COGNITIVE ECOLOGY OF FORAGING: MULTIMODAL SIGNALS AND THE
SPEED-ACCURACY TRADE-OFF

by

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DEDICATION

I dedicate my thesis to my parents, Iffet Kulahci and Vahdi Kulahci.

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ABSTRACT

Many signals in nature are complex and composed of several components. However, the advantages and disadvantages of complex signaling are not completely understood. Although complex signals are advantageous for vertebrate decision-making processes, our understanding of whether similar advantages exist for invertebrates is limited. To investigate how signal complexity influences learning and decision-making in foraging bumblebees (*Bombus impatiens*), I trained them on unimodal (shape or olfactory) or multimodal (shape and olfactory) flowers. Addition of olfactory cues to visual cues improved the learning of bees. A speed-accuracy trade-off in decision-making reflects the difficulty of decisions. The bees trained on multimodal flowers made more accurate decisions without reducing their speed, and had higher target finding rates (correct visits/total decision time). These results suggest that bees forage more efficiently when flowers signal in more than one modality, and support arguments pointing out problems with studying signal components separately.

CHAPTER 1

SIGNAL EVOLUTION AND RECEIVER COGNITION

1.1   Signal evolution: receiver cognition and signal properties

The importance of signal evolution and design has been recognized and studied in many disciplines of biology. However, until recently, most studies of signal evolution have focused on signal production and the information conveyed by signals (Rowe 1999, Johnstone 1996). Although many definitions for signals exist, throughout my thesis, I will use a functional definition. By "signal", I refer to information produced in the form of energy or matter that results in a behavioral response from a receiver (Wilson 1975, Hebets & Papaj 2005). Communication between the signalers and the receivers depends on several stages, consisting of signal production, transmission, reception, perception, assessment and decision (reviewed in Endler & Bosolo 1998). Most of these processes are related to each other, and changes in one can have important consequences for others.

In this review chapter, I will argue that in order to fully understand how signals and receivers evolve and function, we need to include the mechanisms of information processing by the receivers (Chittka & Thomson 2001) in addition to the design and the information conveyed by the signals. Furthermore, signaling in nature rarely is done with only one component, so an approach integrating multiple signal components is necessary for analyzing the function and the evolution of signals (Rowe & Skelhorn 2004).

1.1.1 Signal evolution may be influenced by receiver cognition

Cognitive abilities and limitations influence how animals make decisions among alternative choices, and therefore may have profound implications for the evolution of signals. "Receiver psychology", as introduced by Guilford & Dawkins (1991), refers to the receivers' cognitive mechanisms that can influence signal evolution. The cognitive processes important for signal evolution include detection, discrimination, learning, signal processing, and decision-making. It is important to realize that even though they are strongly interrelated, detection and discrimination are different processes: detection refers to how well a signal is perceived against its background, while discrimination is a process by which animals respond differentially to signals (Rowe 1999).

Detection and learning influence the rates of information processing, and as a result, affect decision-making abilities. At the same time, constraints in these cognitive processes may be a potential driving force in signal evolution. For example, limited attention, or the limited rate of information processing in the brain (Dukas 2002), can create a mismatch between the amount of useful information available in the environment and the amount of information that can be processed. Some signals that animals are physiologically equipped to receive may be ignored as a result of limits on attention, and this can have important implications in situations where animals have to attend to two separate cues from different origins.

One of the contexts in which the limited attention may influence how the mismatch between the available environmental information and the information that may be used is the trade-off animals face between detecting predators and potential mates

(Dukas 2002). For example, male moths respond less to the acoustic signals from predatory bats when they also detect female pheromones (Skals et al. 2005). The tendency to escape from bat signals is reduced when the pheromones signal high-quality females. This observation suggests that as the intensity of the pheromones increase, male attention gets directed more to the female signals. As a result, males ignore the predatory signals. Although the males have the sensory capacity to detect the bat signals, they do not attend to those signals in presence of pheromones of high intensity. At the same time, when the intensity of the bat acoustic signals is increased, the moths' response to the bat signals increases too. These results suggest that the attention of the moths is limited but also influenced by the intensity of signals.

In situations where response from receivers increases the fitness of signalers, selection would favor signalers that produce easily received and processed signals (Rowe 1999). The pressure to produce signals that are easy to detect may lead to divergent selection. On the other hand, if survival of a species depends on not being detected, then signals that are difficult for the predators to detect would evolve. Limited attention may decrease the ability of predators to detect prey items (Dukas 2002), which in turn would increase survival of the prey that are difficult to detect. One of the behavioral consequences of limited attention is that predators seem to search for the prey items using a "search image", by focusing on one type of cryptic prey while ignoring other equally cryptic prey items (Dukas & Kamil 2001, Tinbergen 1960). Predator foraging behavior that results from limited attention may lead to changes in the frequency and the diversity of the prey signals. For example, Bond and Kamil (2002) conducted a long-term study in

which they looked at how blue jay predation influences phenotypic variation in digital moths. The digital moths shown to jays on computer displays included several phenotypes that were all cryptic against the background. When foraging on these cryptic moths, jays selected the moths that were more similar to the ones they had detected on a previous foraging trial, suggesting that their decisions may have been influenced by search images and limited attention (Dukas 2004). The moths in the study had virtual genes coded by algorithms derived from patterns in moth wings. These virtual genes allowed Bond and Kamil (2002) to stimulate the population effects of predation. By comparing moth populations in which jays preyed on moths with another moth population without jay predation, they showed that moth phenotypic variation increased in the population with jay predation. These results suggest that the behavioral patterns resulting from limited attention may have consequences for the evolution of signals.

Sensory biases in receivers may lead to the exaggeration of existing signals, or to the production of new signals, by influencing the perception of and the preference for specific signals (Rowe & Skelhorn 2004). Since animals are not able to process all of the information they are physiologically capable of sensing (Desimone 1998, Dukas & Kamil 2000), the senders may signal in ranges at which their intended receivers may be able to attend (Dukas 2002). For example, males may signal using stimuli that females may pay more attention to, and those males that can exploit the sensory biases of females may be selected. This "sensory exploitation" is an important driving force in signal evolution, but it has not been studied extensively in contexts other than sexual selection (for examples of sensory exploitation in sexual selection, see Ryan 1998).

1.1.2   Influence of signal properties on detection and processing

On one hand, animal decisions and cognitive abilities influence signal evolution; on the other hand, the reverse process also happens. Evolution of animal senses, cognition and learning are influenced by the signals from the environment (Chittka & Thomson 2001). Depending on the properties of signals, detection, discrimination and processing may be diminished or enhanced, and may alter efficiency of decisions.

The level of complexity is one of the most distinguishing properties between signals. Complex signals are made up of multiple components (Hebets & Papaj 2005), and can enhance detection and discrimination by receivers (Rowe 1999). For example, flowers differ in many different dimensions along the visual modality. Some of these visual dimensions include the shape, the hue, the brightness, and the size of the flowers. The combination of these dimensions creates a complex signal, while signals in any one of these dimensions could be classified as a simple signal. In comparison to simple signals, parts of a complex signal may convey different messages, influence efficacy, or interact with each other to produce effects that exceed the individual effects of their components (Hebets & Papaj 2005). Complex signals may also provide detection advantages in situations where components have different detectability under different conditions, at different times or from different distances (Partan & Marler 1999).

Multimodal signals are complex signals composed of more than one sensory modality or channel (Rowe & Guilford 1999, Guilford & Dawkins 1991). Multimodal signaling is widespread in nature. For example, avian courtship displays composed of elaborate visual traits and songs are some of the most widely studied multimodal signals.

Similarly, fruit flies use both acoustic and chemical cues to attract males (reviewed in Partan& Marler 2005). Plants also produce signals in more than one modality; the visual signals of flowers are usually coupled with olfactory cues, and this multimodality may help attract pollinators.

Addition of a new component from a different modality to a signal may present additional constraints and opportunities for both the receivers and the senders (Partan & Marler 2005). For example, data from human studies show that signals with two modalities, such as signals composed of visual and olfactory components, are detected faster than any of the components alone (reviewed in Rowe 1999). This effect is thought to be a result of multisensory (or intersensory) integration in which input from one modality influences processing of others (Small 2004, Calvert 2001, Stein 1998). Studies on multisensory integration in vertebrates suggest that the presence of this ability may have driven the evolution of multimodal signals (reviewed in Rowe 1999). In addition to increased detection, multimodal stimuli may result in increased discriminability (Rowe 1999, Eninger 1952). A similar result was also observed in bees; visual discrimination was enhanced in presence of olfactory stimulus (Kunze & Gumbert 2001).

Although detection, discrimination and processing of multimodal signals are known to have enhancing effects on vertebrates, few examples exist from invertebrate studies (but see Skals et al. 2005, Kunze & Gumbert 2001). Insects have structurally different brains than vertebrates and may have different processing and sensory integration mechanisms. Even though signal processing from individual modalities has been investigated in invertebrates, our knowledge of multimodal signal processing and

integration is almost non-existent (Holldobler 1999). Regions in the human brain that were initially thought to be strictly devoted to processing unimodal signals are becoming recognized as regions that process multisensory stimuli (Ghazanfar & Schroeder 2006). It is possible that similar types of multisensory interactions happen in invertebrate brain regions we currently think of processing only single sensory stimuli.

Despite their influence on detection, discrimination and processing of signals, the role of multimodal signals in communication is poorly understood (Partan & Marler 1999). The mechanisms by which multimodal signals combine during processing are still controversial (Belardinelli & Sestieri 2004). Data from primates suggest it is possible that a combination of signals about an object creates a conceptual representation of the object in the brain, rather than separate representations by single sensory stimulus (Ghazanfar & Santos 2004). Whether enhancing interactions of multimodal signals also happens between unimodal signals is also unclear, although such an effect would depend on whether perception of multiple signals from the same modality are different than multiple signals from different modalities (Rowe 1999).

1.1.3 Learning and signal properties

In order for previous experiences to be adaptive, animals should be able to incorporate information from their previous experiences into their future decisions. Learning and remembering new stimuli, locations, or behavior have major influence on decision-making and choice behavior of animals (Menzel 2001). In some cases, learning may help individuals overcome their previous preferences. For example, specific colors are preferred by naive insect pollinators (Gumbert et al. 1999, Giurfa et al. 1995, Lunau & Maier 1995). However, they can learn to associate other colors with food rewards after visiting a rewarding flower with that color, although the speed of learning may depend on color of the flower (Gumbert et al. 1999, Menzel et al 1993). Likewise, pollinators can also switch to a more rewarding species when there is a drop in reward levels or change in flower densities (Kawaguchi 2006, Thomson 1981, Heinrich 1979).

In situations where decision-making is hindered by limited attention, learning which stimuli to attend to may help animals overcome consequences of limited attention (Dukas 2002). Coping with limited attention is especially important in situations that require dealing with complex signals. As most signaling systems involve more than one component, it would be advantageous for animals to learn the components of the signal that contributes most to fitness.

Although results of some of the earlier experiments (Bitterman 1996, Funayama et al. 1995) have suggested evidence for the independence assumption, which argues that there will be no interaction between the components of a stimulus (Bitterman 1996,

Couvillon et al.1997, Funayama et al. 1995), several recent studies have found signal interactions in learning.

