The Importance of Visual and Olfactory Stimuli During Flower Visits in *Apis mellifera*

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Summary

Flowers attract honeybees using colour and scent signals. Bimodality (having both scent and colour) in flowers leads to increased visitation rates, but how the signals influence each other in a foraging situation is still quite controversial. We studied four basic questions:

When faced with conflicting scent and colour information, will bees choose by scent and ignore the "wrong" colour, or vice versa? To get to the bottom of this question, we trained bees on scent-colour combination AX (rewarded) versus BY (unrewarded) and tested them on AY (previously rewarded colour and unrewarded scent) versus BX (previously rewarded scent and unrewarded colour). It turned out that the result depends on stimulus quality: if the colours are very similar (unsaturated blue and blue-green), bees choose by scent. If they are very different (saturated blue and yellow), bees choose by colour. We used the same scents, lavender and rosemary, in both cases.

Our second question was: Are individual bees hardwired to use colour and ignore scent (or vice versa), or can this behaviour be modified, depending on which cue is more readily available in the current foraging context? To study this question, we picked colour-preferring bees and gave them extra training on scent-only stimuli. Afterwards, we tested if their preference had changed, and if they still remembered the scent stimulus they had originally used as their main cue. We came to the conclusion that a colour preference can be reversed through scent-only training.

We also gave scent-preferring bees extra training on colour-only stimuli, and tested for a change in their preference. The number of animals tested was too small for statistical tests (n = 4), but a common tendency suggested that colour-only training leads to a preference for colour. A preference to forage by a certain sensory modality therefore appears to be not fixed but flexible, and adapted to the bee's surroundings.

Our third question was: Do bees learn bimodal stimuli as the sum of their parts (elemental learning), or as a new stimulus which is different from the sum of the components' parts (configural learning)? We trained bees on bimodal stimuli, then tested them on the colour components only, and the scent components only. We performed this experiment with a similar colour set (unsaturated blue and blue-green, as above), and a very different colour set (saturated blue and yellow), but used lavender and rosemary for scent stimuli in both cases. Our experiment yielded unexpected results: with the different colours, the results were best explained by elemental learning, but with the similar colour set, bees exhibited configural learning. Still, their memory of the bimodal compound was excellent.

Finally, we looked at reverse-learning. We reverse-trained bees with bimodal stimuli to find out whether bimodality leads to better reverse-learning compared to monomodal stimuli. We trained bees on AX (rewarded) versus BY (unrewarded), then on AX (unrewarded) versus BY (rewarded), and finally on AX (rewarded) and BY (unrewarded) again. We performed this experiment with both colour sets, always using the same two scents (lavender and rosemary). It turned out that bimodality does not help bees "see the pattern" and anticipate the switch. Generally, bees trained on the different colour set performed better than bees trained on the similar colour set, indicating that stimulus salience influences reverse-learning.

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Introduction

The honey bee, *Apis mellifera* (or *mellificia*, the correct but rarely-used term), is a hymenopteran insect which lives in social colonies and feeds mainly on nectar and pollen. It collects these from flowers. By transferring pollen from one flower to another on its body, the honey bee facilitates pollination in many angiosperms. It finds flowers by vision, especially colour vision, and by the flowers' characteristic scents.

Even though the honey bee's visual and olfactory capacities, and the neuronal pathways underlying them, have been studied for a long time, there is still no general consensus about *how* bees use multimodal flower signals in foraging. This project aims to follow this question systematically – knowing, of course, that the whole field is far too complex to be covered completely in one large-scale study.

To make sure that our results represent the bees' behaviour under natural conditions, we conducted behavioural experiments with free-flying workers out in the open. Despite having tiny brains, worker bees can easily be trained and tested in experiments; they have astonishing capabilities for learning, they are peaceful and focused, and since they deliver all the rewards they are given to the hive and then come back to earn more, they can be trained for hours on end. In fine, they make ideal subjects for behavioural experiments.

This study looks into whether colour is generally more important for foraging than scent (or vice versa), and how stimulus quality influences the answer to this question. We also studied whether an existing preference of one modality over the other can be reversed, or if it is ingrained in a bee's brain; and finally, we asked whether bees show elemental or configural learning when faced with multimodal stimuli, and how well bees can reverse-learn multimodal stimuli.

1. Scent and Colour Signals: We Only Know that We Know Very Little

To tackle all these questions, we first have to summarize what we know about the perception and processing of scent signals and colour signals, and their interactions. Although the issue has been studied by many different researchers over several decades, conclusive answers are missing.

There is no doubt that presence and absence, quality and quantity of scent and/or colour signals take influence on bees' foraging behaviour (Mota et al. 2011, Leonard and Masek 2014, Kunze and Gumbert 2001, Kriston 1973, Leonard et al. 2011a and 2011b, Hebets and Papaj 2005, Gould and Gould 1988, 173; and many others). It seems clear that a scent and a colour signal together are better than just one of them alone (Kunze and Gumbert 2001). How exactly they influence the perception and processing of one another, however, is very difficult to figure out.

Most older studies cannot be directly compared to one another (e.g., Gould and Gould 1988, 173; Kriston 1973, Bogdany 1978, Mota et al. 2011, Kunze und Gumbert 2001), because different researchers used different kinds of stimuli: for colours, the stimulus could be paper, or plastic or monochromatic light; for scents, some researchers used complex mixtures (usually essential oils), and others used just one single substance, such as geraniol. The fact that the salience and quality of stimulus components influences the outcome of an experiment has been known since Pavlov (1927, 141). Kamin (1968) and Rescorla and Wagner (1972) came to the same result in their audio-visual rat and rabbit experiments. Kriston (1973) discovered the phenomenon in scent-colour bee experiments. Still, it seems that the majority of scent-colour bee experiments so far paid little attention to the quality of the colours and scents used, and no consistent and systematic method has been used between different research teams.

The salience of each stimulus component is an important part of stimulus quality, but so is the bee-subjective similarity between opposing stimuli within the same modality: target and distractor may be equally salient on their own, but this does not necessarily say anything about how subjectively different they are for the animal tested. An otherwise highly interesting study by Katzenberger et al. (2013), for example, took salience of scent and colour stimuli into account, but paid no attention to the subjective similarity between colours or scents.

Due to a lack of technical possibilities, many older studies concentrated less on physiological interactions of signals in the bee brain, but rather on signal detection in the bee's natural environment. Karl von Frisch, for example, claimed that colour was more important as a long-distance signal, and scent only served as a short-distance signal to make absolutely sure of the flower species (von Frisch, 1953, 50-51). For decades, this hypothesis was considered a general truth. But in 1996, Giurfa et al. trained bees to discriminate coloured stimuli from an empty grey background, and found that bees could discriminate colours from grey only at a visual angle of 15° or more; if the stimulus offered green contrast, they could still discriminate the stimulus from the background at an angle of 5°. Bees' vision is therefore much less accurate than von Frisch thought.

Because of the minimum visual angles, flower size is very important for colour discrimination. Giurfa et al. (1996) give an example: "Corollas of most species have diameters of less than 5 cm and, in the best case (α_{min} = 5°), the farthest distance from which these corollas would be detectable is about 45 cm". He concludes that the grouping of flowers into inflorescences or clusters must lead to better visitor rates. This idea is supported by the calculations of Vorobyev and Hempel de Ibarra (2012): "… it is predicted that honey bees can [visually] detect patches of flowers from further distances than individual flowers". Very small flowers which are standing alone make themselves more conspicuous by swinging in the breeze on top of long stems, and harnessing the bee's motion detection to grab its attention (Giurfa et al. 1996). On the other hand, in a group of flowers not only the coloured surface is added up, but also the number of scent molecules in

the air – again, we cannot know which signal grabs the bees' attention first. Tautz (2007, 81) points out that bees always approach flowers against the wind, and suggests that scents may be more important over long distances, as colours and shapes can only be seen up close. The final answer is probably individual in each case, depending on flower species, scent production, green contrast, size of flower patch, and of course weather conditions. In reality, the problem becomes more complex the more is found out about it.

Other researchers (for example Gould and Gould 1988, or Bogdany 1978) have concentrated on the degree of importance colour and scent have relative to one another in a conflict situation. Gould and Gould's study, in particular, was one of the inspirations behind our experimental design. In it, bees were trained on multimodal stimuli (scent, colour and shape) and later tested on new combinations of colour, scent and shape, to figure out the importance of each factor. Scent appeared to be more important than colour. In our study, we will test if this is really generally the case, using different colour-scent combinations with known salience and similarity but keeping the scent components constant. We will also use the data to check for changes in the relative importance of scent and colour depending on the number of rewards before the first conflict test. Kriston's (1973) data implied that scent may initially be the crucial factor initially, with colour becoming more important after the 10^{th} reward. We will check if we can find this "Kriston effect" in our data.

A hotly debated question is whether a compound stimulus is learned and remembered as the sum of its parts (elemental learning) or as a completely new, independent stimulus (configural learning) (Giurfa 2003, review). For both, there is no general consensus. We are going to approach the question by training bees on bimodal stimuli and then testing them on components only.

In the course of the same experiment, we will also reverse-train bees on bimodal stimuli. Reverse-learn experiments with bees on colour-only stimuli (see, for example, von Helversen 1974, or Dyer et al. 2014) have been conducted in the past. Bees have also been reverse-trained on scent-only stimuli (Mota and Giurfa 2010), but not, to our knowledge, on scent-colour stimuli. It is not known whether bimodality will enhance, or rather inhibit, reverse-learning, and we will try to get to the bottom of this question.

Another unexplored question is this: if a bee prefers to use the scent component of a bimodal stimulus for its choices, rather than the colour (or vice versa), is this behaviour fixed, or flexible? Are there bees which tend to ignore one factor throughout their lives, or does choice behaviour change, depending on which signal is available in the current foraging situation? We will study this question by picking bees which preferred one sensory modality and training them exclusively on the other, to see if their behaviour changes.

On a related note, concerning our experimental design: Couvillon and Bittermann (1989) argue that "scentless" can be a scent signal too; however, Koltermann (1969) found that "scentless" is far harder to learn for bees than a scent cue (this is also our experience), and therefore should not be treated equivalent to a scent in an experiment.

2. Foraging for the Hive

The honeybee's family structure is the key to its behaviour. The social structure of a bee colony puts unusual selective pressures on honeybees, which need to be taken into account when designing experiments for bees and interpreting the results.

The worker bees and drones in a colony are siblings. The so-called "queen" is really their mother[1], and is the only reproductive female. She makes sure that this stays so through hormonal manipulation; other than that, she does not "command" her workers. Instead, workers behave according to cues they take from their environment (Tautz 2007, 264). Anthropomorphically expressed, individual bees do what they think needs to be done right now.

The reproductive success of a colony depends equally on the queen's ability to produce eggs, and on her daughters' ability to care for the brood and bring in all the necessary nectar, pollen, minerals, water, etc. (Of course, drones also contribute to genetic diversity by mating with queens from different colonies, but in the context of this work, drones are of little interest.)

Worker bees serve their colony in a number of ways: during the first three weeks after emerging from a cell, they take over cleaning and brood-caring tasks inside the hive. Towards the end of this phase, they guard the entrance to the hive, and also make explorative flights in the area around it, to prepare for the second half of their lives: as a forager in the field (Herold 1965, 70-72). This means that bees spend the first half of their adult lives almost entirely in darkness, where olfactory signals are of great importance. Visual signals become important during the foraging phase from week 4-6 of their lives. It should be mentioned that this timeline is not completely fixed, but can be accelerated or reversed when a drastic change in hive population makes it necessary (see Ray and Ferneyhough 1999).

