $t_{(4)} = 5.099$, $p = 0.007$). Thus we used both reward and punishment for the following experiments.

Experiment 1: individual speed accuracy tradeoffs in colour discrimination

To test within-group speed-accuracy tradeoffs in colour discrimination, fish were trained to associate the colour green with reward and to distinguish a similar hue, turquoise, from the rewarding target. Thirty fish were tested for 20 discrete trials. In addition to receiving a food reward for a correct choice, fish received punishment for an incorrect choice (stirring the water in the signal area for three seconds with a net). The positions of the colour signals were randomised in each trial. The choices and decision time of the fish were recorded.

Experiment 2: individual repeatability in discrimination of similar colours

In order to investigate whether fish showed a stable strategy over time, we tested 15 fish with the same colours after 24 and 48 hours, using a method which was identical to experiment 1. Fish were tested for 20 trials for three consecutive days.

Experiment 3: decision making in pairs

Following the previous experiments, we aimed to determine how fish made decisions in a pair when they presented different strategies. Thirty fish were tested individually with the same procedures as in experiment 1 for 20 trials to gain the references for their strategies. After the experiment, they were paired randomly and housed in the separate area of the same tank with a barrier in the middle. After 24 hours, fish were tested in pairs using the same experimental conditions with similar colours for 20 trials. We avoided the first two hours after the lights were switched on in order to avoid the possible sexual-oriented behaviour.

Data analysis

Based on the time and position data recorded by the Trackit 3D software, we calculated the investigating time that fish spent in front of the colours. Investigating zones were 12 cm (length) by 10 cm (width) by 7 cm (height) in front of the colour signals. Only when fish were moving toward the colour targets were they considered in the analysis. Since the data were not normally distributed, we performed Spearman's rank correlation to test whether speed was correlated with accuracy. Repeatability was calculated following Lessells & Boag (1987) as described in detail in the previous chapter. For fish consensus decision making, we used a one-way ANOVA with Tukey's HSD to test the differences between decision speed and accuracy of careful, impulsive individual and dyads of zebrafish. Eta-square η^2 was provided as effect size for ANOVA. Data were analysed using R (v.2.15.1; repeatability calculated from the rptR package) and JMP (v.7, SAS Institute). Two fish in the pre-training phase had innate preferences to the left (visited the same side for seven trials consistently, which is significantly different from random choices), and four fish in the similar colour pair lost motivation (did not move to the chamber for more than two hours) during the experiment and were excluded from the analysis.

7.4 Results

Experiment 1: fish showed speed-accuracy tradeoffs in discrimination of similar colours

Between individual fish, there were strong correlations between decision time and accuracy (Spearman's rank correlation: r = 0.563, n = 30, *p* = 0.001, Fig 23). The more time that a single fish spent in front of the colour signals, the more accurate was the decision made. When the fish was more impulsive and made fast decisions, the number of incorrect choices was higher.

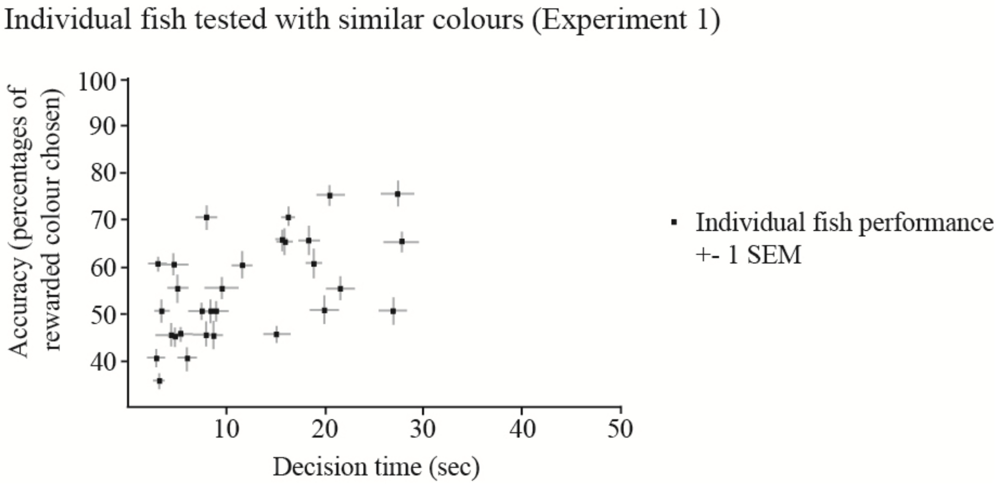


Fig 23 In the similar colour pair, fish showed speed accuracy tradeoffs, while there were significant correlations between investigating time and accuracy. The black dots represent individual fish, and error bars show +- 1 SEM for both decision time and speed.