Signals can interact to either enhance or block learning of each other (Smith 1997, Rescorla and Wagner 1972). For example, if a bee is trained on one olfactory cue, learning about a second olfactory cue may be blocked in additional trials when the bee is presented with a complex signal composed of both components (Smith & Cobey 1994). At the same time, it is possible that when the cues used are from different modalities, there is an enhancing effect on learning. In support of this argument, Gerber and Smith (1998) have found that presence of visual stimulus enhances learning of olfactory stimulus in honeybees. Similarly, olfactory discrimination learning in hummingbird hawkmoths (*Macroglossum stellaratum*) is faster when the task is coupled with differences in color (Balkenius & Kelber 2006). Sound facilitates visual learning in chicks (Rowe 2002), while olfactory cues enhance avoidance learning (Roper & Marples 1997). It is possible that independence assumption holds for multimodal signals, but not for unimodal signals (Funayama et al. 1995). Although empirical evidence suggests that signal interactions may influence learning, the mechanisms responsible are currently unknown (Hebets & Papaj 2005). Additional studies that focus on learning of multimodal and unimodal complex stimuli are needed to understand advantages of complex signals for receivers.

1.1.4 Theoretical aspects of signal detection and discrimination

Studies on decision-making have benefited from the predictions of the Signal Detection Theory (Green & Swets 1966, Wiley 1994), which looks at the uncertainty in signal detection caused by similarity of important stimuli, such as those signaling the presence of resources, with other signals in the environment (Wiley 1994, Shettleworth 1998). Although I will be using pollinator foraging as an example to address how signal detection can be influenced by signal properties, the arguments I present below can be generalized to the other signaling systems as well.

One of the distinguishing features of flowers is their shape. A foraging pollinator, such as a bumblebee, frequently encounters flowers with different shapes. In Signal Detection Theory, it is assumed that the receivers' perception of signals can be outlined with normal distribution and equal variances. Therefore, the density function that shows the probability of encountering a specific signal, such as a flower with a tubular shape, can be represented with a bell-shaped curve across the range of different signal values. It can be assumed that tubular shaped flowers provide the bee with nectar and/or pollen, while circular shaped flowers are non-rewarding.

When two signals are perceived to be similar, the receiver cannot know whether a specific signal value corresponds to the correct signal or to the incorrect signal. The receiver therefore sets up a threshold value (shown in Figure 1.1 with the dashed lines). Any value above this threshold value, towards positive infinity, results in a response from the receiver. With this threshold, four different outcomes are possible. The bee can

respond to the tubular shaped flowers (Correct Detection), respond to the circular flowers (False Alarm), not respond to the tubular flowers (Missed Detection), or correctly reject the circular flowers (Correct Rejection).
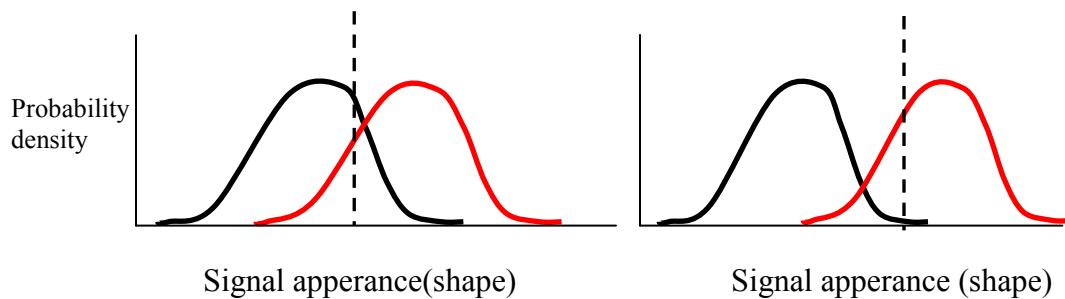
Figure 1.1   1.1a and 1.1b. Signal detection theory and two signals in the environment. In this particular example, red graph represents perception of the tubular-shaped flower that is rewarding, while the black graph represents the perception of the circular flower that is not rewarding. The two signals represented in Figure 1.1a can become more different from each other by increasing the distance between their means.

According to the above options, a visit the bee makes will either be to the correct flower or to the wrong flower. It is important to realize that while deciding between two signals such as these plotted in Figure 1.1a, no matter where the bee places her threshold for response, she cannot maximize the probability of correct detection and minimize the probability of false alarms at the same time. This limitation happens because the probability of the false alarm varies with the probability of the correct detection. False alarms, as well as missed detections, are due to the uncertainty caused by the similarity of signals. If the similarity of the signals can be reduced, the error rates can also be reduced (Wiley 1994, Shettleworth 1998).

One of the ways that similarity between the two signals can be reduced is by reducing the overlap. If the variances are kept the same, then increasing the separation between the means, as shown in Figure 1.1b, would reduce similarity between signals (Ashby & Townsend 1986). Even if the response threshold of the animal stays the same, the separation of the means would increase the ratio between the probability of correct detections and the false alarms. As a result, the receivers may distinguish between the signals more accurately.

If signalers add another component, then the receivers may be able to detect them easier. The additional component can either be from the same sensory modality, such as a color component added to the shapes, or from another sensory modality, such as olfactory cue. Although adding another signal component may be costly for a sender due to increased energetic constraints (reviewed in Partan & Marler 2005), or may be disadvantageous in situations where the second component attracts an unintended receiver or a predator (Roberts et al. 2006), increased detection by the receivers may be one of the reasons why most signals in nature are complex or multimodal.

In order to address whether adding a second component to the signal makes it easier to detect, we need to use General Recognition Theory, which is an extension of Signal Detection Theory for the stimuli composed of two or more signal components (Ashby & Townsend 1986). For example, it is possible to consider flowers that differ from each other in their shapes as well as their odors. When the same density function from the Signal Detection Theory is plotted for a flower that has two components, we end up with a 3-dimensional representation (Figure 1.2a). In this particular example, the x-

axis is shape, y-axis is the olfactory cue. The function relating perception of shape and

olfactory combination is plotted on the z-axis. The two bell-shaped density function

curves represent two flowers, each with shape and olfactory components.
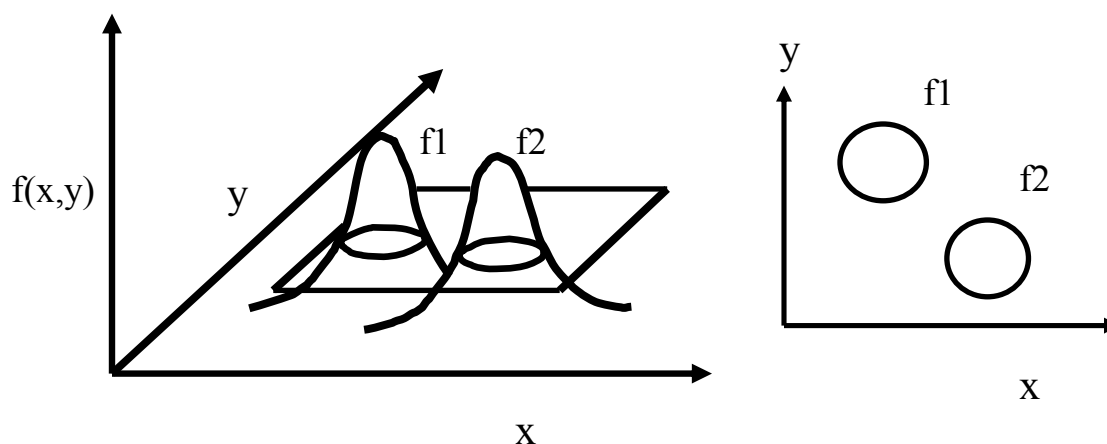
Figure 1.2.   Representation of two stimuli, each with two components (x,y). Using the
examples from the text, the x-axis can be viewed as the shape dimension,
while y-axis is the olfactory dimension. f(x,y) represents how flowers with
shape and olfactory components are perceived by the bee. When a cross-
section of the bell-shaped diagrams for the two flowers (f1, f2) is taken,
equal probability contours shown in 1.2(b) are obtained. Figures re-drawn
from Ashby & Townsend (1986), with permission.

Since visualizing in 3-dimensions is not always convenient, Ashy and Townsend

(1986) take a cross section of the diagram by cutting through the density functions with a

plane. The result is a representation of the two flowers along the two stimulus

dimensions, shape and olfactory. The cross sections produce contours that have equal

probability of occurring. Figure 1.2b shows these equal probability contours obtained by

looking at the cross-sections from Figure 1.2a.

With two components, the resulting flowers can have (1) odor1-shape1 and (2) odor2-shape2 as well as the hybrid forms including (3) odor1-shape2 and (4) odor2-shape1 (Figure 1.3a). General Recognition Theory assumes that the receiver sets up a "decision bound" (Ashby & Townsend 1986) similar to the threshold used in the Signal Detection Theory. For example, a bee choosing a flower based on its shape may use the decision bound that is vertically placed between flowers 1 and 3 (or, 2 and 4). In this case, the point at which the decision bound intersects the x-axis represents the response threshold portrayed in the Signal Detection Theory (Figure 1.1). A similar process can be observed for a bee deciding between two flowers with different olfactory cues (between 1 and 4, or 2 and 3), and the decision bound in that case would be the horizontal one.

However, it is possible that when bees are evaluating which flowers to visit, they use information from both the shape and the olfactory cue. To simplify the argument, I portray this case in Figure 1.3b, where the bee needs to choose between the flowers 3 and 4 that were initially shown in Figure 1.3a. The optimal decision bound in this case would be a diagonal line between the two flowers.

By using distances to decision bounds in this hypothetical example, the relative difficulty of deciding between two stimuli that differ in two dimensions versus between two stimuli that differ in one dimension can be estimated. The distance of a stimulus to the decision bound can be used as an inverse measure of task difficulty (Maddox et al. 1998). As the mean of the stimulus gets closer to a bound, it becomes more difficult to make a decision, while a stimulus farther from the bound results in an easier decision.

y
(Olfactory)

4

2

1

3

x
(Shape)

Figure 1.3a

y
(Olfactory)

x
(Shape)

Figure 1.3b

y
(Olfactory)

x
(Shape)
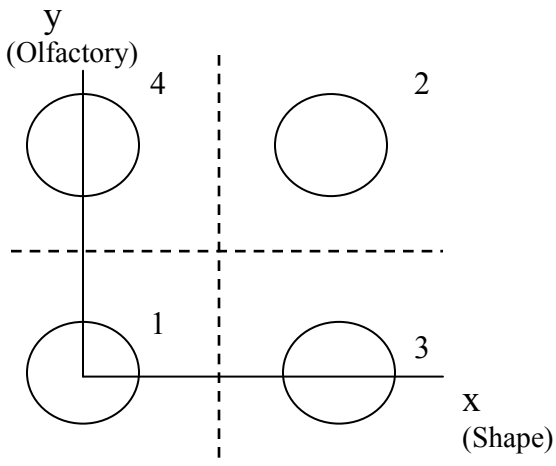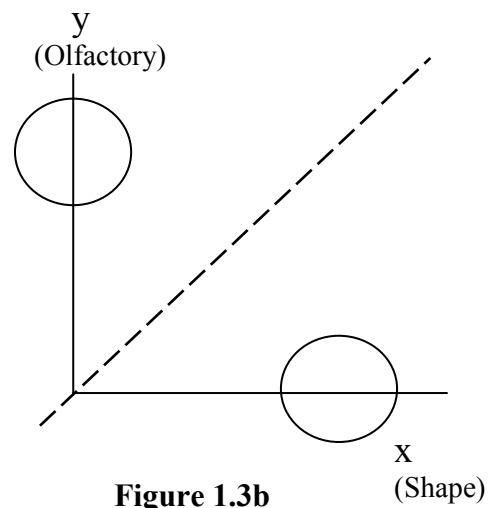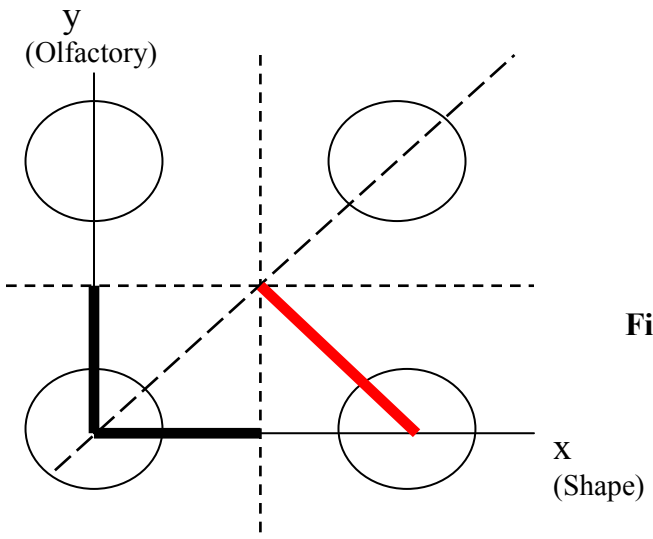
Figure 1.3c