A queen mates with 12-30 males, guaranteeing genetic and behavioural diversity between her offspring. This seems to prevent the birth of both extremely good and extremely bad worker phenotypes, but leads to a more steady "income" of nectar (summarized in Beekman et al. 2003). Foraging behaviour is not only influenced by genetics, but also by individual experience, and this form of adaptation is particularly important due to a bee hive's life cycle: Menzel (2012b, review) points out that the lifespan of a hive's genes is theoretically infinite, as a hive can raise new queens and new workers whenever needed. Therefore, genetic adaptation to a changing environment is not ideal, but can be compensated for through individual learning. Smith et al. (2012) summarize: "Much of the honey bee's impressive learning ability has probably evolved because of the instability of

[1] If the colony's old queen has died, a new queen will be reared by her sisters, the workers. If no eggs are provided from which to rear a queen, workers will start to lay unfertilized (drone) eggs as soon as the old queen's pheromones fade. But in this case, the colony is doomed, and the drones are the last hope to spread the colony's genes.

information about nectar and pollen relative to a honey bee's foraging lifetime". Both genetic differences between individuals and learned foraging strategies are adaptations to the honeybee's unstable environment.

Bees' foraging behaviour can differ in many ways: their strategy can be slow and deliberate, or fast and inaccurate (see, for example, Dyer's 2012 review); bees can be more or less flower-constant; they can be accurate about daytimes, or just come to the experimental setup at any time of the day (personal observation). These different behaviours between individuals, even between sisters and half-sisters, are a form of bet-hedging which keeps the hive prepared to harvest food no matter what the environmental situation around the hive is (Dyer 2012).

3. Flower Constancy

130 million years ago, the first angiosperms developed (Tautz 2007, 55). Honeybees have existed in their current form for 30 million years (Tautz 2007, 4). Today, roughly 17000 plant species are pollinated by honeybees, and 4000 of them are completely dependent on this particular pollinator (Tautz 2007, 57). Everybody is familiar with the idea that bees visit flowers and collect nectar and pollen, but the ecological benefit is really mutual: during flower visits, insects involuntarily carry pollen on their bodies and bring it from the pollinia of one flower to the pistil of another. Pollinating insects and plants are forming a symbiosis – the insects get food, the plants get pollinated. Bees make pollination particularly effective because they show a behaviour called "flower constancy". After finding a particularly good reward on one flower species, they will try to visit this species again and again, which benefits the plant species' pollination rate. Coevolution has led to flowers which are highly adapted to their specific visitors (Waser 1983). For bees, it would be more profitable to have several "favourites" at the same time, but because bees' memory is limited, flower constancy is usually only formed about one species (Waser 1983, Grüter and Ratnieks 2011, review) – fortunately for the flowers.

How did this amazing symbiosis between flowers and bees develop? Kevan and Baker (1983) summarize that early arthropods visited plants to eat their pollen, and that nectar was probably first a chemical to help move germination along. According to paleontological findings, arthropod-based pollination seems to have existed long before the first angiosperms as we know them (Kevan and Baker 1983). At the time when coloured flowers developed, arthropods already had three receptor types, although it is not clear if they used them for colour vision the way most arthropods do now (Chittka 1996). Flower scents, too, were used to appeal to already-existing sensory organs of arthropods (Schiestl 2010). Flowers as we know them most likely evolved as an adaptation to arthropod pollinators. Adapting one's morphological properties to one's pollinator does not only promote flower constancy, but can also help to attract *only* the appropriate pollinator species, and thereby

avoid pollen waste. This specialization led to an enormous variety of flower types (Kevan and Baker 1983).

The most important premise for flower constancy is, of course, that a pollinator must be able to identify its target species correctly. Bees recognize flowers by scent, colour and shape (von Frisch 1953, 44-57), and it seems that flowering as unique as possible would benefit a plant. The possibilities for combining shape, colour and scent seem endless. But bees have only a limited number of sensory receptor types, so flowers can only use certain signals to attract them. Also, plants have to synthesize the coloured pigments and scents, and the tissue which forms the shape of the flowers; and apart from these economic limitations, plants are under diverse other selective pressures (see *5. Floral Morphology* below). So there are, inevitably, similarities between completely unrelated flowers, increasing the danger of mis-pollination. To make oneself look and smell as unique as possible, using a combination of signals instead of just one is a useful strategy (see below, *4. Several Signals – What For?).*

Bees do not exhibit flower constancy under all circumstances, and some could, but do not try very hard to stick to it (Chittka et al. 2003). Flower constancy is also reversible: when a bee realizes that the target species does not reward it any longer, it can try out new flowers, and switch to another species when it finds better rewards there. A bee can even learn to switch from a rewarding flower to a better-rewarding flower. Some bees get frustrated easily as soon as flower constancy stops paying off, and quickly start to choose at random. This is not advantageous for flowers, but in some environmental scenarios, these bees are the most profitable for the colony (for more detail, see Dyer et al. 2014).

Even with the best-performing bees, flower constancy is not absolute[2]: even after multiple rewards on a particular species, a bee may still make occasional visits to other species, just to see if something better comes along (see, for example, Dyer 2012). After all, "[h]ow reliably a particular flower's odor is associated with nectar or pollen can change hour-to-hour and day-to-day, potentially many times within a foraging honey bee's lifetime" (Smith et al. 2012, see also Koltermann 1969, and Kevan and Baker 1983). The same is true for other signals (e.g. Reinhard and Srinivasan 2009, and Kevan and Baker 1983).

Some non-rewarding flowers have no interest in looking unique: by resembling other, rewarding flowers, they can lead these flowers' pollinators astray, and maybe achieve pollination without having to invest anything at all in nectar or pollen rewards. This food-deception phenomenon falls into the category of Batesian mimicry (Dyer 2012). There are also general food deceptors: flowers which do not imitate a specific species, but exploit the bees' curiosity and previously learned general preferences (Schaefer and Ruxton 2011, 175). The reproductive success of this strategy must be strong enough to make up for its great

[2] These possible "deviations" which are useful for the bee are a pain in the neck for a scientist, because they must be taken into account when designing an experiment.

disadvantage: not only the model's but also the mimic's pollen very often ends up on the wrong flower (Roy and Raguso 1997).

Schaefer and Ruxton (2011, 118) point out that "[e]ven in highly rewarding species … the flower may have been depleted by recent visits of competing pollinators", therefore making it necessary for pollinators to show a certain amount of tolerance for rewardlessness before abandoning this species as a food source. This facilitates the existence of unrewarding flowers, if the deception is good enough. If not, bees will quickly learn to avoid the mimic. Therefore, mimics must resemble the model as closely as possible. But producing the same pigments and scents is cost-intensive and complicated. Also, resembling a certain species *too much* could make the mimic too dependent on only one model.

It seems that food-deceptive mimic flowers have found an economical solution for this problem: They imitate flower colour, but not flower scent. No evidence for scent-based food mimicry has been found so far[3] (Kunze and Gumbert 2001, Peter and Johnson 2008, and Galizia et al. 2004). Kunze and Gumbert came up with a plausible explanation: as bees usually learn scent particularly fast, they might learn "this scent is unrewarded" faster than they learn "this colour is unrewarded", and change their strategy faster. Also, as bees learn colour better in the presence of scent – even if both stimuli are scented the same – they might find it easier to detect colour differences they normally would not notice. A perfect mimic would have to imitate both colour and scent perfectly, and this seems to be too difficult to carry out in reality. Finally, Kunze and Gumbert suggest that in a changing environment, one model species might disappear at times, so it is probably advantageous to keep the option of mimicking more than one species.

4. Several Signals – What For?

There seems to be no doubt that multimodal signalling improves flower constancy (reviewed in Schaefer and Ruxton 2011, 138-139). Leonard et al. (2011b) summarize the dilemma bees are facing when making foraging decisions: "If bees use a threshold-based rule to decide whether to accept an individual flower, then any overlap between sensory traits of floral types generates a non-zero probability of making two types of mistakes: false alarms and missed detections", with false alarms leading to a waste of energy, and missed detections making foraging less successful. Multimodal signals seem to reduce both, but how they do this is a very complex field.

There are many ways in which multimodal signalling can be beneficial. The following are of interest for colour/scent-directed bee pollination, summarized by Hebets and Papaj (2005):

[3] Sexual scent mimicry is widespread, see for example Raguso 2008, but not of interest for this work.

- A “redundant signal”: Several signals (possibly for different sensory modalities) provide information about the same thing – in our case, a potential food source. When looking at Batesian mimicry, this theory becomes even more interesting: “Redundant signalling may also prevent signallers from cheating, allowing a receiver to accurately assess a signaller when presented with contradictory evidence”.
- The “efficacy backup hypothesis”: The two modalities have different transmission properties, so if one signal becomes inaccessible or less salient (for example, strong wind drives the scent away from the flower; or the flower is hidden under leaves and cannot be seen), the bee can find the flower through the other signal.
- The “perceptual variability” hypothesis: individuals differ in their ability to detect signals in different sensory modalities. Displaying multimodal signals could be a way of hedging one’s bets with different individuals. A plausible example: many older forager bees lack segments of their antennae (personal observation), which impairs their sense of olfaction (see *6.d. The Olfactory Sensory Organs and Pathway*) and colour signals might become extra useful in this case.
- The “amplifier” hypothesis: one signal makes it more likely that the other will be detected by making it more conspicuous, but without carrying any information when presented on its own.
- The “alerting and attention-altering” hypothesis, stating that “one signal either alerts a receiver to the presence of another signal, … or influences the formation filtering mechanism of the receiver such that the receiver’s attention is focused on the other signal”.
- The “context” hypothesis: one signal serves as a context to help the receiver interpret the signal in the way intended by the sender.
- The “emergence” hypothesis: several signals are processed as a completely unique signal, not the sum of several parts (compare “configural learning” in Giurfa et al. 2003).

These suggestions are not mutually exclusive, and often very difficult to distinguish in nature. Some of these ideas deal with the signals themselves, some with their transmission, reception and processing. Which strategies are really responsible for the peculiarities of multimodal learning is unclear (Kunze and Gumbert 2001, Hebets and Papaj 2005, Leonard et al. 2011a).

Hebets’ and Papaj’s models deal mostly with perception. Leonard et al. (2011a) round up the list with several hypotheses which deal more explicitly with memory functions:

- Their version of the “redundant signal” theory, not simply based on perception but extended to memory retrieval.
- “Attention triggering” - their version of the “alerting” hypothesis from above: “In this scenario, detection of one component brings a different component of the complex display into working memory”, thereby facilitating learning.

- The “attention-consuming” hypothesis, where “a more complex floral signal is able to out-compete other stimuli for access to working memory”.
- Their refined version on the “context” hypothesis above, particularly relating to learning and recall of signals. Here, they also point out that in many cases, flowers use signals known to bees from other contexts (mating, pheromones such as geraniol, etc.) to attract them, and an additional stimulus compound might help the bees to put the well-known signal into an attractive context.