Experiment 2: individual fish showed a consistent strategy over 24 & 48 hours

The previous experiment showed that individual fish presented a speed-accuracy tradeoff, where some fish were more careful and some more impulsive. We further tested whether the strategy was constant over time using the same pair of similar colours as Experiment 1. The fish presented significant repeatability with investigating time and accuracy (repeatability test, speed: R = 6.482, n = 15, $p = 0.003$; accuracy: R = 0.433, n = 15, $p = 0.043$, Fig 24) after 24 & 48 hours.

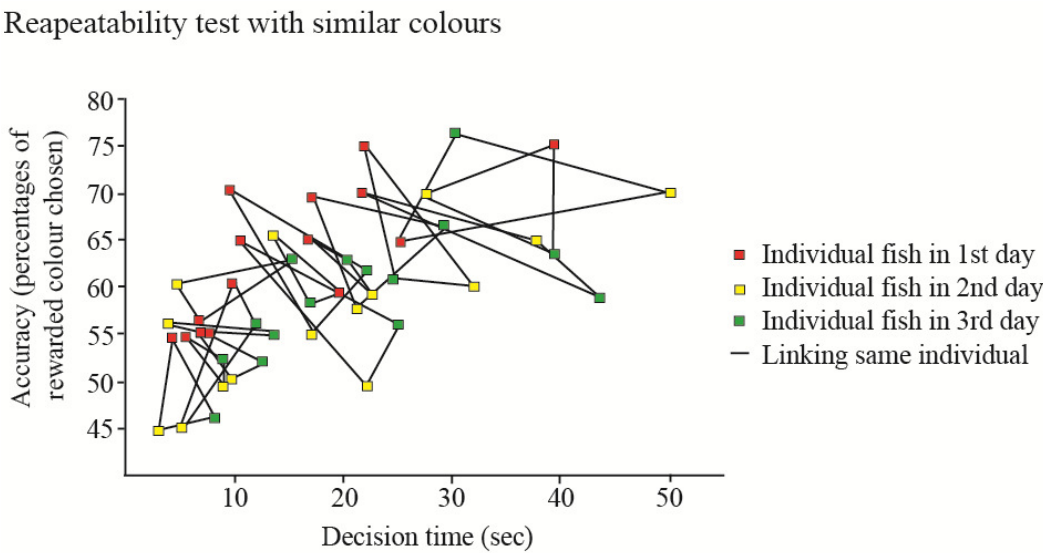


Fig 24 Fish also showed significant repeatability when encountering similar colours. The careful fish made more accurate choices but spent longer decision time remained careful after 24 & 48 hours, and the impulsive individuals also kept their strategy.

Experiment 3: fish foraging in pairs followed the decision of the careful one

We found a within-group speed-accuracy tradeoff in zebrafish, and the strategy was constant within a day for individual fish. We therefore tested how fish with different strategies (careful and impulsive) made decisions together. Zebrafish show shoaling behaviour, and typically forage close to conspecifics. When foraging in pairs, fish also showed significant correlation between speed and accuracy

(Spearman's rank correlation, $r = 0.707$, $n = 30$, $p = 0.003$). Since we wanted to make sure the fish maintained the same strategy, we tested the same fish after 24 hours. We tried to pair a careful fish with an impulsive fish base on their reference performance 24 hours before, and the average group performance based on the previous two sets of experiments. However, due to the limited number of fish we could test per day, we could not pair the fish based on the group performance. The strategy of the fish (careful or impulsive) was determined within a pair, not based on the group. This means the careful fish was only more careful than its partner, but might not be 'careful' from the group point of view. The decision speed and accuracy, when fish were foraging together, were significantly different from the impulsive individuals, but not from the careful individuals (speed: ANOVA, $F_{(2, 39)} = 8.457$, $p < 0.001$, Tukey HSD post hoc test $p = 0.004$, $\eta^2 = 0.237$, Fig 25a; accuracy: ANOVA, $F_{(2, 39)} = 4.0698$, $p = 0.025$, Tukey HSD post hoc test $p = 0.013$, $\eta^2 = 0.083$, Fig 25b). Decision making in a dyad was thus apparently determined by the careful individual.