Figure 1.3.   Equal probability contours of flowers with shape and olfactory dimensions. Flowers 1,3 (and flowers 2,4) have the same odor but differ in shape. Flowers 1,4 (and 2,3) have the same shape but different odors. The dashed lines represent the decision bounds (vertical bound for shape, horizontal bound for olfactory). Figure 1.3b shows two flowers from 3a that differ in both olfactory cues and shapes. In this case, the decision bound is diagonal between the two flowers. Figure 1.3c compares the perceived similarity of flowers using distances to the nearest bound (see text for details). Figures 1.3a and 1.3b modified from (Ashby & Townsend 1986).

In order to compare the difficulty of choosing between multimodal signals to choosing between components of multimodal signals, it is possible to contrast the decisions of a receiver choosing between stimuli 3 and 4, to choosing between 1 and 3 (or, 1 and 4). Figure 1.3c, which is Figure 1.3a and 1.3b presented together, shows the distances to the decision bounds for these two cases. Red line represents the distance to the decision bound for a bee choosing between flowers 3 and 4, while black lines represent the distances to the decision bounds for another bee choosing between 1 and 3, or between 1 and 4. Using Pythagoras' theorem ($a^2+b^2=c^2$), we can see that the distance for the decision between flowers 3 and 4 is longer than the distance between flowers 1,3 or for 1,4. Longer distances between 3 and 4 suggest that deciding between them may in fact be easier.

One of the advantages of the General Recognition Theory is that by using the distance to the decision bounds, we can predict the response times in decision-making. The response time (RT) to identify a stimulus is inversely related to the distance from the stimulus to the nearest decision bound (Thomas 1996, *RT-distance hypothesis* from Ashby & Maddox 1991). The stimuli that are close to the decision bound result in longer decision times (Ashby 2000). From the Figure 1.3c, we can predict that the bee deciding between flowers 3 and 4 will make quicker decisions. As I have argued earlier in the chapter, understanding which signals result in easier decisions has important implications for receivers as well as the senders. Predictions from a theoretical framework, such as that of General Recognition Theory, can be tested empirically to address the question of signal similarity and task difficulty.

1.2 Speed-accuracy trade-off in foraging decisions

1.2.1 The speed-accuracy trade-off

In the previous section, I argued that the complexity and the modality of signals influence receivers' cognitive abilities such as detection, processing and learning. All three of these processes influence how fast and accurately animals make decisions. Decision-making abilities are usually limited by information processing constraints and these constraints may lead to trade-offs. One such trade-off in decision-making involves the commonly observed tight negative correlation between the accuracy of decisions and the speed at which they are made. Because the trade-off is thought to occur due to the advantages that sampling and processing signals for longer time periods provides for the accuracy of the decisions (Rinberg et al.2006), studies of the speed-accuracy tradeoff can be used to address questions related to signal detection and processing. For example, studies on reaction times in humans are frequently used to test models of how information is processed (Abraham et al. 2004, Taylor 1976, Stenberg 1969). The time needed to make a decision is thought to reflect the amount of information the brain has to collect until reaching a critical level or a threshold (Kiani et al. 2006, Uchida et al. 2006, Laming 1968). At this threshold, decision-making is terminated because the necessary number of neurons or neural networks have been activated to reach a decision (Lo & Wang 2006). According to this framework, the decisions that require high thresholds take longer time but are more accurate as a result (Kiani et al. 2006).

Although the speed-accuracy tradeoff was initially recognized in humans (Garrett 1922), it has recently been shown in invertebrates, including bumblebees (Chittka et al. 2003), honeybees (Passino & Seeley 2006) and ants (Franks et al. 2003), as well as other vertebrates including rats (Uchida & Mainen 2003) and mice (Abraham et al. 2004, Rinberg et al. 2006). The speed-accuracy trade-off in these animals demonstrates that the context of decisions can lead to differences in whether speed or accuracy is favored. Stimuli that are perceived to be similar to each other, such as similar colors, present difficult decisions for animals. Easy decisions require lower thresholds and therefore produce accurate and fast decisions. Difficult decisions such as discriminating between two very similar stimuli, on the other hand, are slower and less accurate (Kiani et al. 2006, Palmer et al. 2006, Roitman & Shadlen 2002). Deciding between similar stimuli requires more and noisier information to be processed, so the extra time taken does not result in more accurate decisions in comparison to easy tasks. When forced to make swift decisions between similar odors, mice become less accurate (Uchida & Mainen 2003). When accuracy is favored over speed, ants reduce the speed of their decisions (Franks et al. 2003). Therefore, it is possible that the difficulty of a task can be analyzed by looking at the tradeoff produced by it.

Although humans and other animals can shift between fast and accurate decisions when one is more advantageous than the other, the difficulty of a particular task can only be observed when animals are free to choose when and how to respond. When given the freedom to sample for long periods, humans take longer to solve difficult decision tasks (Pachella 1974). This behavior may also be widespread in other animals. For example,

bees making the slowest decisions discriminate between similar colors at higher accuracies than the faster bees (Dyer & Chittka 2004).

The modality of the cues may have important implications for the speed and accuracy of decision-making. When humans are asked to discriminate between targets that differ in one dimension (such as shape) instead of several dimensions in the same modality (such as shape and size in visual modality), the reaction times are quicker. When the targets differ in two dimensions, humans have to focus on specific features of the targets and analyze each dimension separately. As a result, the reaction times increase (reviewed in Spaethe et al. 2006). As argued earlier, multimodal signals may be processed differently than unimodal signals. It is possible that the reaction times may not be adversely affected when the dimensions that differ are in different modalities (such as visual and olfactory), instead of the same modality (visual). Since receivers' cognitive abilities may influence signal evolution, it is essential to understand which signals lead to increased cognitive constraints.

1.2.2   Foraging in pollinators: a context well suited for decision-making

In order to fully understand behavior, we need to use both mechanistic and functional approaches in our analysis. Foraging is one context in which the synergy between these two approaches has been well recognized (Shettleworth 1998). Animals do not always act optimally, as modelled by optimal foraging models (Heindrich 1983, Varju & Nunez 1991, Schmid-Hempel 1993, Russell et al. 2003, also Sih & Christensen 2001), and cognitive constraints may be important underlying mechanisms for the non-

optimal behaviors (McNamara et al. 2006, Shettleworth 2001, Spaethe et al. 2001, Grunbaum 1998). For example, search times included in the optimal foraging models are traditionally thought to depend on external factors such as distance between patches, or the density of the patches. However, the time needed to detect a food source can also significantly influence search times. A recent study by Spaethe et al. (2001) showed that searching speed of bees depends largely on visual information processing. Similarly, limited attention can influence search rates, especially if the food items are cryptic or similar to each other (Dukas 2002).

Studies of pollinators have been especially useful in understanding optimal foraging. Pollinators usually visit many flowers in a short amount of time in order to collect the nectar they need (Harder 1983), and since flowers are scattered in time and space, having efficient cognitive abilities to detect, process, and learn floral information is essential (Chittka & Menzel 1992). Because of this vital interaction between flowers and pollinators, floral signals and pollinators' cognitive abilities are expected to coevolve (Menzel and Backhaus 1991). Pollination biology presents one of the best-studied systems in ecology and behavior; behavioral, neuroethological and physiological studies address learning and decision-making in pollinators, while ecological and evolutionary studies address the diversity and evolution of floral signals.

Sometimes pollinators show flower constancy by visiting only specific flowers while ignoring other flowers that are also rewarding (Waser 1986). Constancy has advantages for plants since it increases the likelihood of pollen transfer between individuals of the same species rather than pollen movement to heterospecifics (Waser

1983). Several explanations exist for flower constancy. Although none of these explanations is sufficient to explain constancy by itself, the majority involve some type of cognitive limitation, including constraints on memory (Waser 1986, Chittka et al. 1999), or limited attention and search images (Goulson 2000, Wilson & Stine 1996, Waser 1986, Levin 1978).

One recent explanation for flower constancy involves the trait variability hypothesis, which argues that pollinators are limited in their ability to process information about multiple traits (Gegear & Laverty 2005, 2001). According to this hypothesis, pollinators become more constant when flowers differ in multiple traits (or dimensions), such as color and morphology (Bateman 1951, Waser 1983), or size and odor (Gegear 2005). Similarly, other observations of bee foraging suggest bees show higher levels of constancy when flowers are more distinct (reviewed in Chittka et al. 1999) even in one modality. One such case is when flowers have distinct colors (Dyer & Chittka 2004a, Chittka et al. 2001), which presents an easy discrimination task for bees (Giurfa 2004, Lehrer 1999). Bees also generalize and switch between species with similar color (Dyer & Chittka 2004a, Gumbert & Kunze 2001, Gumbert 2000, Chittka et al 1997). Together, these observations suggest that constancy increases when flowers are more different, even if the difference is along one dimension such as color, and that similarities in flowers lead to generalization.

If flowers with distinct signals lead to higher levels of flower constancy, then we can expect divergent selection on flower signals to promote constancy. Phylogenetic studies show that bee visual systems evolved before the evolution of angiosperms

(Chittka & Menzel 1992, Chittka 1996, Gumbert et al. 1999, Dyer & Chittka 2004a, Dyer 2004), so the flower colors are thought to be evolutionarily suited to the visual system of pollinators, especially bees. Bees generalize to similar colors, and the flowers are selected to produce divergent colors that can aid in species identification (Dyer 2004).

However, the pressures from the pollinator visual system may not be the only factors driving floral evolution. Floral colors may also be influenced by evolutionary constraints such as pleiotropy (Chittka et al. 2001). Pleiotropy is the control of one gene over many traits, and can lead to indirect selection on one of the traits. For example, Armbruster et al. (1997) showed that pigments that give flowers their colors are also found in leaves and stems. Expression of these pigments in parts other than the flowers may be essential for survival of plants (Chittka et al. 2001). A complete understanding of flower-pollinator interactions should include such alternatives.