Despite all these different approaches, there is still one problem: “While it is clear that the ability of a receiver to learn and remember a complex signal can be affected by inter-signal interactions, the underlying mechanism is not known” (Hebets and Papaj 2005). Gerber and Smith (1998) add: While in pollination theory scent and colour signals often occur as redundant signals supposed to make the bee more certain about flower identity, from the bee’s perspective they often serve very different functions. “They inform honeybees about different and complementary aspects of flowers (location, blooming status, nectar availability and rate, species) and might be relevant to different motor programs (flight, orientation, choice, landing, PER [proboscis extension reflex, see below]) … Their meaning might therefore be complementary rather than redundant” (Gerber and Smith 1998).

Kulahci et al. (2008) worked with bumblebees, starting out on the interesting hypothesis that by processing two signals of different modalities, two different brain pathways could be used simultaneously (“parallel processing”), rather than consecutively, as might be the case with two signals of the same modality, leading to a decrease in choosing time. Their results showed that multimodality did not reduce choosing time, but increased accuracy significantly. They offer several possible explanations: one signal may serve the bee at close range, the other at a distance (see *1. Scent and Colour Signals* above); or maybe the signals are not processed simultaneously but sequentially in any case.

5. Floral Morphology and the Limitations of Flower-Animal Communication

Plant morphology is an immensely complex field. Plant-animal communication is not only influenced by the sensory systems of the pollinators, but also by other selective pressures, such as predators and competition for resources. The chemical properties of nectar do not only function as rewards for pollinators, but in some cases also have defensive functions against microorganisms. Pigments can also serve in chemical defense: anthocyanins are fungicides and help to reduce UV light stress as antioxidants, and UV-reflecting isoprenylated phloroglucinols can poison herbivores. The possibilities plants have in signal-production are also influenced by climate and soil quality (summarized in Schaefer and Ruxton 2011, 6-16, 134-135).

Even when a signal has only one function to fulfil, the possibilities are still limited by physics. For example, only a limited area of the light spectrum can be used in animal vision:

wavelengths below 300 nm cause tissue damage in the animal, and wavelengths over 800 nm cause too much photoreceptor noise to be effective (Neumeyer 1991). Honeybees cannot perceive any wavelength above 600 nm, which limits the frame for colour morphs further (see below, *6.a. Chromatic and Achromatic Vision*).

5.a. Floral Colour and Markings

It makes no sense to try and attract insects with wavelengths they cannot see. Consequently, very few bee- and bumblebee-pollinated flowers employ the colour red, which humans can see but bees cannot (Schaefer and Ruxton 2011, 133) – a purely red flower appears black to bees. But red flowers do not necessarily appear black-only: poppy, for example, reflects UV light and appears UV-coloured to bees (Kevan and Baker 1983). Schaefer and Ruxton (2011, 133) summarize: "If the sensory differences among pollinators are pronounced, floral colour can lead to considerable ethological isolation" in pollination, facilitating flower constancy. But it is not enough to consider the limitations of the pollinator's visual system: "[T]he limited diversity of floral colours can be understood by the limited combinations of pigments and surface structures available to impart colours in petals and sepals".

The most common flower pigments are carotenoids, anthocyanins and betalains. Their advantage is that they look very similar from all angles. Only in low-light conditions, iridescence (changing of colour when the observer shifts their point of view) can be an advantage (Schaefer and Ruxton 2011, 30).

Carotenoids are terpenoids which reflect red to yellow wavelengths of light. They appear in many tissues, and are responsible for the yellowing of leaves after chlorophyll has degraded, but are usually not visible due to chlorophyll. There are over 600 different carotenoids, most prominently orange carotenes, and yellow xanthophylls (Schaefer and Ruxton 2011, 31). Anthocyanins look blue, red and purple-black to the human eye. They are phenol-based molecules whose appearance depends, for example, on temperature, hydroxylation, methylation, co-pigments, concentrations, and acidity of the vacuoles they are in. Flavonoids, which are produced through the same production path as anthocyanins and often appear in the same tissues, influence the colour of flower tissue by absorbing UV light. Betalains do apparently not occur together with anthocyanins, but reflect a similar spectrum of light wavelengths. They originated from tyrosine (Schaefer and Ruxton 2011, 31-33).

Many flowers are not uniformly coloured, but have patterns or spots – sometimes in UV wavelengths, so that a flower which appears unicolour to us is dappled with UV spots to bees (see, for example, Tautz 2007, 78). These can either serve as honest food signals, they can mimic rewards such as pollen, or they can mimic potential mates, as they do in some *Ophrys* species (Schaefer and Ruxton 2011, 130). But they are only useful at close distances

(see for example Wehner 1981, 314-315; and *6.a. Chromatic and Achromatic Vision* below). Finally, it should be noted that some flowers change their colour after pollination has occurred (Schaefer and Ruxton 2011, 135-136), so that colour is not necessarily stable under all circumstances.

5.b. Scent and Scent Distribution

The field of olfactory signalling in pollination is not as well-explored as colour signalling, because it is far more complex and difficult (Schaefer and Ruxton 2011, 135; Kevan and Baker 1983). This is at least partly because olfactory signals are even more chemically diverse, less stable, and harder to measure – colours stay inside the plant, scent is spread through the air and decays over time. Sadly, "[c]ompared to visual traits, there is considerably less evidence on how abiotic factors influence odour bouquets" (Schaefer and Ruxton 2011, 146) – the distribution of scent in a natural environment is extremely hard to estimate.

Plant scents are made up of volatile organic compounds. Many of these substances occur in vegetative parts of the plant, indicating that they first served other physiological functions (Schaefer and Ruxton 2011, 33-36). Schiestl (2010) writes that 87% of volatile organic compounds are the same in insects and angiosperms – as opposed to gymnosperms, where there is no overlap: "These patters suggest that plants use the insects' own chemical language to influence their behaviour … these examples suggest adaptive evolution, since the plant gains a fitness benefit from mimicking chemical signals that are important for the insects' own reproduction and survival."

Flower scents are usually emitted by the epidermis of flower petals. They are "typically lipophilic molecules with high vapour pressure; that is, they can pass the membrane freely and evaporate into the atmosphere" (Schaefer and Ruxton 2011, 39). There are several chemical classes of odorants which typically occur in flowers. Terpenoids, mostly mono- and sesquiterpenes, fulfil defensive as well as olfaction-related functions. Some of them are derived from cut-up carotenoids, offering a potential for multimodal functions. Another class are phenylpropanoids and benzenoids, based on benzene rings. They are formed through the phenylpropanoid pathway, another product of which are anthocyanins. Also, some odour compounds are derived from amino acids; some are aliphatic; some contain sulphur and primarily attract carrion flies (Schaefer and Ruxton 2011, 38-40).

As flower scents usually consist of very complex mixtures, they are detected by a number of different receptors. The signals from those are ascribed different amounts of importance to the bee's olfactory system. Olfactory "noise" can disturb the reception of scents, either by influencing the sensitivity of the receptor, or by masking scents (Schaefer and Ruxton 2011, 34-35). As Schaefer and Ruxton express it (34): "The olfactory system functions by counting the binding events of molecules to which receptors are sensitive. By integrating binding

events over relatively long times, neurons are able to include even low-probability binding events in generating their response … Temporal resolution is thus lost… and traded for higher specificity." On a behavioural level, however, bees must find the right balance between nitpicking and generalizing between scents. Guerrieri et al. (2005) point out that "under natural conditions, the blends of volatiles … vary widely in quantity and quality both in time and space. To cope with such changes in an efficient way, a 'flower constant' forager should be able to generalise its choice to the same kind of floral sources despite fluctuations in their [the flowers'] volatile emissions".

Colour only depends on light to reach a potential pollinator. In case of the bee, the limitation lies in the bees' relatively large visual angles for colour recognition (see Giurfa 1996). Scent distribution, on the other hand, is heavily influenced by air movement (Schaefer and Ruxton 2011, 135). Molecules of different chain lengths travel at different speeds: Smaller molecules are more volatile, and therefore offer themselves for long-distance communication (Schaefer and Ruxton 2011, 35). This can become useful "[b]ecause animals are assumed to maintain stimulus identity even in spite of changing concentrations of volatiles", so "such different qualities of volatiles might encode distinct information" (Schaefer and Ruxton 2011, 138), possibly making it easier for animals to estimate the distance to the source of the scent. Unlike colour, scent can also provide information about *when* something occurred[4], because scents begin to decay after a certain amount of time (Schaefer and Ruxton 2011, 35).

Since not all animals' olfactory systems are equipped with the same receptors, scents can be used as "private communication channels" to attract animals that benefit the plant, while excluding others (Schaefer and Ruxton 2011, 34). For honeybees, scent can serve as a food signal long before a bee encounters the flower itself: it can be carried into the hive and there support a scout bee's dances. Recruits are given a scent sample of what to look out for (von Frisch 1965). Since dances only give the followers the direction in which to fly, and a rough idea about the distance in which the food source lies, knowing the scent can be very important. There is no way this can be done with colour (Schaefer and Ruxton 2011, 137, Kriston 1973). Reinhard et al. (2004) point out that flower scent brought into the hive can also refresh the memory of foragers who remember this food source from an earlier time.

Finally, pollination is not only influenced by natural plant scents, but also by "chemical footprints" left on flowers by previous pollinators (Schaefer and Ruxton 2011, 137, Giurfa et al. 1994). The importance of chemical footprints for subsequent pollinators is debatable, because chemical footprints often stay on for very long, and therefore do not give reliable information whether a flower is still empty, or if new nectar has been produced (Schaefer and Ruxton 2011, 119).

[4] For example, a flowering event or a pheromone mark left by a conspecific

6. The Visual and Olfactory Sense of the Honeybee

6.a. Chromatic and Achromatic Vision

The eyes of the honeybee are apposition eyes, best-adapted to medium-speed flying in daylight, and consist of 6000 ommatidia (single eyes) each. They are convex and seated on the sides of the head, allowing the bee an all-round view of its surroundings, and also a good view upwards and downwards (Wehner, 1981, 324-327). Since composite eyes cannot be shifted in their sockets the way lens eyes can, the only way a bee can change its visual angle is by moving its whole body (von Frisch 1953, 81). Having compound eyes, a bee's visual acuity is limited by the interommatidial angle (Vorobyev and Hempel de Ibarra 2012, review, and Dyer and Williams 2007). This angle is smallest in the front of the eye and around the vertical "equator", giving these regions the greatest visual acuity; colour is likely perceived equally well all over the eye (summarized in Lehrer 1998). Altogether, a bee's picture of the world is strongly blurred compared to ours.

Like us, bees have colour vision, although theirs is different from ours. Neumeyer (1991) defines colour vision as "the capability of a visual system to respond differently to light differing in wavelengths only". To do this, an organism needs at least two types of receptors, sensitive to different wavelengths, as "the central nervous system can gain information about colour only by comparing the outputs of different photoreceptor types" (Neumeyer 1991). An organism with only one receptor type only has achromatic vision.