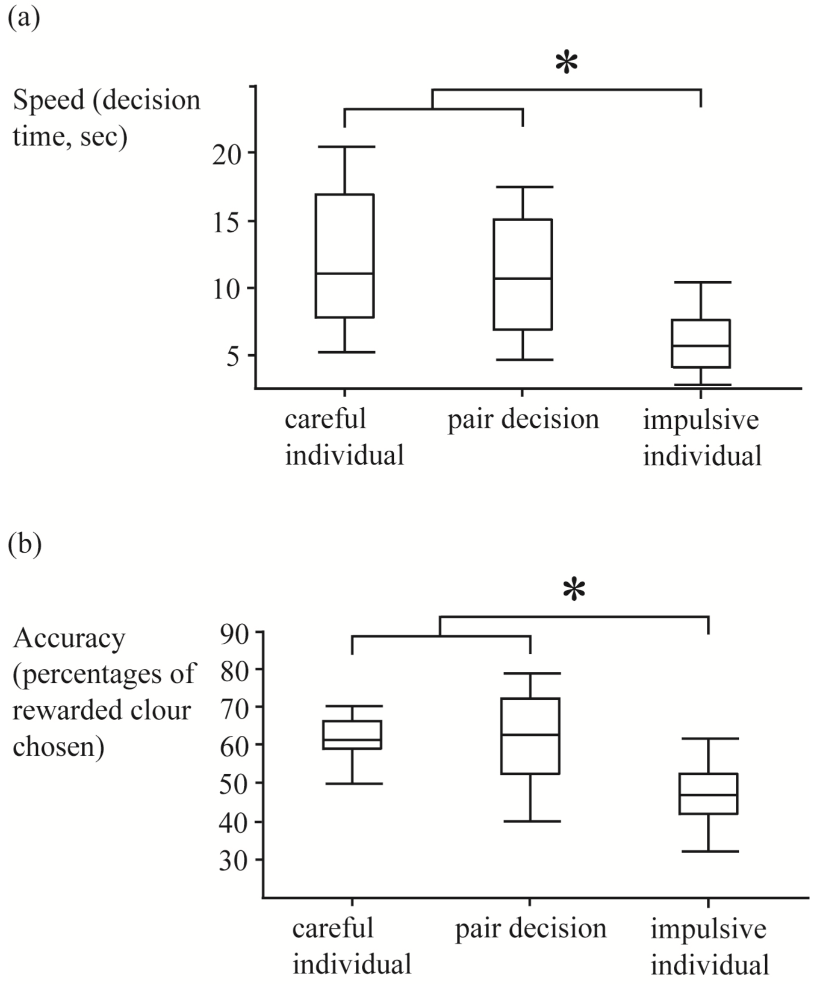


Fig 25 When foraging in pairs, the group decisions were more close to the careful individual in both investigating time (a) and decision accuracy (b).