1.2.3   Bee foraging and the speed-accuracy trade-off

Despite many studies on pollinators, the role of multimodal signals in foraging behavior of pollinators remain largely unexplained (but see Kunze & Gumbert 2001). In particular, the integration of signals from different modalities is important for adaptive foraging behavior (Chittka & Raine 2006, Raguso & Willis 2002). The speed-accuracy trade-off is important as a mechanism that determines behavioral outcomes, but it can also have important consequences for fitness of individuals or even colonies, such as in the case of social insects. In studies of foraging decisions and learning abilities of

bumblebees, the traditional approach has been to look at accuracy, defined by the percentage of correct visits to the rewarding flowers, as a measure of fitness. However, a recent study by Burns (2005) suggests that speed of decisions may also be an important factor in determining bees' nectar collection rates.

The behavior of few animals is as thoroughly studied as that of bees. Their experiences can be controlled under lab conditions and they can be trained to discriminate between artificial or natural flowers with different cues (olfactory cues: Laloi et al. 1999, Laloi & Delegue 2004, Gegear 2005; visual cues: Heinrich et al. 1977, Odell et al. 1999, Gegear 2005). Learning also influences their flower choice in nature; bees are known to shift their preferences according to changing levels of rewards (Kawaguchi 2006, Thomson 1981, Heinrich 1979). They also show the speed-accuracy trade-off while foraging under lab conditions (Dyer & Chittka 2004b, Chittka et al. 2003). Although bumblebee learning has been studied well, the relative significance of floral cues from different modalities in flower selection is still not clear (Andersson & Dobson 2003).

Bee sensory systems and learning abilities have been addressed in several behavioral and electrophysiological studies. Honeybees and bumblebees have trichromatic color vision. The sensitivity peaks for *Bombus terrestris* are at 328 nm (ultraviolet), 428 nm (blue), and 536 nm (green) (Peitsch et al. 1992), while the sensitivity peaks for *B. impatiens* are at 352 nm (ultraviolet), and 450 nm (blue) (Briscoe and Chittka 2001). These sensitivity peaks and their interaction at neural levels determine which colors bees can detect (Giurfa and Lehrer 2001), which have important

implications on not only which flowers bees choose to forage on, but also on the diversity of flowers pollinated by bees.

Although less ephemeral and less informative than visual cues (Raguso 2001, Bradbury & Vehrencamp 1998), olfactory cues also aid in flower approach and associative learning (Raguso 2001, Metcalf & Kogan 1987, von Frisch 1954). Floral olfactory cues are usually composed of flowery scents including terpenoid and benzenoid compounds, but can also include aliphatic hydrocarbons that mimic insect pheromones (Proctor et al. 1996). The ability of bees to discriminate smells depends on structural similarity of the olfactory cues (Laska et al. 1999). Sensory neurons in the antennae allow bees to pick up the odor plumes, and the olfactory information is processed in the antennal lobe before being sent to mushroom bodies, which are the sensory integration places in insect brain (summarized in Raguso 2001). In addition, olfactory cues left by bees can attract or deter conspecifics to specific flowers (Cameron 1981, Stout 1998).

Signaling in more than one modality could be advantageous for plants if it leads to better learning and decision-making abilities of pollinators. According to trait variability hypothesis, flowers differing in two traits (such as multimodal flowers that differ in olfactory and visual traits) might be more difficult to learn/process in comparison to flowers that differ only in one of these components. This prediction would mean that bees would take longer to reach accurate decisions when foraging on multimodal flowers. However, alternatively, if the multimodal signals provide detection, discrimination or processing benefits, bees will make accurate decisions faster when foraging on multimodal signals. Although we have a good understanding of the difficulty

of detecting different visual and olfactory signals for bees, we do not yet fully understand if and how multimodal signals differ from each other or from signals in one modality. Studying the speed-accuracy trade-off produced by multimodal signals may provide insight into not only the detection and processing of signals, but also into our understanding of how cognitive processes influence flower constancy.

In the next chapter, I present a study in which I investigated the role of complexity and modality of signals on decision-making. I looked at the speed-accuracy trade-off in foraging decisions of bumblebees (*Bombus impatiens*) that were trained to forage on artificial flowers differing either in one modality (shape or olfactory) or in two modalities (shape and olfactory).

CHAPTER 2

COMPLEX SIGNALS AND THE SPEED-ACCURACY TRADE-OFF IN

FORAGING DECISIONS

2.1   INTRODUCTION

The ability to use information is essential for the survival of organisms.

Extracting biologically relevant information from signals in the environment requires

efficient signal detection and processing abilities. Studies on decision-making have

benefited from predictions of the Signal Detection Theory (Green& Swets 1966, Wiley

1994), which looks at the uncertainty in signal detection caused by the perceived

similarity of signals (Wiley 1994, Shettleworth 1998). The theory predicts that one of the

ways to increase the accuracy of decisions is by increasing the perceived difference

between the signals.

However, Signal Detection Theory is somewhat limiting because it looks only at a

single signal component. Most signals in nature are complex signals, which are signals

that are composed of several components (Hebets & Papaj 2005). Although adding

another signal component may be costly for a sender due to increased energetic

constraints (reviewed in Partan & Marler 2005), or may be disadvantageous in situations

where the second component attracts an unintended receiver or a predator (Roberts et al.

2006), increased detection by the receivers may be one of the reasons why most signals in

nature are complex. Complex signals may aid in detection, discrimination and processing

abilities of receivers (Rowe 1999). Multimodal signals, which are signals composed of

components from two or more sensory modalities, may provide further detection and processing benefits in comparison to unimodal complex signals (reviewed in Partan & Marler 2005, Rowe 1999).

In order to understand how two or more components may be perceived by the animals, the predictions from General Recognition Theory, which is an extension of Signal Detection Theory for signals with multiple components (Ashby & Townsend 1986), may be useful. Under the assumption of equal variances in signal perception, the theory can be used to compare the difficulty of deciding between two signals that differ in two components to the difficulty of deciding between its two components. The signals that differ in two components may be perceived as more different from each other, and present an easy discrimination task.

It is possible to collect data on the perceived similarity of signals with one component by incorporating results from neurobiology, physiology of animal sensory systems, and the behavioral studies. But how can we study the similarity of complex, especially multimodal signals? The speed-accuracy trade-off presents a unique opportunity for empirically addressing this question. The commonly observed tight negative correlation between the accuracy of decisions and their speed, also known as the speed-accuracy tradeoff, was initially recognized in humans (Garrett 1922) but has recently been shown in invertebrates including bumblebees (Chittka et al. 2003), honeybees (Passino & Seeley 2006) and ants (Franks et al. 2003), as well as in other vertebrates, including rats (Uchida & Mainen 2003) and mice (Abraham et al. 2004, Rinberg et al. 2006). Difficult decisions such as discriminating between two very similar

stimuli are slower and less accurate (Kiani et al. 2006, Palmer et al. 2006, Roitman & Shadlen 2002). So by using the speed-accuracy trade-off, we can address whether adding another component to a signal improves signal detection and/or processing. If it does, we would observe improvement in the accuracy and the speed of animals' decisions.

In this study, I investigate the role of complexity and modality of signals on decision-making processes in foraging bumblebees (*Bombus impatiens*). If the speed-accuracy trade-offs are only influenced by signal detection and processing, we would expect that bees foraging on flowers that differ in two cues (shape and olfactory) will make faster and/or more accurate decisions in comparison to bees that forage on flowers that differ either in shape or in olfactory.

2.2   METHODS

2.2.1   Study system and setup

Bumblebee (*Bombus impatiens*) colonies were obtained from Koppert Biological Systems, Michigan, USA. The colonies were kept in their original nest boxes, and were connected to a Plexiglas buffer box (10 x 11.5 x 2.5 cm) that had a manually controlled gate that allowed bees to be released individually to the plywood experimental arena (120 x 75 x 36 cm) with Plexiglas top. Except during the training and testing sessions, the bees foraged in the arena on "Beehappy" solution obtained from Koppert, and frozen pollen was put daily into the nest box. I used three colonies for the experiments.

The bees were presented with four rewarding and four non-rewarding artificial flowers arranged in a vertical setup. The flowers were constructed by drilling 200 µl

holes into clear acrylic rods (US Plastic, extruded square acrylic tubing) that were embedded into a poster board (73 x 36 cm) placed vertically in the experimental arena, 80 cm away from the entrance. These rods were then placed into the center of the shapes (6 cm in diameter) cut from standardized colored HKS-N papers (Hostmann-Steinberg K+E Druckfarben, H. Schmincke& Co., Erkrath, Germany). For the experiments in which visual stimuli were manipulated, I used yellow (3N) flowers that were cut in circles or crosses. The poster board was covered with green (54N). All of the flowers were covered with transparent acetate sheets and cleaned regularly with 30% alcohol to eliminate any olfactory cues left by foraging bees. Figure 2.1 shows the reflectance spectra of the flowers and the background used in the study, in addition to the reflectance spectra of the flowers through the acetate sheets.

For the olfactory experiments, I used 2 μl of peppermint and clove essential oils (obtained from Aura Cacia, Frontier Natural Products, Norway) diluted in 1:100 pentane. These odors have been used in previous experiments with *Bombus* (Gegear & Laverty 2005 - clove and peppermint oils, Kunze and Gumbert 2001 - clove), and can easily be discriminated from each other. The diluted oils were placed in Eppendorf centrifuge tubes (1.5 ml) embedded into the poster board behind each flower. I poked small holes into the flowers and the overhead sheets to allow odors to be transmitted to the bees. Since chemical cues from previous foragers affect flower choices of bumblebees (Dornhaus & Chittka 1999), this design allowed me to reduce the possibility of odor transfers that might occur between the forager bees and the other bees in the hive. It is possible, however, that some of the olfactory cues in the arena were transferred to the

hive through the cuticle of foragers. Bumblebees may be detecting olfactory cues from longer distances than visual cues (Kunze & Gumbert 2001). Placing the odors behind the flowers was essential for reducing the likelihood of this possibility as well.
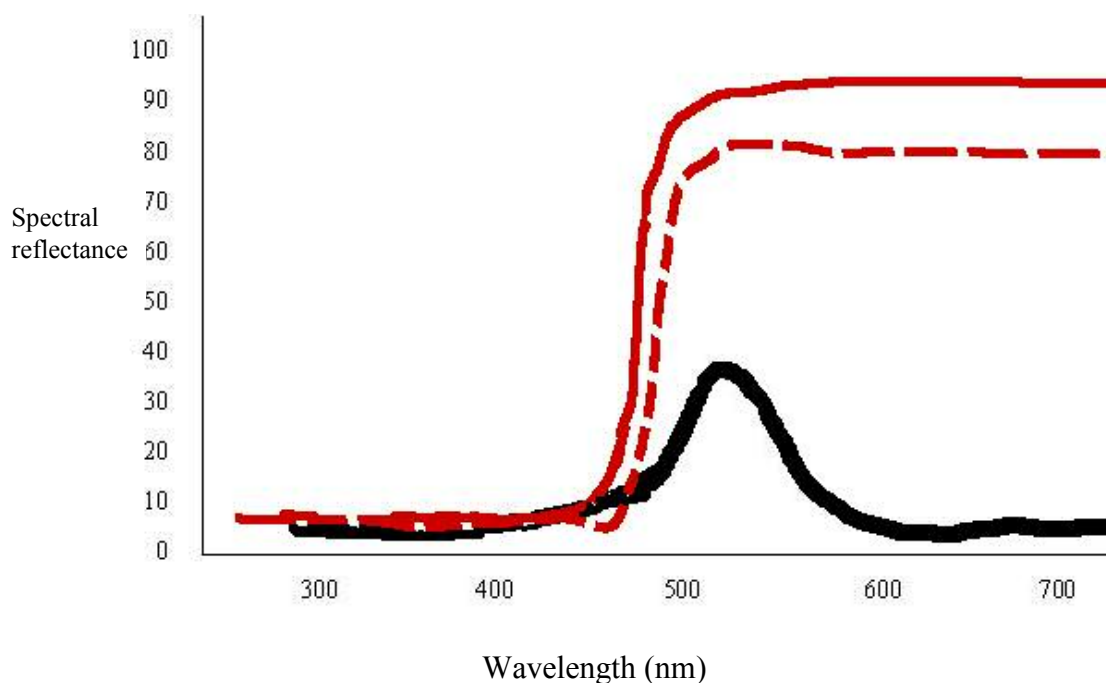
Figure 2.1.   Percent reflectance spectra of the flowers and the background. Black line is the reflectance of the green background HKS-54N. The red line is the reflectance for yellow flowers HKS-3N, while the red dashed line is for yellow flowers as seen through acetate.