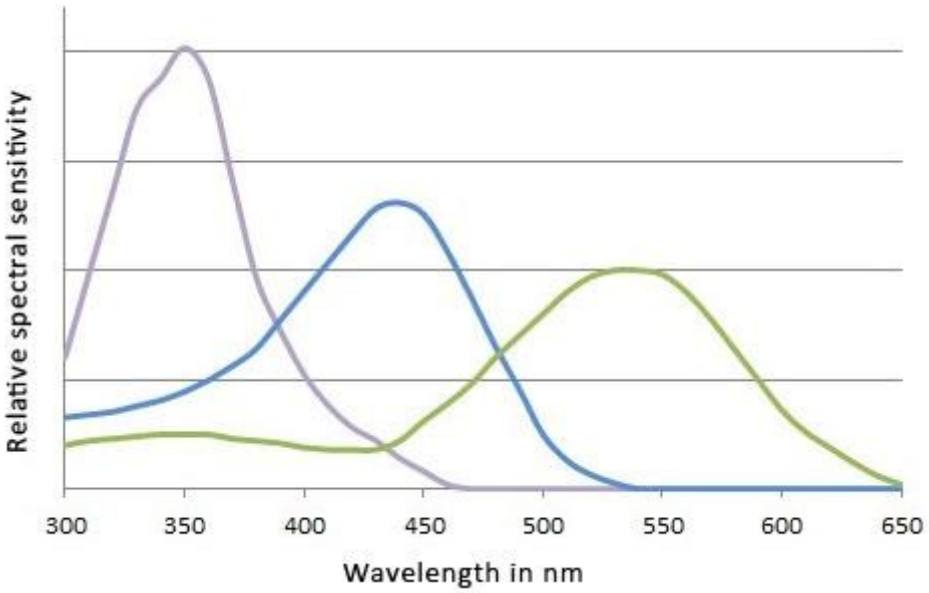

Fig. 1: Relative spectral sensitivity of the honeybee's eyes to lights of different wavelengths. Purple: Ultraviolet receptor, blue: blue receptor, and green: green receptor.

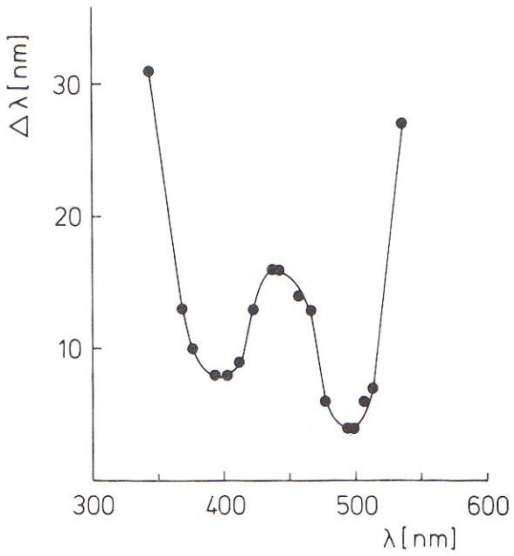

Fig. 2: The delta-lambda-function of the honeybee. At the two minima – areas of overlap between two receptors - the honeybee's colour discrimination is most accurate (from "Evolution of the Eye and Visual System", Neumeyer 1991; after von Helversen 1972).

Bees have three types of photoreceptors: blue, green and UV. The UV receptor absorbs wavelengths from 300 to 460 nm; its maximum spectral sensitivity lies around 344 nm (Fig. 1). The blue receptor absorbs wavelengths from 300 to 550 nm, with its maximum sensitivity at 436 nm. The green receptor absorbs wavelengths from 300 to 660 nm, and its sensitivity is highest at 556 nm (Vorobyev and Hempel de Ibarra 2012). With these, bees can discriminate colours very well. Because there are three receptors, the delta-lamba function of the honeybee (Fig. 2) has two minima: two areas of optimal discrimination, lying at roughly 400 and 500 nm in the ultraviolet-blue and blue-green area, where sensitivities of two receptors overlap (von Helversen 1972).

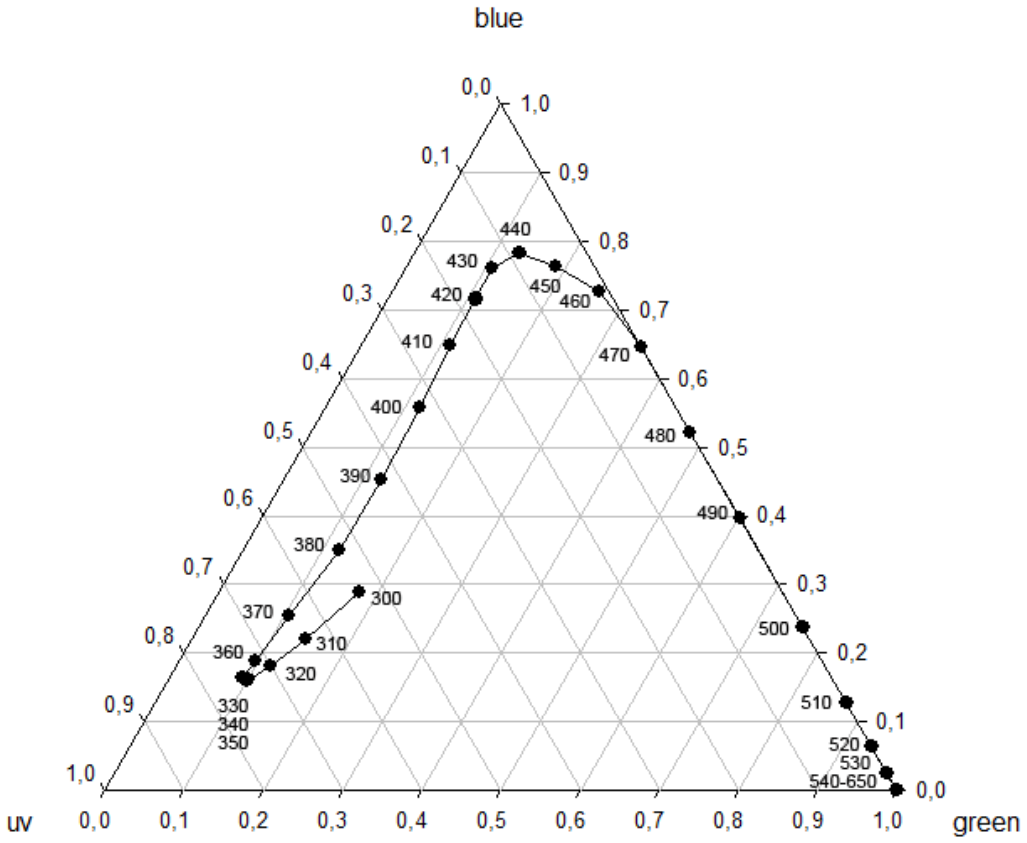

Fig. 3: Loci of spectral colours in the honeybee's visual perception space. Each locus is labelled with the corresponding wavelengths in nm.

The honeybee's colour space is three-dimensional, due to the bee's 3 receptor types, but it is often practical to depict bee colour vision in a two-dimensional plot which only takes excitation ratio of the receptors into account, and omits the factor brightness (Fig. 3).

It is not difficult to determine the properties of colour stimuli for an experiment, and to compare relative similarity: colours close together in the bee's colour space are similar in hue. The further away from the centre a colour is, the more saturated it appears to the bee. A peculiarity of colour vision is that "if we mix yellow with red light, we will see orange … but we will be unable to distinguish the mixture from monochromatic orange light" (Chittka and Brockmann 2005, review) – in other words, the eye only perceives one colour result, and the organism is unable to tell its components apart. In other sensory modalities, such as hearing and olfaction, this is different (Chittka and Brockmann 2005).

The green receptor is also responsible for achromatic vision and motion vision, and makes it possible to detect objects which stimulate it from further away than objects which only stimulate the other receptors: the green receptor allows detection from a 5° angle, the other two only from a 15° angle (Vorobyev and Hempel de Ibarra 2012, Giurfa 1996). Using only colour vision, a bee would have to fly very close to objects before it could detect them. The green receptor allows it to tell green objects from "not-green" or "not-just-green" objects from further away, and everything not-green on a background of green foliage is likely to be

a flower, thus making foraging easier (Giurfa 1996). Tautz (2007, 79-80) points out that bees seem to turn “more colour-blind” when flying back to the hive, as they are not collecting any more. This phenomenon does not apply to patterns, only to colours, suggesting there might be more than one pathway for input from different photoreceptors, serving different functions.

The honeybee’s visual system most likely has two different pathways: a high-resolution pathway for the input from the L-receptor alone, which is therefore achromatic; and a low-resolution pathway for input from all three types, which is chromatic. Consequently, small objects are more easily visible using only the L-receptors, and for larger objects, bees use chromatic vision (Vorobyev and Hempel de Ibarra 2012). Dyer et al. (2011, review) also point out that L receptors respond faster than S and M receptors.

Bee use polarized (short-wavelength) light for orientation, especially in unknown territory (Tautz 2007, 77, 90). Polarized light can only be perceived by the ommatidia at the upper rim of each eye. Its perception is separated from colour vision (von Helversen and Edrich 1974; Vorobyev and Hempel de Ibarra 2012).

How does a bee process input from different receptors into colour vision? The bee’s visual system has three types of neurons: broad-band neurons which respond to many different wavelengths; narrow-band neurons which only process information from one photoreceptor type; and colour-opponent neurons which probably form the basis for colour vision, by showing “combination-sensitive excitatory and/or inhibitory interactions between two or more photoreceptor classes”, but their exact functions are still relatively unexplored (Dyer et al. 2011). Dyer et al. also point out that there seems to be one “fast” colour processing system for coarse information; and a pathway that takes time to establish because circuits have to be modified through learning, which mediates decisions between similar colours.

6.b. From Eye to Brain: A Systematic Overview of the Bee’s Visual Pathway

The bee’s photoreceptors are located in the ommatidia of the compound eyes, with L-receptors making up six of an ommatidium’s eight receptor cells. Their output is passed on into the lamina ganglionaris, and from there, into the medulla. The medulla consists of eight layers of cells, arranged from distal to proximal. The distal three layers are innervated by lamina neurons and S- and M-receptors (summarized in Dyer et al. 2011). The outer layers of the medulla are mostly made up of narrow- and broad-band neurons, the inner layers mostly of colour-opponent neurons. From both layers, signals are passed on to the protocerebrum, and from the inner layer only to the mushroom body. Also, neurons project from the medulla into the lobula, which in turn has outer layers (they are probably involved in achromatic motion detection and mediate mostly narrow- and broadband responses, similar to the outer layer of the medulla); and an inner layer, which is colour-sensitive (and

mediates colour-opponent responses, again, like in the medulla). Despite these apparent parallels between medulla and lobula, they react specifically in terms of temporal response.

The outer lobula layers project to the posterior protocerebrum (which is connected to the motor system), the inner to the mushroom bodies. Both layers also project to the anterior lateral protocerebrum, and from there to the mushroom bodies, posterior protocerebrum and the central complex. From an ipsilateral mushroom body, visual signals are also passed on to the contralateral mushroom body.

Colour is processed by the mushroom bodies, whose neurons "display colour sensitivity, colour opponency and temporally complex patterns including adaptation and entrainment" (Dyer et al. 2011) – hinting at the multiple functions of the mushroom bodies, especially the collar and basal ring (we will talk about them further below) – and also to the lateral protocerebrum. Even here, colour vision and motion vision seem to be processed along different paths; like the mushroom bodies, the protocerebrum also receives olfactory input, and Paulk et al. suggest that the protocerebrum may integrate complex information from the bee's surroundings, beside the mushroom body (Paulk et al. 2009).