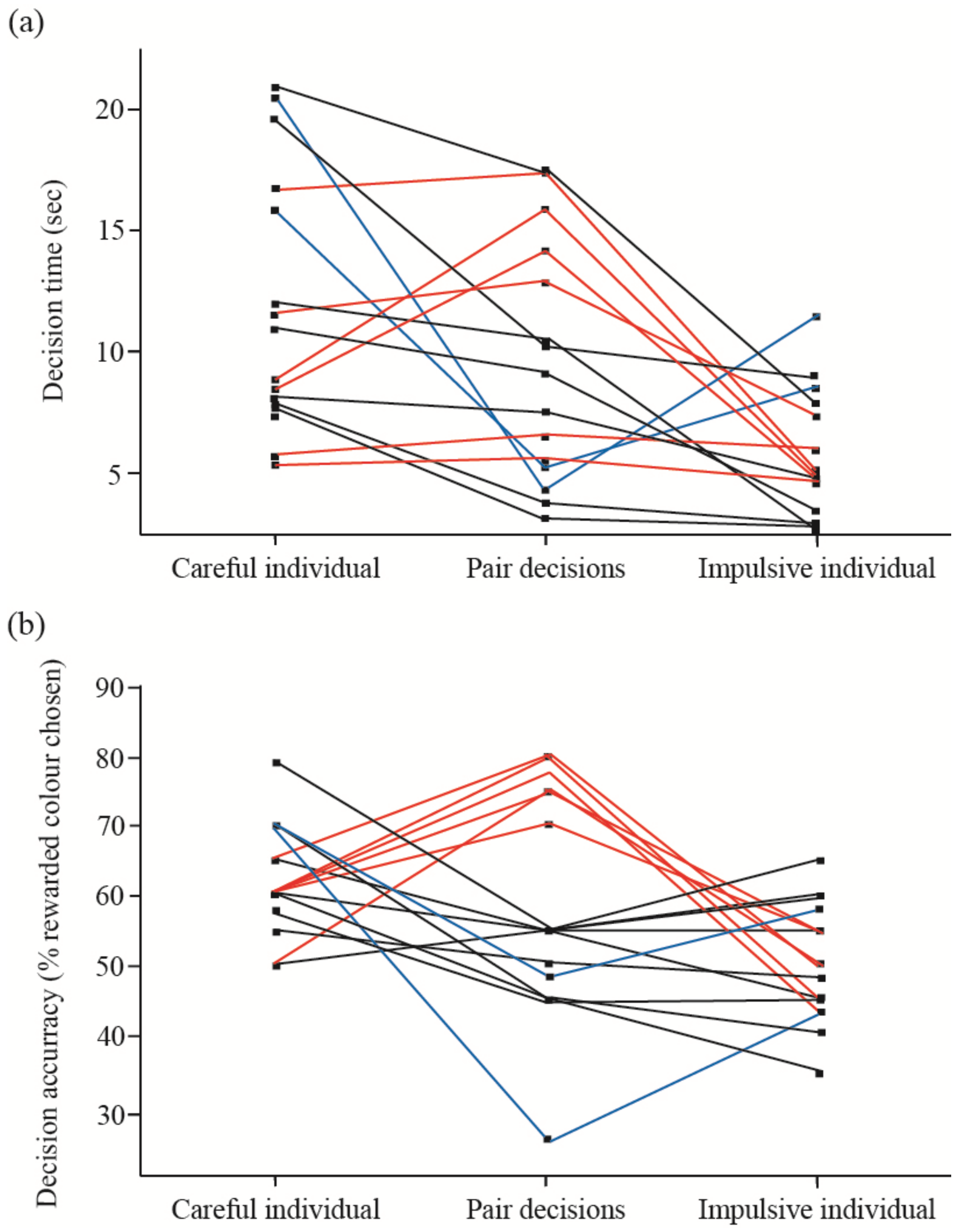


Fig 26 Individual behaviour in each pair for both (a) decision time and (b) accuracy is shown. We tested the fish individually on the first day and tested in pairs on the second day. Each point shows individual fish performance, or performance of a dyad of fish. The line links fish in a pair. The red lines indicate the fish increased speed or accuracy when in a pair, and the blue lines show decreased speed or accuracy.

7.5 Discussion

In the present study, we showed that speed-accuracy tradeoffs exist both between individuals as well as between pairs of zebrafish, using a colour discrimination paradigm. The individual speed-accuracy tradeoff is consistent over time in zebrafish, which is constant with a key criterion of animal ‘personality’ (Gosling, 2001, Sih et al., 2004a). In the similar colour pair, we found significant correlations between decision time and accuracy in fish foraging choices. Zebrafish demonstrated constant individual differences in behavioural traits, as some fish made more accurate decisions but took longer to make the decision, while others were more impulsive and spent a shorter time investigating. Such a tradeoff has been investigated in humans for a long time, but it was not until recently that reports have been presented for other animals (Chittka et al., 2009). Behavioural polymorphisms within a species have been identified in eusocial (Oster and Wilson, 1979) and nonsocial animals (Sokolowski, 1980, Benus et al., 1991, De Bono and Bargmann, 1998), and individual variation plays a key role in animal evolution.