Before the training trials, bees were allowed to visit the clear acrylic rods embedded in the poster board *ad libitum*. In this pre-training phase, the colored paper flowers were removed from the rods to eliminate any associations of food and color.

Each rod had 30% sucrose solution as reward, and the rods were refilled every hour. Bees that visited the rods were individually marked with enamel paints on their thorax.

<u>2.2.2   Training</u>

I trained bees on three different discrimination learning tasks. A minimum of 10 bees were trained in each experiment, and no bee was used in more than one experiment. The groups were trained on 1) shape differences (crosses as rewarding, circles as non-rewarding), 2) olfactory differences (peppermint as rewarding, clove as non-rewarding), 3) olfactory + shape differences (peppermint & cross as rewarding, clove & circles non-rewarding). I switched the rewarding and non-rewarding stimuli after five bees were trained in each group. The rewarding flowers were filled with 30% sucrose solution, while the non-rewarding flowers were filled with water.

During training trials, marked bees were released individually to the experimental arena. A trial began when a bee entered the experimental arena, and ended when the bee returned to the nest. If a bee did not visit flowers within10 minutes, the trial was terminated and she was returned to the nest until the next trial. During training trials, individuals were allowed to visit flowers freely while I recorded the number of visits to rewarding and non-rewarding flowers. In addition, I kept records of the handling time, measured as the time between landing on a flower and leaving the flower.

Between the trials, I re-arranged the flowers to prevent bees from learning the location of rewarding flowers. Rearranging the flowers was done so that the same individual did not receive more than two rewarding flowers in the same location

consecutive trials. I also replaced the rods and cleaned the flower overhead sheets to prevent bees from responding to scent marks left by the previous bees. In order to proceed to the testing phase, bees had to complete a minimum of 3 trials and achieve 80% correct choices on their last 10 visits.

2.2.3   Testing

Once bees learned to discriminate between the rewarding and the non-rewarding flowers, the testing phase began. In most cases, testing immediately followed the last training trial. In the few cases in which the bees did not visit the arena after the last training trial on the same day, bees were tested the following day. Some studies have shown that bumblebee foraging skills decline overnight (Keasar et al. 1996, but see Chittka 1998), so I ran another training trial immediately before the testing trial in those cases. During the testing trials, all flowers were non-rewarding. As in training trials, bees were allowed to visit flowers freely as I videotaped their visits using a Sony DCR-HC48 Mini DV Camcorder stationed next to the nest entrance, directly across the testing array. Bees were tested twice. Between the trials, I changed the location of all flowers and refilled the flowers with water.

I also conducted morphometric measurements with digital calibers. Previous studies have documented that larger bees learn faster (Worden et al. 2005). To see whether bees' learning and decision-making abilities were influenced by their body size, I measured head width (eye to eye), thorax width (intertegula span), and forewing length (average of left and right wings) of the bees that reached the 80% criterion.

2.2.4   Video analysis

I analyzed the videos of testing trials frame-by-frame. In my recordings, each second corresponded to 30 frames. From the frame-by-frame analysis, I quantified the following variables: 1) time of landing, defined by the first contact with the flower, 2) time of takeoff, defined by the last leg of bee leaving the flower, 3) whether or not the bee was "searching", (facing the testing array) between visits to subsequent flowers, 4) whether the visits were to previously rewarded (correct) flowers or previously non-rewarded (incorrect) flowers. The decision time for each visit was determined as the time difference between leaving a flower and landing on another flower. Decision time was first calculated in number of frames, and then converted into seconds. A few times, the bees stopped searching for flowers but remained in the testing area during the trials. Most of these instances corresponded to walking around the arena or staying at rest. In those cases, resuming flight was assumed to be correlated with resumed search activity, and decision times were calculated starting from flight time instead of leaving the previous flower.

2.2.5   Data analysis

The number of visits required for bees to learn the discrimination task in each of the three groups was analyzed with ANOVA using R statistical analysis software (CRAN). In addition to the treatments, the learning analysis included shape of the flowers and the colony the bees belong to. For each bee, the average choice time and the

percentage of correct choices were calculated and entered as data points in regressions analyzed with ANCOVA for differences in slopes and y-intercepts. In both cases, the models started with all the relevant variables, but the interactions and the variables without significant effects were removed from further analysis, starting with the least significant interaction. The variables with significant effects were entered into a post-hoc Tukey analysis completed with JMP Statistical Software (SAS Institute Inc.)

During the testing, all of the flowers were non-rewarding. The bees' behavior may change after they visit a non-rewarding flower that has previously been rewarding in other trials. I used logistic regression to test whether the decision time, the treatment groups, the colonies bees belong to, and flower shape influenced the accuracy of the first visits. Analysis of body size correlations with learning, speed and accuracy were carried out separately from the rest of the analysis.

To test whether treatment groups influence bee foraging efficiency, I looked at the rate at which bees found the target flowers in each group. The target finding rate was calculated by dividing the number of correct visits during the testing trials by the total amount of time bees spent searching for flowers during the test trials.

2.3 RESULTS

2.3.1 Learning

Across the three treatment groups consisting of shape, olfactory, and multimodal, I trained 44 bees, only 33 (n=10, 12, 11 for shape, olfactory and multimodal, respectively) of which reached the learning criterion (80% for the last ten visits). Figure 2.2 shows a typical learning curve observed in each of these groups. The mean number of visits required to reach the learning criterion did not depend on whether the crosses or the circles were rewarding (ANOVA, $F_{1,29} = 0.0391$, $R^2 = 0.001$, $p = 0.85$, Figure 2.3a), or whether olfactory or peppermint flowers were associated with rewarding flowers (ANOVA $F_{1,29} = 4.196$, $R^2 = 0.1809$, $p = 0.0546$, Figure 2.3b), so the shape and the odor variables were removed from the model. Colonies differed in their learning ability ($F_{2,28} = 7.264$, $R^2 = 0.3416$, $p = 0.003$), but the group bees were trained was independent of this learning ability ($p = 0.76$ for the treatment and colony interaction), so the interaction variable of treatment and colony was removed from further analysis.

The number of visits required to reach the learning criterion was influenced by the treatment group (shape, olfactory, multimodal) in which bees were trained ($F_{1,29} = 8.60$, $R^2 = 0.228$, $p = 0.007$; Figure 2.4). The difference between visual and multimodal groups (mean$\pm$ s.d.: 28.9$\pm$ 11 and 16$\pm$ 5.4 for visual and multimodal) was significant as revealed by the post-hoc Tukey test. However, the post-hoc Tukey test showed no statistically significant difference between the number of visits required to reach criterion in olfactory treatment (mean$\pm$s.d.: 20$\pm$ 10.8) and multimodal treatment, or between visual and olfactory treatments.
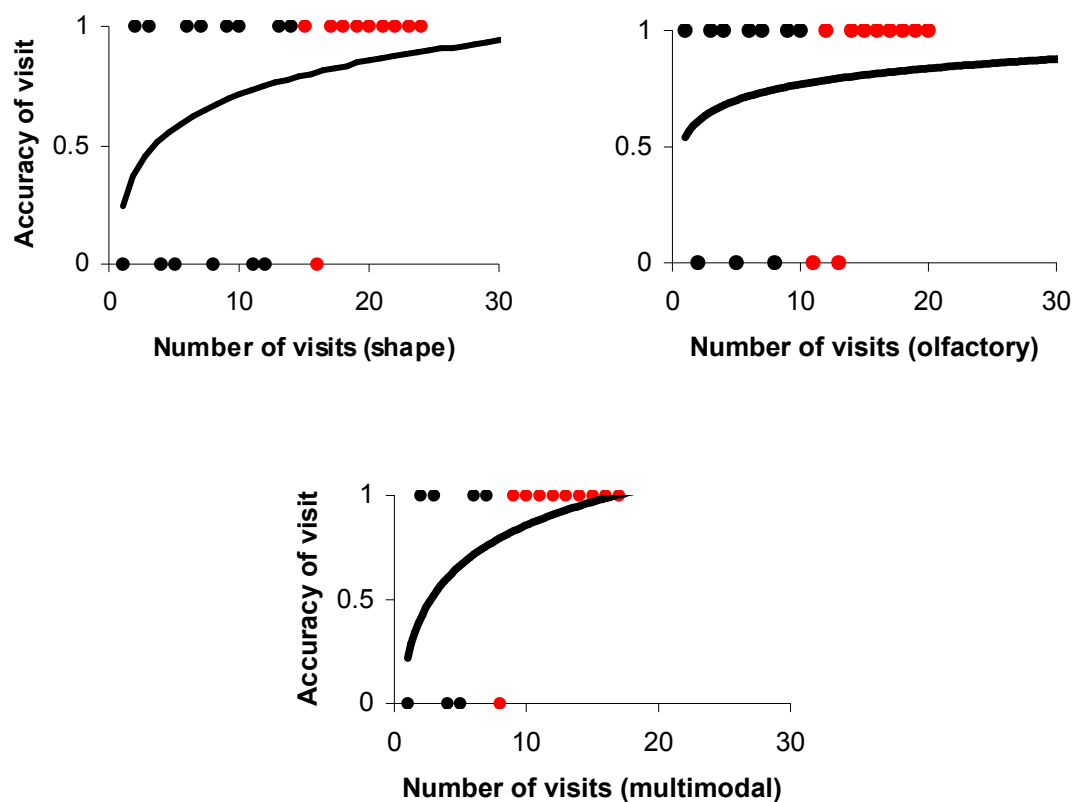
Figure 2.2.   Example of learning curves from bees in three treatment groups. First graph shows visits of a bee trained in shape differences, the second graph shows visits of a bee trained in olfactory differences, while the third graph is for multimodal differences (shape+olfactory). Each data point in the graphs represents a visit bee makes to a flower during the training trials. On the y-axis, 0 represents incorrect (not rewarding) flowers, and 1 represents the correct (rewarding) flowers. Each graph shows data that are pooled across several trials. In order to proceed to the testing phase, the bees needed to obtain 80% accuracy in their last 10 visits (in red).
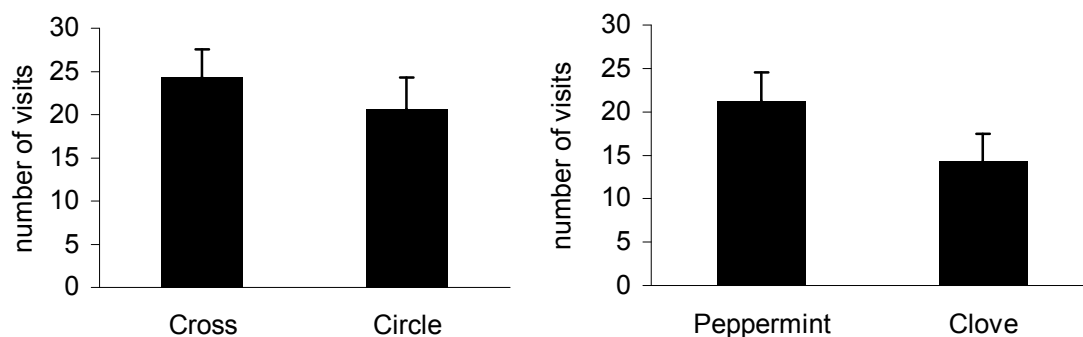
Figure 2.3.   Mean number of visits required to learn different shapes and odors for the unimodal flowers. There was no statistical difference in how fast different shapes are learned (ANOVA, $F_{1,29}$ = 0.0391, R^2 = 0.001, p = 0.845), or how fast different olfactory cues were learned (ANOVA $F_{1,29}$= 4.196, R^2= 0.1809, p= 0.0546).
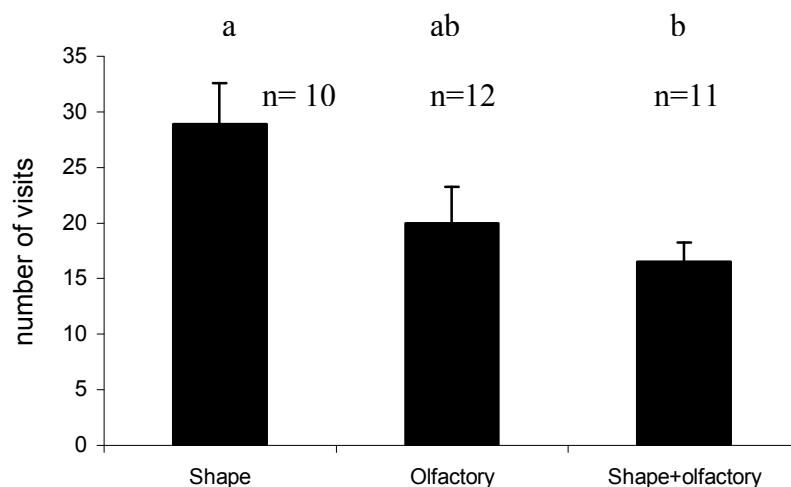
Figure 2.4.   Mean number of visits ($\pm$ s.e.) required to learn flowers in each treatment group. The bees were expected to reach 80% criterion in their last ten visits. The treatment group influenced how many visits were needed to reach the criterion ($F_{1,29}$ = 8.604, R^2= 0.228, p = 0.0065). However the only statistically significant differences were between means of shape and shape+olfactory, as revealed by the post-hoc Tukey test. Values with the same letter are not statistically different from each other.