6.c. Behavioural Thresholds for Colour Experiments

Depending on training method and stimuli, a bee's behavioural threshold for colour discrimination can vary greatly (see, for example, Giurfa 2012 and Dyer 2012). In our experiments, this becomes important as we use two colour sets of different similarity. Colours that appear very different to the bee can be learned using only absolute conditioning, where the bee is only confronted with the target stimulus during training, and does not encounter the distractor stimulus except in non-rewarded tests.

But even though bees have colour-constancy – the ability to recognize the same hue under different illumination conditions (Neumeyer 1981) – they can only learn to discriminate between very similar colours through differential conditioning (Dyer and Chittka 2004). This means that the bee is familiarized with the target stimulus *and* the distractor stimulus during training, and knows that the latter is unrewarded before the first test. Giurfa (2012) writes: "[I]t may be that such … pre-training triggers attentional processes that allow better focusing on the targets". Dyer et al. (2011) write that the phenomenon "suggests different levels of behavioural plasticity in bee colour decision-making for either dissimilar or similar colours": either the visual system slowly "tunes" its sensory neurons to the difficult task when faced with similar colours, or the sensory neurons are perfectly capable of solving the task, but the higher-level neurons have to adjust (Dyer et al, 2011). In any case, the process is slow and gradual.

In our experiments, we used only differential conditioning for the very similar colour set, and also gave those bees longer training phases to make sure that they had the opportunity

to learn the colours of the stimuli, not only the scents (for similarity between colours, see below, Fig. 7).

6.d. The Olfactory Sensory Organs and Pathway

"The role of the olfactory system is to decode the complex eddies of volatile molecules in the environment and shape them into pieces of relevant information", summarizes Sandoz (2012, review). The olfactory system in the honeybee begins with the antennae, and ends with processing of the information in the brain. While the visual system is especially well-studied at a behavioural level in experiments with free-flying bees, olfaction has been studied especially in laboratories with harnessed bees, which allows easy access to the brain.

The antennae are a bee's organs for scent detection (von Frisch 1922). The main olfactory receptor neurons are located in the surface of the outer 9 antennal segments. These receptors are called *sensillae placodeae.* There are 60.000 per antenna (Dostal 1958, Sandoz 2012), and they lie inside small pores in the antenna surface. After the honeybee's genome was decoded, it turned out that bees have 163 olfactory receptor genes, and therefore likely 163 different receptor types (Robertson and Wanner 2006). The number of receptor genes matches the number of glomeruli in the antennal lobe (AL, see below), so that one glomerulus likely corresponds to one receptor type (Robertson and Wanner 2006).

The axons of the receptor neurons per side make up the antennal nerve, which runs to the AL. Just before reaching the AL, each antennal nerve splits into six tracts, T1 to T6. T5 and T6 are composed of mechanosensory neurons, and run past the AL. T1-T4, which are of greater importance for our study, project into the AL. The AL is the primary olfactory centre of the bee brain, and consists of 165 glomeruli, "anatomical and functional units", formed by approximately 4000 inhibitory local neurons, which conduct the first computations (Sandoz 2012).

From there, approximately 800 projection neurons carry the processed information on to higher-order brain areas (summarized by Rybak 2012, and Sandoz 2012). Multiglomerular projection neurons form the mediolateral antennal proto-cerebral tracts (ml-APTs), which run into the lateral protocerebrum. Most projection neurons are uniglomerular, and these form the median and lateral APT (m-APT and l-APT). The l-APT receives information from T1-glomeruli and runs through the lateral horn and into the mushroom body calyces. The m-APT receives information from T2-T4-glomeruli and runs through the mushroom body calyces, and on to the lateral horn. This makes it possible for the m- and l-APTs to carry different parts of the bee's odour perception spectrum (Rybak 2012, Sandoz 2012).

The honey bee has one mushroom body (MB) per brain hemisphere. Like in many social insects with complex tasks in life, the bees' MBs are very large, containing 40% of the bee brain's neurons. The mushroom bodies are most likely responsible for higher sensory

integration, learning and memory-forming (Rössler and Groh 2012), and for processing inputs from different sensory modalities (Sandoz 2012).

Anatomically, a mushroom body consists of a peduncle, vertical (α) and medial (β) lobes, and two calyces (unlike in *Drosophila*, which has only one calyx per MB). Physiologically, it is made up of Kenyon cells (KCs), whose axons form the peduncle. The MBs get input from projection neurons coming from the AL to the calyx. Here, the projection neurons' boutons make synapses with the KC dendrites, forming microglomeruli. The calyces receive input from both visual and olfactory projection neurons: the lip especially from olfactory neurons, and the collar mostly from visual neurons. The basal ring (the bottom of the cup) receives input from both and from other sensory systems (Rössler and Groh 2012).

To avoid a reaction to unimportant signals, Kenyon cells have only weak synaptic strength, so that only strong and continuous input manages to excite them. To facilitate discrimination between different scents, the more signals from different components reach the microglomeruli, the more the answer is suppressed (Sandoz 2012).

Each scent evokes its own excitatory pattern in the glomeruli, which is the same between different individual bees, and chemically similar odours lead to similar patterns in the glomeruli. It seems likely that similar glomeruli patterns lead to subjective similarity for the honeybee, but there is no actual evidence yet (Guerrieri et al. 2005).

While bee-subjective colour similarity can be evaluated relatively easily (see *6.a. Chromatic and Achromatic Vision*), constructing the bee's olfactory space is almost impossible. Chittka and Brockman (2005) summarize: "Odors are hardly presentable on a physical continuum (like the wavelengths of light); they are multidimensional entities … Theoretically, a perceptual space might have as many dimensions as there are distinct receptor types – or it might have as many axes as there are glomeruli with distinct response profiles. Is it possible that olfactory space in bees, then, has several dozen dimensions?"

The most important attempt to map bee-subjective scent similarity to date is Guerrieri et al's (2005) study. They checked for similarity between 16 different scents through behavioural experiments and found that chemical similarity led to generalization (and therefore subjective similarity) between scents, depending on carbon chain length and functional groups. They then constructed a three-dimensional olfactory space, using chain length and functional group category as axis parameters. This three-dimensional space described their results approximately, but it remains to be seen if the model will suffice for future studies. Similarity between a scent pair has been shown to differ, depending on which scent is used as the target and which as the distractor; it also seems that bees can learn the components of a scent mixture, but at the same time perceive the mixture as a unique stimulus (reviewed in Chittka and Brockman 2005). All of these factors make it enormously difficult to construct an olfactory space for the honeybee.

In our study, we are using multi-component essential oils (see *Material and Methods*). While the components are known to us (sadly, listing them in this work would be a violation of company secrets) we still know nothing about their relative subjective similarity, or their associative strength, or how quickly they disperse. We therefore kept scents and scent concentrations constant throughout the experiments, and varied only colours.

6.e. The Mushroom Bodies and the Processing of Multimodal Signals

Leonard and Masek summarize: "The integration or modulation of multimodal sensory inputs might happen at a peripheral level, at the output neurons converging to final behaviour, or at any intermediate point along the processing path" (Leonard and Masek 2014). With everything we currently know, present research focuses especially on the mushroom bodies, which are known to be important centres for multimodal processing (see, for example, Dyer et al. 2011). Its microglomeruli show enormous plasticity, depending on the task they have to fulfil (Rössler and Groh 2012, Sandoz 2012). It is interesting that their volume begins to grow with the onset of foraging activity at age 20-21 days, the time when visual input gains much greater importance than before. Queens, which do not need to cope with outside life after having mated, decrease the size of the MB collar and increase the size of the lip. Likewise, long-term memory formation causes changes in microglomeruli density in the lip, but not the collar (summarized in Rössler and Groh 2012).

Apart from sensory input, the microglomeruli are also linked to "modulatory systems, in particular octopaminergic and dopaminergic neurons … which play an important role in associative learning. The precise connection of neuromodulatory and other extrinsic neurons into the microglomeruli microcircuits requires further investigation", as Rössler and Groh (2012) put it. Tang and Guo (2001) also showed that *Drosophila*'s mushroom bodies are indispensable for decision-making in conflict tests, which makes it likely that they fulfil the same task in the bee's brain. Therefore, the mushroom bodies are probably the place where most of the neural processes relevant to this work – associative learning, olfactory and visual processing, and decision-making – take place, but we know very little about how or where they do.

More unfortunately, "almost nothing is known about the multimodal function of the basal ring" in the MB (Rössler and Groh 2012), except that integration of visual and olfactory information probably takes place there. Giurfa (2012) writes that the MB's "[o]utput neurons are multimodal thus suggesting that crosstalk and information exchange, necessary to higher forms of cognition, could take place within these structures", and he also suggests that the MBs might mediate attentional processes and problem solving. The output of the mushroom body is passed on to extrinsic neurons through the α- and β-lobes to the protocerebrum, and from the peduncle to the lateral horn via a multimodal output neuron called PE1, but what happens in the lateral horn is also unknown (Rössler and Groh 2012, Rybak 2012). Menzel (2012a) summarizes that "we are still far from understanding even the

basics of gross organization … of [the olfactory system's] anatomical structure, its coding properties and plasticity".

7. Learning in the Honeybee Brain

How do bees store information? Like in many other organisms, such as *Drosophila* or *Aplysia*, short-term memory (STM) formation requires protein modification, but long-term memory (LTM) formation depends on the expression of genes (summarized in Müller 2002, and Müller 2012).

Hättig (2009, 4-8) gives an overview over the physiological process: Memory is generally stored as a change in synaptic strength. To initiate the strengthening of a synapse during learning, it takes a coincidence detector which becomes active when a) a cell is depolarized, and its internal Ca^{2+} concentration is consequently increased, and b) it becomes stimulated by a neighbouring cell through neurotransmitters. Both signals together activate adenylate cyclase (AC), which turns adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). cAMP is a second messenger. It activates protein kinase A (PKA), which modifies proteins by phosphorylating them and changing their properties. So far, we have looked at the formation of short-term memory. The alterations made in proteins can easily be reversed. To achieve long-term memory, PKA has to be strongly active for longer periods of time. Then it can reach the nucleus and activate cAMP response-element binding protein (CREB). CREB, together with a cofactor protein (CBP) can then influence the expression of downstream genes.

Studies have shown that bees' memory can be manipulated through cooling, electric shocks, or anaesthesia after bees have been trained for only one reward (summarized in Müller 1996). After more than one reward, these methods become ineffective quickly – apparently, repeated acts of learning lead to anaesthesia-resistant memory.

Where in the bee brain do these processes take place? Müller (2002 and 2012) summarizes, using the relatively well-studied scent learning as an example: The pathway for the unconditioned stimulus (US, e.g. sucrose) must at some point converge with that of the conditioned stimulus (CS, a scent signal). In the honeybee, this takes place in the antennal lobes and mushroom bodies. Both are innervated by the ventral unpaired median maxillare 1 (VUMmx1) neuron, which plays an important part in US processing; and both AL and MBs receive CS input. Despite these similarities, ALs and MBs seem to function largely independent of one another during learning, and contribute in different time frames (Müller 2012).

If VUMmx1 is stimulated by octopamine, the cAMP cascade is initiated in the ALs. As described above (*6.d. The Olfactory Sensory Organs and Pathway*), input from the antennae into the AL causes a scent-specific activation pattern in the AL's glomeruli. VUMmx1 likely

reaches all the glomeruli, but “induces a rather general activation” (Müller 2002). VUMmx1 also arborizes to the MB calyces, but sucrose stimulation does not lead to PKA activity here. Dyeing experiments have indicated that a Ca^{2+} pathway, rather than the cAMP pathway, is responsible for learning here (Müller 2002).