The reason why animals maintain the same strategy over time is still unclear. Theoretical models indicate that variations in behaviour may enhance the evolution of cooperation (McNamara et al., 2004), and studies in eusocial animals show that differences in colonies may enhance group fitness (Mattila and Seeley, 2007). Such differences may be essential for colony extension to cope with rapidly changing environments, where the optimal strategy shifts according to conditions (Burns and Dyer, 2008). Flexibility in strategies can be energetically costly and quickly out of date when the environment changes again. In the previous chapter we showed that animals do not necessarily choose the optimal strategy; however, the more beneficial options change over time. This may explain why animals maintain constant individual differences over time. However, further experiments are needed to explore the evolutionary basis of animal ‘personality’, and the genetic and neural basis underlying constant individual variation.

Constant individual variation can influence animal consensus decisions. When fish were put in pairs during the colour discrimination task, the group decision was closer to that of the careful individual who made more accurate choices but took longer to do so. However when looking at individual pairs, we can see that there were many careful individuals also increasing the decision time and accuracy. Thus the result that the group decision was closer to the careful individual may not be caused by the impulsive individual following the careful individual, but by increases of decision time and accuracy in a dyad.

We demonstrated how animals with different consistent individual behaviour traits make judgments together. Accurate estimations and decisions are essential for animals, and group-living species are able to balance personal and social information received from other group members (Dall et al., 2005). Individual variation can affect social interactions and even benefit the group (Pike et al., 2008, Krause et al., 2010), and average judgements of the social group can be more ideal compared with individual opinions (Surowiecki, 2005). In the present study, zebrafish in pairs did not exhibit ‘better’ strategies (for example, decreased investigating time but with higher accuracy). It may be that in the current experimental setup the careful strategy was better for zebrafish. Another explanation may be that the impulsive individual hesitated to make a rapid decision in exchange for remaining in the pair. The energetic gain may be similar between ‘impulsive-careful’ axes. In such cases when slowing down investigations, making more accurate choices may balance their strategy efficiency.

Here, we demonstrated for the first time that zebrafish showed constant speed-accuracy tradeoff with regard to foraging strategy, and how individuals with diverse strategies make consensus decisions. During the colour discrimination task, some fish made constant ‘careful’ decisions while others were always ‘impulsive’. When pairing ‘careful’ and ‘impulsive’ fish together, they made joint ‘careful’ decisions with higher accuracy but longer decision time. Pairs of fish are the starting point of investigating zebrafish consensus decision making and how individual differences affect social groups. The zebrafish model also provides a powerful tool to examine the neurological and genetic basis underlying animal consistent individual behavioural differences in animals and their impact on the social world.

Chapter 8 Discussions and contributions

8.1 Selective and divided attentions in bumblebees

In this thesis, we have explored the ability of bees to disentangle shape from colour in bumblebees in the context of predator detection, and their ability to divide attention between foraging and predator detection tasks. We found that when bumblebees were attacked by yellow spiders, they formed a search image of spider shape, but disregarded the spider's colour. The bees formed a colour-independent search image of their predators, and they also changed their foraging behaviour to minimise exposure to predation threat. This ability has significant ecological benefit since some species of crab spiders, such as *Misumena vatia,* are able to change their body colour depending on the environment (Chittka, 2001, Morse, 2007, Insausti and Casas, 2008). The bees were able to learn complex shape cues that can be generalised to other colour forms of the spider (Stach et al., 2004). Many animals possess innate predator avoidance responses, but in the current study, naïve bees did not avoid flowers harbouring cryptic spiders. This finding supports previous observations (Ings and Chittka, 2008) that bumblebees do not appear to have a strong innate avoidance response to spider shapes.