Body size of test bees were analyzed separately from the rest of the analysis. Bees of different colonies varied in head width ($F_{1,22}$ = 5.585, R^2= 0.202, p= 0.0274), thorax size ($F_{1,22}$ = 6.798, R^2 = 0.236, p= 0.016) and wing length ($F_{1,22}$ = 11.54, R^2= 0.344, p= 0.0026, Figure 2.5). However, none of the differences in body size influenced learning ($F_{7,16}$ =0.8199, R^2=0.264, p=0.584 overall; head width: p=0.288; thorax size: p=0.526; wing length: p=0.403).
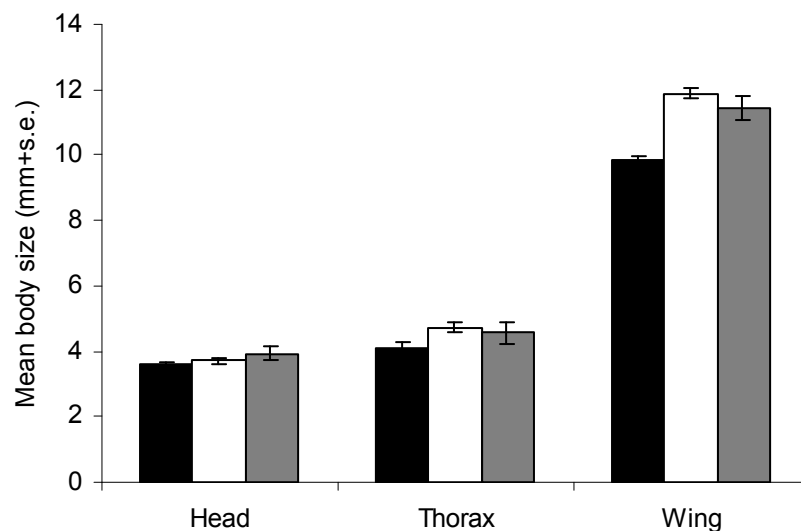

Figure 2.5.   Intercolony differences in body size. Body size (mm, mean±s.e.) differed across three colonies. Black bars represent the first hive, empty bars represent the second hive, and the grey bars represent the third hive. Head size ($F_{1,22}$ = 5.585, p= 0.0274), thorax size ($F_{1,22}$ = 6.798, p= 0.016), and wing length ($F_{1,22}$ = 11.54, p= 0.0026) all differ across colonies.

2.3.2   Testing and the speed-accuracy trade-off

I tested 31 of the 33 bees that reached the learning criterion (n= 8,12,11 for shape, olfactory and multimodal, respectively). Overall, during all of the testing trials, the mean percentage of correct choices was 67.26 % $\pm$ 11.58 (mean % $\pm$ s.d.) across all groups, and the flower type bees were trained on influenced their accuracy ($F_{1,29}$=5.95, R^2= 0.17, p=0.021). Bees trained on multimodal flowers had the highest accuracy (73.32 % $\pm$ 9. 53), followed by bees trained on shape cues (64.5 % $\pm$ 14.94) and olfactory cues (63.56 % $\pm$ 9.17). Post-hoc Tukey test revealed that there were significant differences between accuracy of bees trained in shape versus multimodal groups, as well as between accuracy of bees trained in olfactory versus multimodal groups. However, there was no significant difference between accuracy of bees trained on shape versus olfactory stimuli. The results of an ANOVA with contrasts also showed that the difference in accuracy between unimodal (shape and olfactory) flowers and multimodal flowers is significant ($F_{1,28}$= 4.97, p = 0.033; Figure 2.6).

The speed of decisions was also influenced by the treatment groups ($F_{2,30}$= 5.635, R^2= 0.2866, p= 0.008, Figure 2.7). Results of post-hoc Tukey showed statistical difference in speed of decisions between bees trained on shape differences and on multimodal flowers. Bees trained on shape made slower decisions (mean$\pm$s.d.:3.49 $\pm$ 0.79 sec) than bees trained on multimodal flowers (mean$\pm$s.d.:2.59 $\pm$ 0.55 sec). Post-hoc Tukey showed no statistically significant difference between decision times for olfactory (mean$\pm$s.d.: 2.87 $\pm$ 0.45 sec) and multimodal flowers, or between visual and olfactory flowers.
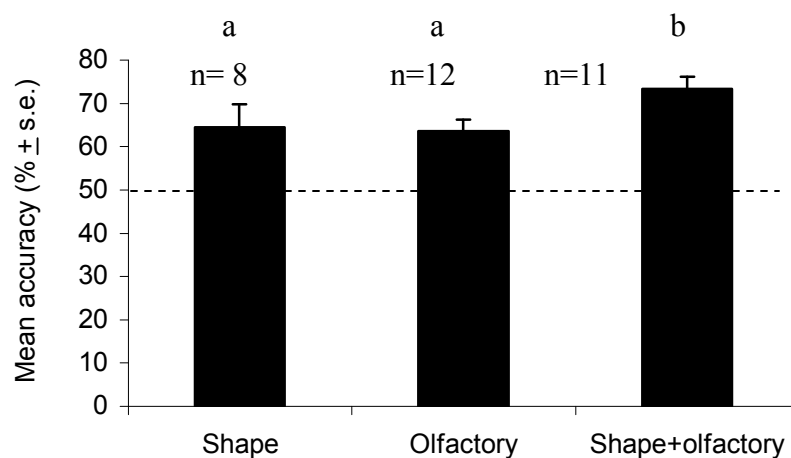
Figure 2.6. Mean accuracy (% ± s.e.) of bees in each group. The dashed line at 50% indicates random choice. Accuracy was influenced by the treatment ($F_{1,29}$ =5.95, R^2= 0.17, p=0.021). A post-hoc Tukey test revealed differences between unimodal (shape, olfactory) and multimodal (shape+olfactory) accuracy. This difference was also supported by the ANOVA with contrast ($F_{1,28}$ = 4.97, p = 0.033). Values with the same letter are not statistically different from each other.
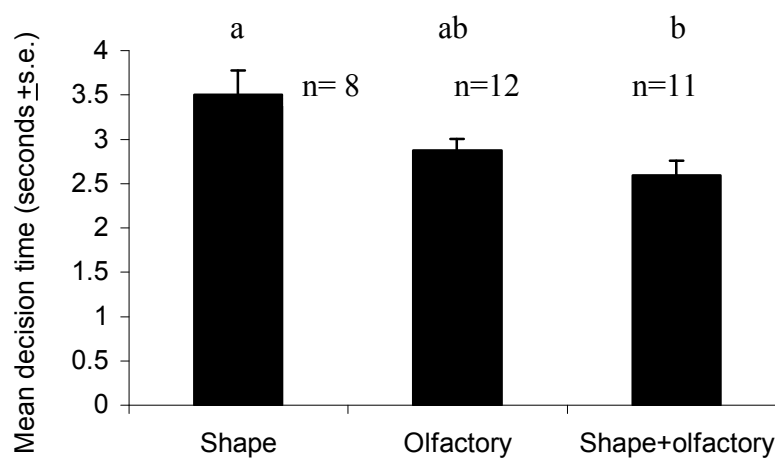
Figure 2.7. Mean decision times of individuals trained in different treatments (second ± s.e.). Decision times were influenced by the treatment group ($F_{2,30}$= 5.635, R^2= 0.2866, p= 0.008). A post-hoc Tukey test revealed difference in speed between shape and multimodal flowers. Values with the same letter are not statistically different from each other.

In the olfactory and the multimodal groups, the accuracy of choices was significantly positively correlated with the time bees took to reach those decisions (Pearson's correlation r = 0.6, t=2.374, p = 0.039 for olfactory; r = 0.664, t=2.663, p = 0.026 for multimodal). In both groups, the bees taking longer to decide had higher overall percentage accuracy. However, the correlation between accuracy and time in the shape group was not significant (r = 0.475, t=1.323, p = 0.234).