It seems that “a temporal pattern of PKA activation” (Müller 2002) is required for LTM formation to take place in the AL. “However”, Müller (2002) continues, “the complete sequence of events that connect the training procedure with CREB-mediated gene expression required … has not been identified yet” – which still applies (Müller 2012). But he suggests that there are likely several signalling cascades involved. In the MBs, LTM is induced if glutamate is administered directly after conditioning, but the exact cascades are not known yet (Müller 2012). Müller (2012) also states that the AL and MB might not be the only components involved in LTM formation, and that “it remains unclear yet in which neuronal network the honey bee brain and by which molecular mechanisms LTM is maintained”.

8. Basic Problems in Multimodal Bee Experiments

The main reason why it is so hard to find out more about multimodal scent and colour processing in honey bees is that bees can learn scent while harnessed and fixed, with easy access to their brains, but not visual signals (the exception being Gerber and Smith 1998). Eleven years ago, Giurfa optimistically announced that visual learning would soon be studied physiologically in live bees, just like scent; but this prediction has not yet come true (Giurfa 2003). Visual signals can only be learned if the bee can move freely, but in free flight its brain is inaccessible (see, for example, Giurfa 2012). The only known solution would be to amputate a bee’s antennae (Giurfa 2012), but this would defeat the purpose of scent-and-colour learning. Finding a paradigm where both scent an colour can be tested through the proboscis extension reflex (PER) seems to be one of the most important aims for the future of bee research, but at the moment, the only available method is non-invasive and means working with free-flying bees.

It is not known why bees cannot learn visual signals while harnessed, or why they can as soon as their antennae are removed. Maybe bees’ visual systems are built to bring in relevant information only when the bee is moving through the air, since it does not need its visual system much when crawling inside the hive (where it is dark) or on flowers (where scent could guide its way).

A famous problem is this: what does a laboratory study tell us about an animal’s behaviour in its natural environment? On the other hand, experimenting under natural conditions adds uncontrollable factors (in our case: weather, temperature, natural flowers all around which might distract bees, and others). We weighed one against the other, and decided to stay as close as possible to natural conditions to be able to draw conclusions to

honeybees' real-life pollination activities. We therefore stuck with the traditional method of operant conditioning with free-flying animals.

Another problem is the question of which stimuli to use. Knowing an animal's receptor types for a sensory modality, or knowing the pathways in the brain, or where the sensory information is processed, tells a researcher nothing about *how* this animal perceives the world around it. Perhaps an animal does not use all the receptors it has, or photoreceptors which appear physiologically the same can fulfil different tasks. The only way to find out which stimulus components can be used for a bimodal experiment is through behavioural experiments.

Koltermann (1971) pointed out the importance of using scents and colours which lie within the framework of the bee's natural foraging activity, and we used colours and scents which are as close as possible to the kinds of flowers bees encounter in nature. To avoid using stimuli for which bees have different preferences, we relied on scents and colours which had been tested in previous behavioural experiments extensively, and had been found to have a similar innate "popularity" with bees. We decided to use colours for which bees have a natural preference (Giurfa et al. 1995): hues of blue, blue-green, and yellow.

Blue and blue-green are both extremely popular with bees, yellow is attractive but not quite as attractive as the other two. We worked with one very similar colour set (unsaturated blue and blue-green), and one very dissimilar colour set (saturated blue and yellow). Pairing saturated blue and yellow meant that bees were not initially faced with two stimuli of the exact same preference (see Giurfa et al. 1995), but there was no equally popular colour pair dissimilar enough for these experiments. Any worries that an innate preference of blue over yellow could influence our results were quickly dispersed: after the initial training phase, the first tests showed that there was no preference for any stimulus colour (see *Results*).

We used lavender and rosemary scents because a previous study (Koltermann 1973) showed that *Apis mellifera carnica* has a very similar preference for these two scents. We did not have the same brands of essential oils as Koltermann did, but our results showed that after the initial training phase, there was no preference for either scent, so our products were just as good for the purpose as Koltermann's.

9. The Aims of This Study

Our experiments can be divided into three basic questions:

When faced with conflicting colour and scent information, do bees decide by colour or by scent? We will conduct two basic test series: one with two saliently different colours (saturated blue and yellow), the other with saliently similar colours (unsaturated blue and blue-green); both paired with rosemary and lavender scent. We expect that the difference in

salience of the colour sets – and therefore, the degree of difficulty to discriminate colour - will influence choice rates.

We will also use the data from this experiment to check for each group whether the bees' preferences for scent or colour changed after additional training. This was inspired by Kriston's 1973 experiments, where bees initially paid more attention to scent, and later more to colour. We will look for evidence of a similar effect here.

In the second test series, we ask: *Can bees which prefer to use one sensory modality in foraging be induced to abandon this modality, and rely on the other?* To do this, we will train naturally scent-preferring individuals on colour-only stimuli, and conflict-test them afterwards. We will also train naturally colour-preferring individuals on scent-only stimuli, followed by conflict-testing. Our hypothesis is that bees' remarkable learning skills and behavioural flexibility will enable them to switch from one modality to another.

The third test series deals with the question: *Can bees reverse-learn bimodal stimuli which differ both in scent and colour?* Here, we will also check if bees recognized a bimodal stimulus' components when they encounter them alone: before every reversal, we will test the bees on the bimodal stimuli against one another; the colours against one another; and the scents against one another. It has been shown that bees can reverse their preference once or twice, and then deliver random results, when trained and tested on colour or scent only. In experiments with monomodal stimuli, bees do not "learn to learn", i.e., to anticipate the switch, as a human subject would. Our hypothesis is that a bimodal signal may be saliently strong enough to enable bees to switch more often, or even to enable them to learn the switching principle.

Here, like in the first test series, we will use two colour sets of different salience. This is because we want to study the influence of stimulus salience on the ability to switch preference; but also because we want to see the influence of salience on component learning.

Material and Methods

General Materials and Methods

All experiments used the same basic training methods and materials, but the training and testing protocol was different in every series of experiments. We only used free-flying forager bees (*Apis mellifera carnica*) which were kept in a normal hive in a garden area on the campus of Mainz University. The bees were trained and tested individually in an outdoors setting.

After each experiment, the bee was caught in a small container or matchbox and frozen for 3-4 minutes. This would daze it sufficiently so that we could examine its antennae with a magnifying glass, and count if and how many segments of the antennae were missing. This was a way of making certain that their sense of smell was not significantly impaired. According to Dostal (1958), lack of only few segments does not impair a bee's olfactory sensitivity much – the relation is not linear but logarithmic – so we decided to keep data from bees which still had ¾ or more of the total number of those antennal sections which carry the olfactory receptors. Fortunately, a trained eye can see if an antenna is damaged even without a magnifying glass, before the experiment begins, so that we had to sort out very little data ex post.

Feeding Station and Setup

Bees flying away from their hive are very hard to recruit for experiments, because they seem to feel an urge to get high into the air as quickly as possible. Therefore, it is advisable to build a feeding station, where foragers come reliably to feed, and can more easily be "abducted" for an experiment (see, for example, von Helversen 1974).

Our feeding station consisted of a wooden pallet, which served as an even base. On top of that we put a wooden chair, which we adjusted with a water-level, as we needed an absolutely even and horizontal basis. On top of the chair we put a feeder house made of ply-wood, which consisted of a roof, a back wall, a floor and two props on the sides. Inside the feeder house, we placed the actual feeders: Two upside-down storage jars filled with sucrose solution (made from household sugar and tap water), standing on two level plastic tiles with grooves cut into them. The sucrose solution would flow into the grooves, making it easy for the bees to drink (Fig.4).

Fig. 4: Bees visiting the feeder and sucking sucrose solution from the grooves in the plastic tile. Some bees can be seen landing, or flying away.

The feeders were usually equipped with 12% to 25% sucrose solution, depending on weather, time of year and flowering of the surrounding vegetation. During July and August, when most trees had stopped flowering but the weather was hot, the feeder would be frequented so heavily that we often had to dilute the sucrose solution to 5%, just to be able to work. In May and October, however, it took 25% to get enough visitor bees (for similar experiences, see Schwarz 1954).

The feeder was put up 12 meters from the hive. The experimenting table stood 6-7 meters from the feeder (Fig. 5). With this setup, recruited bees could easily find the table, but unwanted “visitors” rarely disturbed the experiments.

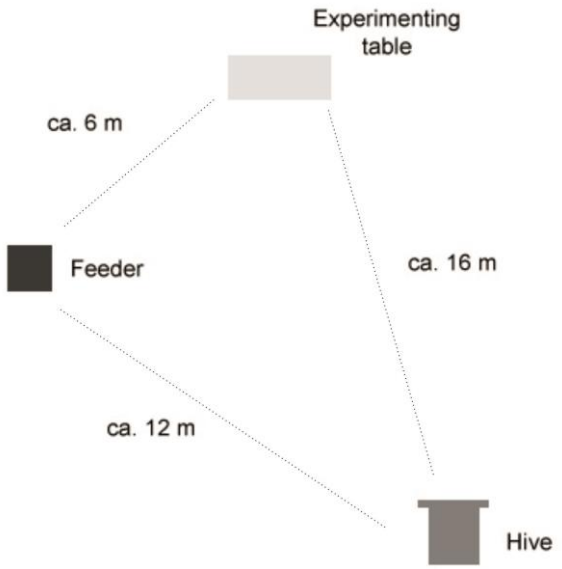

Fig. 5: A bird’s eye view sketch of the experimental setup.

Arena and Stimulus Presentation

The experiment arena consisted of a vertical grey disc, diameter 60 cm, and small hanging devices with landing platforms (hangers, Fig. 6). The disc could be rotated and the hangers could be moved to different positions on the disc to avoid training the bee to a certain spot.

On the hangers, coloured and/or scented paper stimuli were mounted with double-sided scotch tape. We always used four hangers at the same time, two with target and two with distractor stimuli. Bees were trained to land on the platforms below the stimuli and wait for their reward, which consisted of 30% sucrose solution.

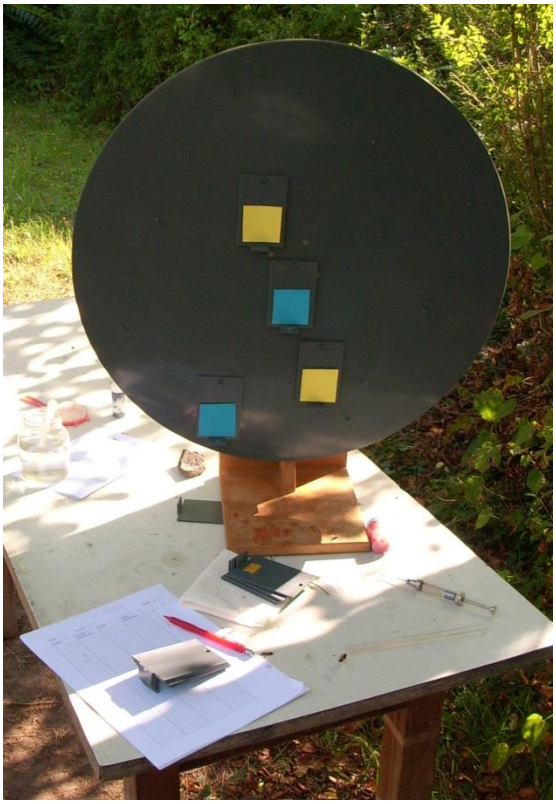

Fig. 6: The arena for training and testing. Four hangers can be seen on the circular, rotatable PVC disc; blue and yellow stimuli are mounted on them with double-sided scotch tape. Nothing unnecessary should be on the table during the experiments, as bees tend to get distracted by interesting new things. For the safety of the experimenters, the table should always be kept meticulously clean from splashes of sucrose. Otherwise, bees will often crawl all over the table, end up being squashed by accident, and might sting the experimenters. For the same reason the glass of sucrose solution should be kept somewhere out of reach for the bees, for example in a drawer or box (the glass in this picture contains water).