We also demonstrated that bees were able to divide their attention to attend simultaneously to two discrimination tasks. We presented bees with food searching and predator avoidance tasks, which they had to solve simultaneously when foraging. The bees had to discriminate between two similar flower types and they had to avoid camouflaged robotic spider predators. The bees were able to solve both tasks concurrently, but when the cost of making an error was rather low, they chose to prioritize predator avoidance. The reason that the bees prioritised their attention to predation risk is not because of attentional limitation; rather, it serves to maximise foraging rate. In addition, even when they were able to divide their attention between two tasks simultaneously, that behaviour came with some consequences such as a lower ability to attend to predator detection. These results are consistent with research on humans, which has found that the value of visual information can influence attentional priority (Anderson et al., 2011). Divided attention in visual search has been studied extensively in human and non-human primates (Treisman and Gelade, 1980), but there is limited research on other animals (Dukas, 2002), especially for invertebrates. We have shown that the bees were able to divide their attention, in a manner similar to what has been found in humans (Pashler, 1999). When encountering stimuli which were difficult to handle, the bees showed limited capacity to process information and divide their attention between tasks.

8.2 Speed-accuracy tradeoffs in bumblebees and zebrafish

I tested the within group speed-accuracy tradeoffs in both bumblebees and zebrafish. Both species showed significant correlations between speed (decision time) and accuracy, which means that some individuals spent more time investigating and made more accurate choices, while others decided rapidly but were more error-prone. In animal studies testing stimulus discrimination, the researchers were usually only concerned about investigating the accuracy but not the speed of making decisions (Chittka et al., 2003); however, an evaluation of decision accuracy needs to be put in the context of the time allowed to make the decision. In humans, there is a long history of considering both parameters (Woodworth, 1899) and speed accuracy tradeoffs have been considered in the context of factors but have been little studied in other animals. It is clear, however, that such tradeoffs also exist in animal decision making, and indeed in various behavioural contexts, such as foraging (Chittka et al., 2003), predator-prey interactions (Ings and Chittka, 2008, Burns and Rodd, 2008) and nest site selection (Franks et al., 2003).

Moreover, speed-accuracy tradeoffs can occurs at multiple levels. When conditions change, the animals may sacrifice speed or accuracy in favour of the respective other dimension depending on the situation (Chittka et al., 2003, Palmer et al., 2005). For example, when the task becomes difficult, the subject may be slower and a less accurate performance might result (Palmer et al., 2005), or when punishment exists, they may trade speed for accuracy (Chittka et al., 2003). In bumblebees, we found that when the discrimination task became more difficult, the animals traded accuracy for maintaining the same decision making time. It is interesting that they chose to sacrifice accuracy but not decision making time. Perhaps this was because in my study design there was no strict punishment when bees chose the wrong colour. The bees gave more serious consideration to the surveying time than they did to making highly accurate decisions. When the task is difficult for the animal, and the costs of making errors are affordable, lowering accuracy or even making choices at random may become the preferred solution (Burns, 2005). As can be seen in humans and other animals (Zenger and Fahle, 1997, Franks et al., 2003), bees are able to survey the environment and change their emphasis on either speed or accuracy depending on diverse conditions.

8.3 Consistent individual behavioural differences

In the present set of studies (chapter 6 and 7), we found that both bumblebees and zebrafish exhibit consistent individuality in their speed-accuracy tradeoffs. We tested target animals with colour discrimination tasks and looked at the within-group speed-accuracy tradeoffs. The animals exhibited diversity in foraging strategies, where some individual were more ‘careful’ (made more accurate decisions, but required longer decision making time) while others were more ‘impulsive’ (faster choices but less discriminating). In addition, such individuality was stable over time. The repeatable individual behavioural differences over time have been described in many animal species (Gosling, 2001, Sih et al., 2004a) This suggests that the ecological and evolutionary consequences of constant individuality are favoured by selection. Even though this topic has interested a large number of researchers, the formation, ecology and evolutionary basis of behavioural polymorphism is still unclear. Flexibility in strategies can be energetically costly if the animal has to manipulate signals from the habitat and make correct decisions, and when the environment is changing rapidly the judgement may become out of date quickly. Diversity can enhance group fitness in animals (Mattila and Seeley, 2007). Such differences within a group may be essential for the group’s ability to cope with rapidly changing environment, where the optimal strategy shifts with the conditions (Burns and Dyer, 2008).