The correlation between speed and accuracy in the olfactory and in multimodal flower groups supports the hypothesis that there is a trade-off between speed and accuracy in these two groups, and this trade-off may also be present in the shape group as well. Figure 2.8 shows the speed-accuracy tradeoffs observed in the three groups. An ANCOVA of the three groups shows that the y-intercepts differ from each other ($F_{3,27}$ = 5.987, R^2= 0.399, p=0.002). The multimodal treatment yielded the highest y-intercept (43.549 % of correct choices), followed by olfactory (33.008 % of correct choices), and shape (28.702% of correct choices). The homogeneity of regressions assumption of ANCOVA was not violated and there was no overall difference in the slopes of the regressions (treatment x speed: 0.9629). A post-hoc Tukey test showed that there was no significant difference between the shape and olfactory groups' speed-accuracy trade-offs. However, the test also showed that the speed-accuracy trade-off in the multimodal treatment was different than other two groups. ANCOVA with contrasts comparing multimodal to unimodal (shape, olfactory) flowers supported the results of post-hoc Tukey test ($F_{1,28}$ = 8.65, p =0.006). Since Figure 2.8 shows that the regression line for olfactory lies between shape and multimodal groups, I separately analyzed the differences

in the speed-accuracy trade-off between the olfactory group and the multimodal group. I found that the y-intercepts of the trade-offs from these groups were statistically different ($F_{3,19}$= 7.371, R^2= 0.464, p=0.0018).
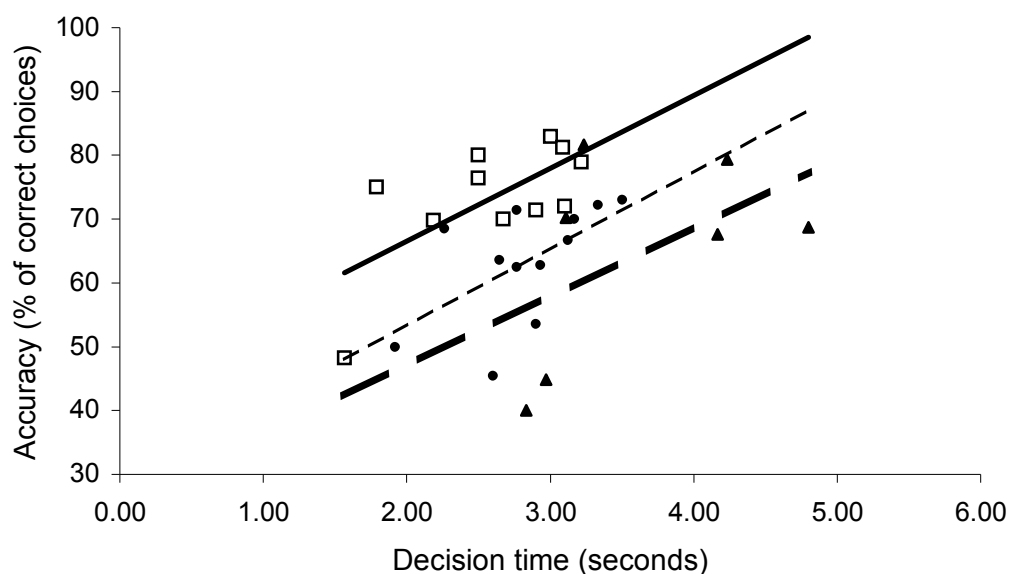
Figure 2.8.   The speed-accuracy trade-off of each group. Triangles and the dashed thick line represent the shape treatment. Bullets and the dashed thin line represent the olfactory treatment. Empty squares and the continuous line represent the multimodal treatment. The lines were parallel, but the y-intercepts from unimodal flowers (shape, olfactory) were different from that of multimodal flowers ($F_{3,27}$= 5.987, R^2= 0.399, p=0.002).

In addition to analyzing the overall performance by looking at the average speed and accuracy of each bee, I analyzed data for their first visits in each trial since the behavior of the bees may change after visiting non-rewarding flowers that were initially rewarding. The variables that were initially in the model included the decision time for

the first decision, accuracy of the first decision (correct or incorrect), average accuracy

reached in that trial, and average decision time for that trial. The time taken for the first

decisions and the accuracy of the first decisions were entered into a multiple logistic

regression, with the first visits as the response variable. The time taken for the first

decisions in each trial did not significantly influence the first visit accuracy (multiple

logistic regression, $z = 1.246$, $p = 0.212$). However, the accuracy of the first visits was

strongly influenced by the treatment group to which bees belonged ($z = 2.882$, $p = 0.003$).

Figure 2.9 shows the mean ($\pm$ s.e.) of the accuracy of the first visits. The data are pooled

across invididuals from the same treatment groups. The accuracy of the first visit had no

significant effect on the overall accuracy achieved in that trial ($F_{1,55} = 0.6343$, $R^2 = 0.011$,

$p = 0.4292$) or the average time for decisions in that trial ($F_{1,55} = 0.9165$, $R^2 = 0.016$, $p =$

0.3426).
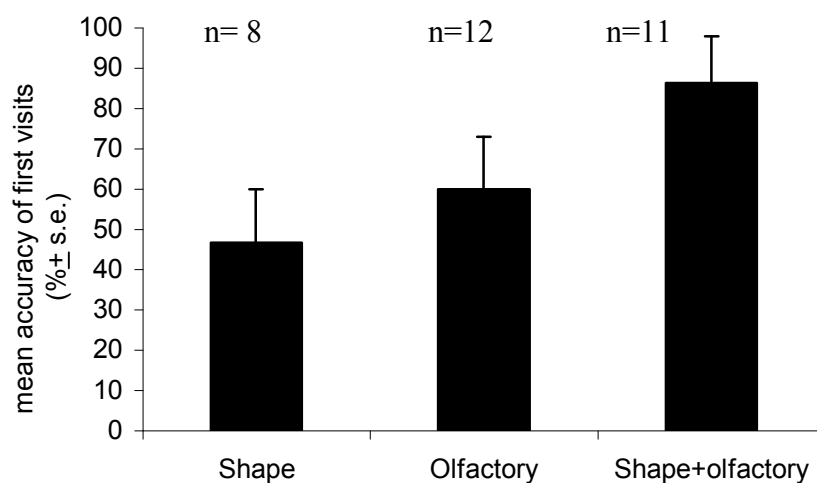
Figure 2.9.   The accuracy of first visits is influenced by the group bees were trained in
($z = 2.882$, $p = 0.003$). The graph shows mean accuracy (% $\pm$s.e.) for the
first visits, with data pooled across individuals from the same treatment.

Body size had no effect on the accuracy of decisions ($F_{3,19}$=0.047, R^2= 0.069, p=0.702 overall, thorax: $F_{1,19}$= 0.001, p=0.96; wing: $F_{1,19}$= 0.006, p=0.93; head: $F_{1,19}$= 1.421, p= 0.24). Speed was influenced by head size (Figure 2.10), but not by thorax size or wing length ($F_{3,19}$= 1.766, R^2=0.069, p=0.877 overall, thorax: $F_{1,19}$= 0.04, p= 0.82; wing: $F_{1,19}$= 0. 03, p= 0.86; head: $F_{1,19}$= 5.21, p= 0.03- after removing nonsignificant variables).
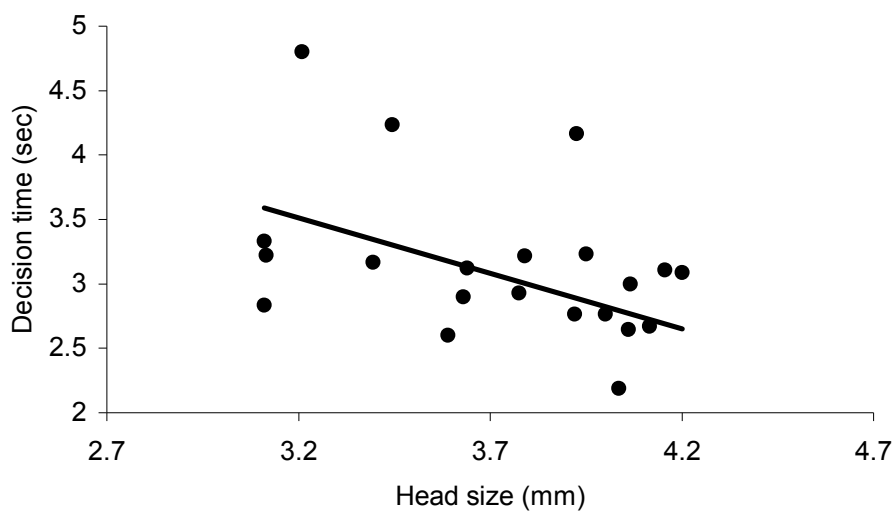
Figure 2.10. Head width and decision time. Bees with larger heads, measured from eye to eye, made quicker decisions ($F_{3,19}$= 1.766, R^2=0.069, p=0.877 overall, head: $F_{1,19}$= 5.21, p= 0.03). Other body measurements did not have a significant effect on the number of visits required to reach learning criterion, the speed or the accuracy of decisions.

2.3.3   Target finding rate

Bees' target finding rates (number of correct visits/total decision time) were

influenced by the trade-off between speed and accuracy (F $_{2,28}$= 72.7, R^2= 0.848,

p<0.001). In addition, the treatment groups had significant effects on the target finding

rates (ANCOVA, F $_{2,28}$ = 9.4606, R^2= 0.4212, p= 0.0008, Figure 2.11). Post-hoc Tukey

test revealed that the multimodal flowers lead to higher rates than either of the unimodal

(shape, olfactory) flowers, and that there is no statistical difference in target finding rate

between shape and olfactory groups.

Figure 2.11.   Target finding rate was influenced by the treatment group (F $_{2,28}$ = 9.4606, R^2= 0.4212, p= 0.0008). A post-hoc Tukey test showed differences between shape+olfactory and other groups, but no differences between shape and olfactory groups. Values with the same letter are not statistically different from each other.

2.4 DISCUSSION

Bees learned the flowers that differ only in shapes slowly. However, the learning criterion was reached quicker when the flowers differed in both shape and odor. During the testing trials, the speed of decisions followed a similar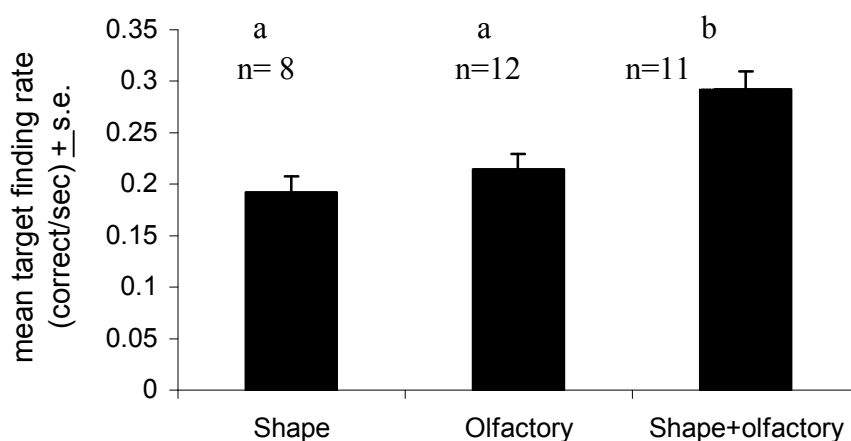 trend to learning of flowers; bees made faster decisions when the olfactory cues were added to the shapes, and the multimodal flowers resulted in more accurate decisions than either the shape or the olfactory cue-only flowers. As a result of changes in speed and accuracy, the speed-accuracy trade-off in different groups was influenced by whether the flowers were unimodal or multimodal. This trade-off between speed and accuracy also influenced the target finding rate of bees. Bees trained on multimodal flowers had higher target finding rates. I also found differences between colonies in learning speed and body size. However, any differences I observed in learning were independent of intercolony body size variation.