Stimuli and Stimulus Production

The stimuli consisted of 5x5 cm squares of cardboard paper. We used two different sets of colours, provided by different brands:

- Heyda 4716215, a saturated yellow (green and UV-coloured to the bee), and Heyda 4716233, a saturated blue – two very different colours for the bee (Fig. 7 and Table 1).
- Baehr 219 22 32, an unsaturated blue-green, and Baehr 219 22 37, an unsaturated blue – two very similar colours for the bee (Fig. 7 and Table 1).

Also, for scent-only experiments, we used Baehr 387 22 82, a type of grey which resembled the plastic of the arena and hangers (Fig. 8 and Table 1). Presenting the scent alone without the cardboard (for example, in a tube) was not an option because the dose of scent presented to the bee would have been completely different from the rest of the experiment. By using paper with similar hue and brightness to the background, we made sure we had the same amount of scent but only a very weak visual signal for the bee.

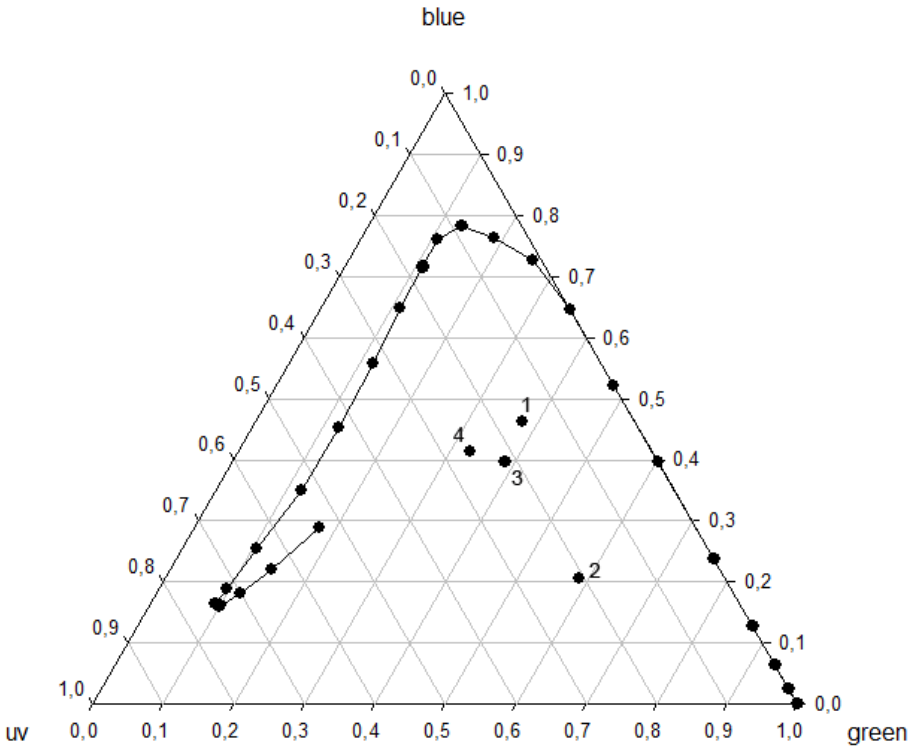

Fig. 7: Locations of the coloured paper stimuli in the honeybee's colour space. 1: saturated blue, 2: saturated yellow, 3: unsaturated blue-green, 4: unsaturated blue.

The locations of the saturated colour pair 1 and 2 are clearly further apart than those of the unsaturated colour pair 3 and 4 (Fig. 7), showing that they are bee-subjectively more different.

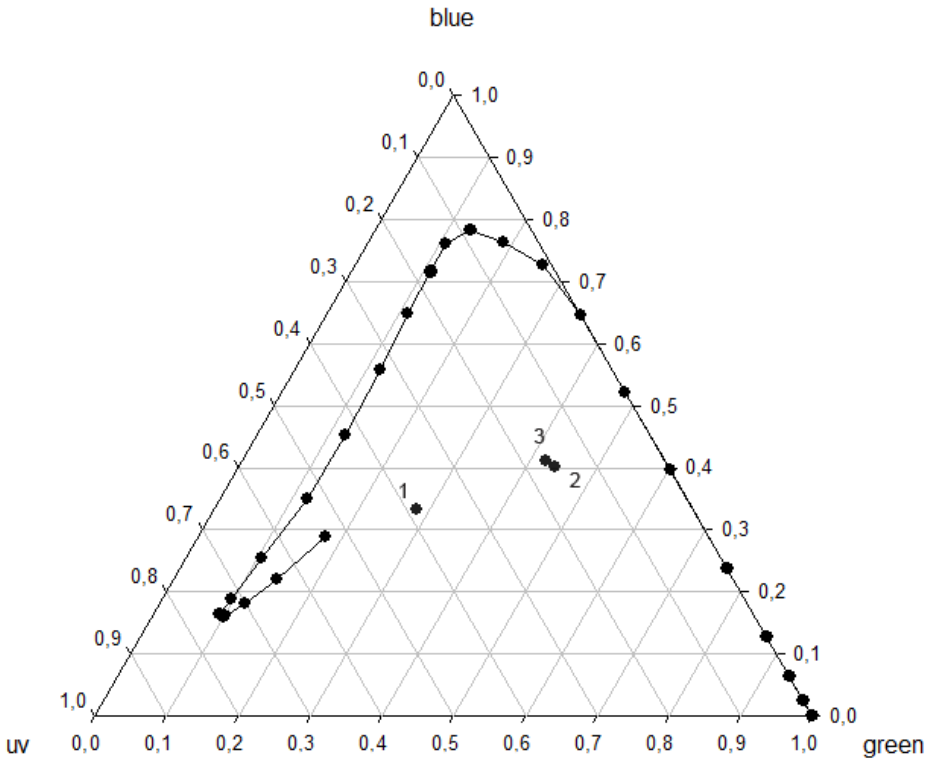

Fig. 8: Locations of the background material (2 and 3) and the grey paper stimulus (1) in the bee's colour space.

Incidentally, there were two different kinds of hangers, made of PVC with almost identical visual properties: one was a little lighter than the other (Fig. 8 and Tab. 1). But we always used four hangers of the same sort, and never mixed, so as to avoid giving the bee an undesirable visual cue. Both disc and hangers were made from PVC which looks dark grey to the human eye, but offered the bee's eye less stimulation in the UV part of the spectrum than the blue or green part. The background therefore looked mostly blue-green to the bee. The hue of the grey paper stimulus is further apart from both of them (for explanation, see next paragraph).

Tab. 1: Relative brightness of stimuli (white boxes) compared to the background (hangers and disc, grey boxes).

Colour	% brightness
Saturated yellow	33,3771353
Saturated blue	29,1283399
Unsaturated blue	35,348226
Unsaturated blue-green	38,6333771
Grey	12,6806833
Lighter hangers	9,48313622
Darker hangers and disc	6,30749014

Tab. 1 shows that all stimulus colours except neutral grey were much lighter than the plastic background of the hangers, meaning that they were all very easily distinguishable from the background. The grey stimulus was very similar in brightness, making it inconspicuous against the background, even though it was different in hue. Finding a grey paper which was more similar in brightness *and* hue was not possible, since "human-grey" paper makers usually do not take differences in ultraviolet reflection into consideration. Baehr 387 22 82 was the closest choice on the market.

To add the scent component, the paper squares were kept overnight in airtight food storage boxes (brand "Hega" in 2011 and 2012, and "Emsa" in 2013) with natural essential oils (Symrise 106509, lavender, and Symrise 660789, rosemary). The boxes each contained a certain amount of oil, calculated by volume: for the preliminary experiments, this number varied logarithmically, but for the actual experiments, it was always 20 µl/litre of air in the box (50 for lavender, see *Results* for explanation).

The scents were dripped on filter paper, which was then put at the bottom of the box on a small plastic plate (Fig. 9). Above it, there was a grate, on which the stimuli were placed, so that they never touched the oil, but absorbed the scent through the air. After 24 hours, the stimuli were ready for use. If they had not been used after 72 hours, they were discarded.

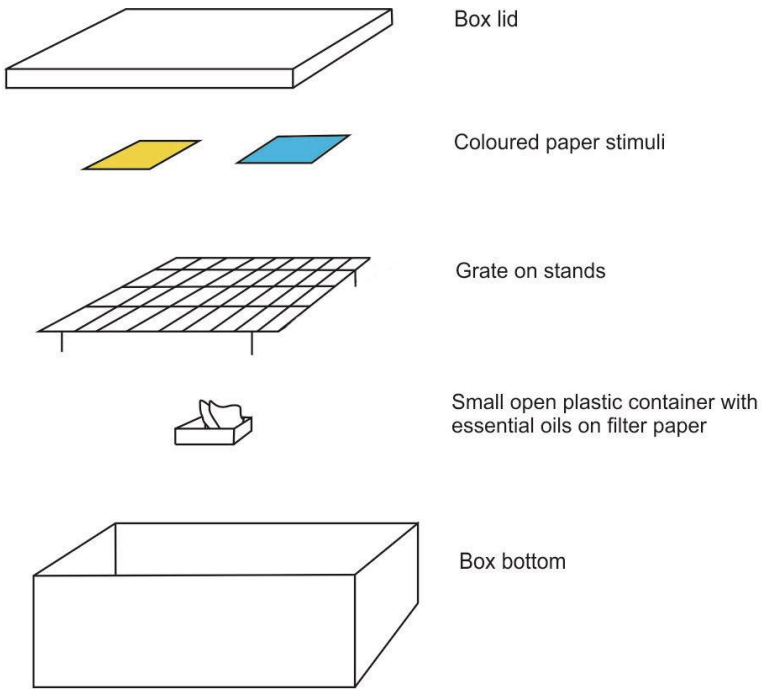

Fig. 9: How to make coloured paper stimuli absorb essential oil molecules through the air: essential oils are dripped on filter paper, which is placed at the bottom of an airtight box in a small open container. A grate on stands is then placed on top of the filter paper, so that the grate and the paper are not touching. Paper stimuli are placed on the grate, and the box is closed.

During the experiments, stimuli were used for 1-2 hours, and then placed in the boxes again. If necessary, they were reused after several hours of "scent-refreshing", but if possible, they were used only once. At the end of each day, all stimuli were discarded. Bees were at all times carefully prevented from walking on the stimuli to leave additional scent cues.