8.4 Behavioural diversity and consensus decisions

In chapter 7, we demonstrated how dyads of zebrafish with distinct behavioural traits made decisions together. We found that zebrafish exhibited consistent between-individual differences in speed-accuracy tradeoffs, wherein some individuals were more ‘careful’ and others were more ‘impulsive’. Therefore, we paired the ‘careful’ and ‘impulsive’ individuals and made them discriminate between two similar colours. The combined decisions were closer to the decisions made by the careful individuals. Using pairs of animals is the simplest way to begin a study of animal group decisions. Living in social groups is a widespread phenomenon in the animal kingdom and provides many ecological benefits for group members (Alexander, 1974, Krause and Ruxton, 2002). Staying close to each other may provide useful social information, such as foraging, mating and vigilance cues (Brown, 1986, Westneat et al., 2000, Elgar, 2008), or yield benefits with respect to conserving energy or heat (Weimerskirch et al., 2001, Gilbert et al., 2009). Unlike solitary individuals, animals living in groups have to integrate information collected by group members and make their decisions jointly. Within a group, individual behavioural, morphological and sensibility individual differences may exist among group members, and individuals can also gather different personal information during the investigation of resources and the environment. Integrating the personal information and judgements made by individuals can shape the movement of the group. In many studies, certain behavioural trait differences are known to be important factors shaping consensus decisions, and certain individuals are more dominant than others in the collective decision making process (Couzin et al., 2005, King et al., 2008, Stueckle and Zinner, 2008, Conradt et al., 2009). For example, bold individuals are more likely to take the lead in a group and have a larger impact on making choices (Harcourt et al., 2009, Kurvers et al., 2009). Personal differences within a group can benefit group members. Diversity between individuals may enhance colony fitness in eusocial insects (Mattila and Seeley, 2007, Oldroyd and Fewell, 2007) or increase mating or foraging efficiency (Sih and Watters, 2005, Dussutour et al., 2008, Nicolis et al., 2008). Nevertheless, few studies have looked into how individual personality traits motivate social networks, especially for animals other than human beings. In the present study, we looked at how a pair of animals with diverse behavioural strategies made choices together. This provides a starting point for investigating zebrafish consensus decision making and how individual differences affect social groups.

8.5 Future work

In the present thesis, we showed consistent individuality in bumblebee foraging behaviour from bees within the same colony. The mechanisms behind behavioural diversity are fascinating future research directions. Testing individuality with other social bee species with different colony formations may provide a better picture with regard to the ecological benefit of constant behavioural diversity. Modelling colony fitness and how group members cope with different environments over time may provide a clue for the origin and formation of stable individuality. The interaction between individuals of different strategies within the colony is another interesting research issue. Is the percentage of the careful-impulsive individuals the same within and between colonies? If we remove careful or impulsive individuals, will the remaining bees in the colony change their strategy?

Zebrafish have been one of the most popular model species in developmental and molecular biology for decades (Grunwald and Eisen, 2002, Sison et al., 2006). The zebrafish model also provides a powerful tool to examine the neurological and genetic basis underlying consistent individual behavioural differences in animals and their impact on the social world. Since the genes that regulate behavioural characteristics are largely unknown, identifying the genetic polymorphism that influences animal 'personality' and how the individual differences alter group choices can be a possible future topic.

8.6 Key contributions

This study makes the following key contributions to the current body of literature:

a. We showed that bumblebees can disentangle the shape of a predator from its colour. The bees were able to form a colour-independent search image of the shape of the predator they encountered.

b. We demonstrated that divided attention exists in bumblebees. The bees were able to divide their attention between foraging and predator avoidance tasks at the same time.

c. We found that plasticity in speed-accuracy tradeoffs exists in bee foraging strategies. When the predator on the flower was cryptic, the bees traded accuracy for maintaining the investigating time. When punishment appears for errors in a colour discrimination task, bees trade speed to sustain the same foraging accuracy.

e. We demonstrated between-individual speed-accuracy tradeoffs in both bumblebees and zebrafish. Some individuals perform more 'careful' choices than others, where they made more highly accurate choices but spent a longer time investigating those choices. Other individuals were more 'impulsive' and spent less time investigating but they were more error-prone.

f. We showed that 'personality' exists in both bumblebees and zebrafish. Animals showed consistency in speed-accuracy tradeoff strategies tested with colour discrimination tasks.

g. We tested the speed-accuracy tradeoff by examining dyads of zebrafish engaged in colour discrimination tasks. When tested in pairs of 'careful' and 'impulsive' fish, the combined decisions were closer to those of careful individuals.

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