There are two interesting findings from this study. First, the results from learning experiments suggest that shape differences may not be very informative when the bees are learning which flowers to visit, at least under lab conditions. When odor was added to shape differences, the number of visits to reach learning criterion decreased significantly. This decrease, when taken together with the lack of significance between the number of visits required to learn olfactory and multimodal flowers, suggests that bees may be paying more attention to olfactory differences rather than shape differences when learning which flowers to visit. This result is not surprising, since earlier studies have

documented that bees learn shapes slowly (for honeybees, reviewed in Gould 1993).

However, it is possible that even shapes that were previously thought to be difficult to

discriminate for bees (i.e. convex shapes) can be learned (Lehrer & Campan 2005). Most

flower-pollinator interaction studies focus on the importance of floral symmetries and

how the pollinator preferences for specific shapes and/or symmetries drive floral

evolution (Rodriquez et al. 2004, also reviewed in Endress 2001, Neal et al. 1999).

Flowers rarely signal with one component or with one modality, and the learning results

from this study suggest that it would be valuable to consider possibility of interactions

between two modalities. For example, several studies have pointed out the effects of

multimodal signals on learning (Kunze & Gumbert 2001, Gerber 1998, Gerber & Smith

1998, also Rowe 1999). Gerber and Smith (1998) have found that presence of visual

stimuli enhances olfactory learning in honeybees, and used this result to argue against the

independence assumption in learning, which predicts that there will be no interaction

between learning of visual and olfactory stimuli. Similarly, Kunze and Gumbert (2001)

found that learning of visual cues in bumblebees is enhanced when both flowers were

scented with the same olfactory stimulus.

     The second interesting finding is that the flowers that differed in both shape and

odors resulted in more accurate decisions when compared to the flowers that differ either

in their shape or odor. However, the increase in decision accuracy did not result in a

decrease in the speed of the decisions. Speed-accuracy trade-offs are more pronounced

when a task is difficult; difficult decisions such as discriminating between two very

similar stimuli take longer times and result in less accurate decisions (Kiani et al. 2006,

Palmer et al. 2006, Roitman & Shadlen 2002). It is also known that most easy tasks do not result in a trade-off between speed and accuracy (Dyer & Chittka 2004). Within the multimodal group, there was a significant correlation between speed and accuracy of decisions. Although choosing between multimodal flowers was still challenging enough to produce a tradeoff, it was easier than choosing between visual or olfactory flowers. The higher accuracy on multimodal flowers, the y-intercept differences in the speed-accuracy trade-off between unimodal and multimodal flowers, and the significantly higher target finding rates on multimodal flowers suggest that multimodal signals are advantageous for bumblebees' decision making.

What is the mechanism responsible for the influence of the multimodal signals on the speed-accuracy trade-off? One possibility involves efficacy backup, in which components of a complex signal provide detection and/or discrimination benefits in different environments (Hebets 2005, Hebets & Papaj 2005). Signals may provide the same information to the receiver, but aid in detection amidst environmental noise (Partan & Marler 1999). This hypothesis would predict a lower probability of responding to each of the components than to the whole signal. However, efficacy backup signals are assumed to act independently without interacting with each other (Hebets 2005). A similar hypothesis, the efficacy tradeoff, states that components of a signal may overcome different problems, such as being detectable at different distances (Hebets & Papaj 2005). Although it is possible that visual and olfactory cues are detected at different distances (Giurfa et al. 1996), the relative short distance used in this experiment, and the design of the experiment in which olfactory cues were placed behind the flowers,

reduces the likelihood of this possibility. Another mechanism that may be responsible for the observed effect of multimodal signals is a possible interaction between olfactory cues and visual cues to direct the receiver's attention, reducing effects of limited attention (Hebets 2005). Although my study was not specifically designed to differentiate among the above possibilities, the influence of multimodal signals on decision-making suggests that the components of a multimodal signal may in fact be interacting with each other.

Target finding rate depended significantly on both the speed and the accuracy of decisions, and whether the bees were visiting multimodal versus unimodal flowers. Bees in the multimodal flower group found targets at a significantly higher rate than bees in unimodal flower groups. It is also important to note that because the trade-off between speed and accuracy influences the target finding rate, looking only at one of these variables in behavioral studies may be misleading. Similarly, Burns (2005) also points out the importance of decision speed in a model that predicts how speed-accuracy trade-off influences nectar collection rate.

The results of the speed-accuracy trade-off, as well as the target finding rate suggest that multimodal signals may be easier to respond to. The ease of detection and/or discrimination may be one of the reasons why many organisms use multiple components instead of becoming more distinct in just one dimension, even if it may be costly to add an additional component to its display (Partan & Marler 2005). In General Recognition Theory, the distance to the nearest decision bound can be used as a measure of task difficulty (Maddox et al. 1998); the stimuli that are farther from the bound represent easy tasks. Using this argument, I suggested in the Introduction that two multimodal signals

will be perceived as more distinct from each other than their individual components. The shapes used in this study were circles and crosses. Bees discriminate shapes based on their outer boundaries (Lehrer & Campan 2005, Srinivasan 2006), so discriminating between circles and crosses is easy for a shape discrimination task. Similarly, the clove and the peppermint scents have previously been used in several bumblebee olfaction experiments (Gegear & Laverty 2005), and may present an easy task. Still, the results from the speed-accuracy trade-off and the target finding rates point to detection and/or decision-making advantages of the multimodal flowers. These results suggest that addition of a second component, even when signals are different from each other in one dimension, may still be advantageous.

Another way to approach the question of why signalers use multicomponent signals would be to consider the environmental variation that the signalers have to overcome in order to transmit their signals. The signals not only need to stand out against other signals in the environment, but they also need to do so in a variety of environmental conditions. The efficacy trade-off hypothesis (Hebets & Papaj 2005) mentioned earlier also uses this argument. One component may be detected from a longer distance, or be displayed at different times than other components. Alternatively, two components may increase the diversity of the receivers that can respond to the signal. In this case, if some species are better at detecting one component than the other, then a second component could be beneficial. As argued in the Introduction, multimodal signals aid in learning and detection of signals across several species, and it is possible that pollinators perceive flowers as multimodal sensory units (Raguso & Willis 2002, Gegear & Laverty 2001).

However, different species of pollinators may use different cues to varying extents. For example, *Manduca sexta* uses olfactory cues for long-distance detection of flowers, but requires visual cues to feed (Raguso & Willis 2002), whereas *Vanessa indica* uses only color contrast to locate flowers (Omura & Honda 2005). Even closely related species such as honeybees and bumblebees show differences in how different modalities are used while foraging (Gegear & Laverty 2001). Knowing differences in signal use among receivers is crucial for understanding signal evolution and function.

Search times were shorter when olfactory cues were added to the shape differences. Although some contradictory data exist (Odell et al.1999), it is possible that olfactory cues are detected from a longer distance than visual cues (Kunze & Gumbert 2001). This may be the reason why multimodal flowers were detected faster than visual flowers. Although I tried to control for this effect by using a short distance between arena entrance and testing array, as well as by placing the olfactory cues behind the flowers, the possibility of detection range differences between two modalities can not be ruled out without further experiments that control how quickly the olfactory cues are detected.

The analysis of body sizes suggest that bee head size may correlate with faster decisions, possibly due to faster detection times. In previous studies, size of compound eyes have been shown to correlate strongly with body size (Spaethe & Chittka 2003, Land 1981, Synder& Menzel 1975). Also, in a study looking at eye morphology of different-sized bumblebees and their ability to detect artificial flowers, Spaethe and Chittka (2003) showed that larger bees have improved visual resolution. Similarly, larger bees may have higher sensitivity to odors (Spaethe 2001). Head size was important in

explaining the decision speed, but it had no influence on individuals' accuracy or learning. Recent studies have documented that larger bees learn faster (Worden et al. 2005). In my experiments, body size measurements did not correlate with learning. However, there were significant colony differences in body size and learning. Similarly, Raine et al. (2006) did not find an influence of body size in learning, but found strong intercolony differences in learning ability. Differences in learning speed may be attributed to intercolony genetic differences (Raine et al. 2006), which have also been shown in honeybee olfactory learning experiments (Bhagavan et al. 1994).

Finally, the results of this study have implications for the flower constancy behavior shown by some pollinators. The speed-accuracy trade-off for multimodal flowers suggests that detecting and/or processing information about multimodal flowers is easier than it is for unimodal flowers. Similarly, by using predictions from General Recognition Theory, it can be argued that a comparison between multimodal signals and their components would show that multimodal signals present an easier discrimination task than their components. The results of the speed-accuracy test, and the predictions from the theory contradict the underlying assumption behind the trait variability hypothesis (Gegear & Laverty 2001), which attributes the high levels of constancy on flowers that differ in multiple traits (such as visual and olfactory) to the increased cognitive limitations, such as difficulty in processing, learning, and remembering.

I suggest that there are two reasons why the assumption of increased cognitive limitations with increasing number of traits does not hold in my study. First, my study presents a discrimination task, and half of the flowers in the study were unrewarding.

This design is in contrast to flower constancy studies in which all of the flowers are rewarding, and bees can freely switch between different flowers. The increased levels of costs associated with visiting the unrewarding flowers may have aided bees' learning.

Second, in the flower constancy experiments in which the flowers differ in two traits (such as visual and olfactory traits), the foraging array includes four different types of flowers. Out of these four flower types, two flowers share the same scent but differ in visual properties, while other two share the same visual properties but differ in scent (similar to the four flower types in Figure 3a). In my experiments with multimodal flowers, I only had two flower types, none of which shared a trait with another type of flower. In other words, the flowers differed in both visual and olfactory cues from each other (similar to the two flowers in Figure 3b). This difference is important because when a bee visits a flower with multiple traits, she can be learning the flower as a compound stimulus instead of learning the individual components of the flower. When objects are learned as compound stimuli, it becomes difficult for animals to distinguish the components of stimuli when they are separated from each other (reviewed in Rowe 1999). Due to this effect of learning compound stimuli, bees specializing on one of the flowers may not visit other flowers on the array when floral traits differ in more than one component. It would be valuable to repeat my experiments with testing arrays similar to those of flower constancy studies, to see how bees are responding to the compound stimuli and whether compound learning may be influencing flower constancy.

These differences in design may account for why learning was not adversely affected by multiple floral traits. However, the speed-accuracy trade-off and the target

finding rate both suggest advantages of multimodal signaling. It is unlikely that the design differences between my study and constancy studies account for this result that contradicts assumptions of trait-variability hypothesis. It would be valuable to re-evaluate whether multiple traits result in increased cognitive limitations for pollinators. One possible way is to include the speed-accuracy trade-off in further constancy studies.

In conclusion, I emphasize the importance of including the speed-accuracy trade-off in studies of decision-making. Although I focused on a foraging context, similar investigations can be applied to any type of decision animals make. Several recent studies have suggested that decision times in addition to accuracy of decisions should be taken into account when physiological mechanisms of perception are important in understanding behavioral results (Dyer & Chittka 2004, Chittka & Spaethe 2007). My results on the target finding rates support the argument that focusing on accuracy alone might be misleading. Even though it is not clear whether multimodal flowers provide advantages in bee learning, the higher target finding rates and the significant differences in speed-accuracy trade-offs with multimodal flowers suggest multimodal signals enhance decision-making processes of bumblebees. The mechanisms responsible for this enhancement are not entirely clear, but it is obvious that multimodal signals have important implications for receiver's cognitive abilities, and may also play a special role in signal evolution. Lastly, these results are in agreement with the growing body of literature suggesting that complex signals will be better understood when their components are studied together instead of in isolation.

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