Recruiting Process

To train a bee to visit the experimental table, we put a drop of 30% sucrose solution onto a Plexiglas spoon and made the bee taste it. The best way to do this is to touch its antennae with the fluid, since bees can taste with their antennae, and getting to the proboscis is more difficult. Once the bee had tasted the strong solution, it would usually climb onto the spoon and begin to drink there. Now it could be carried to the experimental setup, where it could be fed further, and marked with a special permanent colour which we had made specifically for the purpose (1 part linseed oil mixed with a generous amount of painter's pigment or pulverized painter's chalk, then stirred into 2 parts natural shellac dissolved in alcohol, and poured into an airtight container). The colour was brought onto the bee's thorax or abdomen using a small piece of wire, or a spruce needle. After marking, the bee was allowed to fly back to the hive.

We would usually bring several bees to the experimental table. The first bee which came back on its own accord was chosen for the experiment, the rest was discarded. Unfortunately, the discarded bees would often remember the setup hours later, come back and disturb the experiment. To avoid this disturbance, we initially trained bees to land not on the stimuli, but a completely unrelated object: the small jars in which we kept the marking colours. When we had chosen a bee for the experiment, the jars were removed, and the bee was trained on the normal stimuli. If any discarded bees came back but did not find the jars, they would give up quickly.

To train a bee on the actual target stimulus, we would take the bee up on the spoon and bring it to the hanger on which the stimulus was mounted, and on which there was another drop of 30% sucrose solution. The distractor stimulus was equipped with a drop of water of a similar size. The bee was taken up with the spoon and placed under the target. Here, it would drink, and afterwards be picked up with the spoon again and held away an arm's length, so that it was forced to fly back to the disc itself.

We placed the bee under the target up to 8 times. If it had not learned to land on the landing platforms then, it was discarded. Otherwise, we kept on training it, but gradually gave it smaller and smaller drops on the landing platforms. The reward on the spoon stayed the same. This way, the bee quickly accepted that it had to land on the platform without immediate reward, but would be picked up with the rewarding spoon (unless, of course, it had landed under the distractor). Around the 14^{th} or 15^{th} reward, we could leave out the

drops on the platforms entirely. The bee would now wait for the rewarding spoon, provided it came within one or two seconds. We went through this complicated procedure because bees which are rewarded with a drop of fluid lying under the target and distractor tend to pay no attention to the stimulus (as we found out in some shot-in-the-dark experiments at the very beginning of this work).

Training and Testing

During training, landing was rewarded on the target stimulus, and unrewarded (but not punished) on the distractor stimulus. Tests were always unrewarded.

We recorded not only rewarded and unrewarded landings, but also aborted landings on either stimulus. During training, a "rewarded landing" was only a landing where drinking sucrose and target stimulus came together. If, for example, the reward did not appear quickly enough and the bee flew away, this was only counted as an abort, because the bee had no chance to learn. During tests (which were unrewarded in any case), any touch to the platforms was counted as a landing.

As an "abort", we defined the behaviour of flying close to a stimulus without touching the platform (and possibly flying very close to its surface to bring the antennae close to the source of the smell), and then flying away again to try a different stimulus: in other words, visiting a stimulus but deciding against landing on it after closer inspection. With aborts, bees displayed insecurity about whether this stimulus was the correct one or not. We therefore used aborts to measure choice insecurity, and recorded both aborts on correct and incorrect stimuli.

To describe the procedure of training and testing, we use a code of formulae also used, for example, by Couvillon (1988). The letters A and B always describe colours, X and Y always stand for scents. A, X or AX are always the original target stimuli, B, Y or BY the original distractors. Since all our experiments were counter-balanced, we always trained equal numbers of bees on every possible AX combination. Some examples:

AX versus BX – two colours, both with the same scent.

AX versus BY – stimuli differ both in scent and in colour.

AX^{+} versus BY – AX is rewarded, BY is neither rewarded nor punished.

Recording and Statistics

All decisions the bees made (landings and aborted landings) were written down in check sheets and later typed into Microsoft Excel. Only the binomial test which determined the

participation thresholds for the learning and conflict tests (see next paragraph) was done in SPSS v 20. All other statistical tests were made using R 2.15.1, as were the box plots. The latter were reworked in Corel Draw X6 and Photoshop CS5 to make the labelling of the axes clearer, and to add significance indicators.

All box plots shown in this work were constructed as follows: boxes encompass the data from the 25% to the 75% quartile, the whiskers encompassed data which deviated from those quartiles by 1.5 x the interquartile range (IQR), or less. All other data points were outliers.

Wherever necessary, data was tested against chance level, which was always 50%. The chance level line can be seen in all boxplots, and is not explained in the graphs themselves.

Exact p values are not given in the graphs, but can be found in Appendix B.

The ternary plots which display the bees' colour space and the reflectance properties of the stimuli were created using Sigma Plot V 11.0 and, if necessary, reworked in Photoshop CS5 to add labels, or to show the bee's visual perception space and the loci of the stimuli in the same plot.

Experimental Design and Binomial Test

Often, we had to test whether a bee fulfilled the requirements to take part in the full experiment (e.g., had it learned the task at all? Did it show a preference for a certain stimulus?). We used a binomial test to find a good compromise between the maximum number of unrewarded landings a bee would make, and the minimum number of landings it took to get a statistically significant result.

Using SPSS v 20, we found our compromise at 15 landings (which a bee will normally perform unrewarded before getting impatient), out of which 12 (80%) had to be correct or in favour of a certain stimulus to produce a significant result. Therefore, we used this rule in all tests where bees had to meet certain requirements to be trained and tested any further.

In experiment E3 (where we looked at reverse-learning and learning of bimodal stimulus components), there were a bimodal test, a scent test and a colour test in a row. This would have been 45 landings in total – too many for most bees, so we had to reduce the number of landings per test. Here, the binomial test said that when N = 10, a significant result is only reached when at least 9 landings are in favour of one stimulus, a difficult, but still feasible rate. In between the different tests, the bee was allowed to drink until it had had enough, and returned to the hive: 30 landings with two big rewards in between. This was tolerable for the bees.

Preliminary Experiments

Before tackling the exciting questions, we had to make sure of all the relevant qualities of the stimuli we were going to use: the salience of colour, scent, and the combinations of both.

P.1. Stimulus Colours

For the colour stimuli alone, this was relatively easy, as the brightness of and the wavelengths reflected by the papers could be measured and then compared to the properties of bees' photoreceptors. Thus, we could determine how "saliently different" and how easily detectable the colour sets are for the bees. We used a spectral photometer (Spectro 320-121, Instrument Systems, Munich, 2008, with a deuterium halogen light source DH-2000, and the corresponding software, SpecWin, version 3.4.0.46) for measuring the results, which we then illustrated using Sigma Plot V 11.0.

P.2. Stimulus Scents

For the scents alone, we had to determine where the behavioural threshold lay. This could only be done through practical experiments. We trained bees to fly to a scentless stimulus, A, and avoid the scented stimulus, AX[5]. We then decreased the dose of scent X logarithmically. By the time a bee could not reach 80% correct decisions any more, even after several attempts, we knew that we had found her behavioural threshold.

Leaning on the results of Fischer (1957), we suspected that the threshold for lavender would be higher than for rosemary, and performed many irregular shot-in-the-dark experiments to get an approximate idea whether this was true for our scents. In the end, we decided to run a regular experiment using 2.5 times as much lavender oil as rosemary, since this ratio seemed to be roughly equally salient for the bees.

We began our experiment by scenting paper with 1µl/litre of air (rosemary; for lavender, 2.5 times as much), which we knew from experience bees could easily detect. Around the point where we suspected the threshold (between 0,001 and 0,0001 µl/litre of air), we added a half-logarithmic step in between (0,0003 µl/litre of air), to get a more exact result.

[5] We used scentless A as the rewarded stimulus so as not to confuse the bees. The decreasing concentration of scent might have irritated the bee if AX had been the rewarded stimulus, but by teaching them to avoid scent X altogether, we gave them simpler "instructions".

As we could not measure 1/100 of a µl with normal pipettes, we had to dilute all the lower doses of the essential oil with paraffin oil (Carl-Roth-GMBH, Karlsruhe; the method was also used by Ribbands 1955, and Fischer 1957).

We trained twelve bees in total, six bees on each scent, and tested them following this protocol:

- 20 rewards on A^+ versus AX, scent dose 1 µl/litre
- Test: A versus AX, 15 landings

If the bee passed this test, it was then trained on the next lower dose. If not, it got more training to 1 µl/litre: another 10 rewards, then a test; if necessary, this could be repeated until the end of the 5^{th} test. If a bee did not pass the 5^{th}, the experiment was finished. However, at this stage, all bees passed easily.

- 10 rewards on A^+ versus AX, scent dose 0,1 µl/litre
- Test: A versus AX, 15 landings (4 repetitions of training and testing if necessary, as above).

- 10 rewards on A^+ versus AX, scent dose 0,01 µl/litre
- Test: A versus AX, 15 landings (repetition if necessary, as above).

- 10 rewards on A^+ versus AX, scent dose 0,001 µl/litre
- Test: A versus AX, 15 landings (repetition if necessary, as above).

- 10 rewards on A^+ versus AX, scent dose 0,0003 µl/litre
- Test: A versus AX, 15 landings (repetition if necessary, as above).

- 10 rewards on A^+ versus AX, scent dose 0,0001 µl/litre
- Test: A versus AX, 15 landings (repetition if necessary, as above).

So, bees were given 60 rewards on the first dose (the initial 20 + 10 + 10 + 10 + 10, with a test following after each block), and 50 on every subsequent one (10 + 10 + 10 + 10 + 10, with a test following after each block).

The first reason for these “five test chances” is that bees can learn to solve difficult tasks better when given more training. Smith (2012) reviews the phenomenon, which has also been found in other scent discrimination tasks: “The heightened responses to both odors [unlike in our experiment, his stimuli were both scented] continue over several trials until there is a rapid increase in response to the CS+ and a decrease in response to CS-. The behavior is as though they have finally attained an ability to differentiate one odor from the other, which leads to a precipitous change in behavior (an ‘aha!’ effect)”. The second reason for the many different training and testing phases is based on personal experience: bees often become reluctant in their first non-rewarded test if the task is difficult, and may start

to choose at random. Once they get used to landing on unrewarded stimuli for short periods of time – followed by rewarded phases – their performance becomes reliable. These precautionary measures are especially necessary for difficult tasks.

P.3. Learning Rate of Stimulus Components

Here, we tested whether our combined stimuli were salient enough for further experiments. We tested

a) how well the blue and yellow paper stimuli (Heyda brand) absorbed lavender and rosemary scent

b) how well the blue and blue-green paper stimuli (Baehr brand) absorbed lavender and rosemary scent

c) how quickly bees could learn to discriminate the blue and yellow paper stimuli if they were scented with the same scent.

For the similar (blue and blue-green) paper stimuli, we already knew that it takes bees roughly 60 rewards to learn to discriminate them reliably – when unscented (Reinhardt, diploma thesis, 2010). Since added scents make discrimination easier, if anything (Kunze and Gumbert 2001), we decided to always use 60 rewards of training, without any further preliminary experiments. We scented all papers (similar or dissimilar) with 20 µl/litre of air for rosemary, and 50 for lavender, which was above the behavioural threshold, to give bees the opportunity to learn the scents easily.

For experiments a) and b), we trained and tested:

- 20 rewards on AX^+ versus AY
- Learning test of 15 decisions, AX versus AY
- 10 rewards on AX^+ versus AY
- Learning test
- 10 rewards on AX^+ versus AY
- Learning test

For the dissimilar, blue and yellow papers, we trained 16 bees, four for every possible combination (example: blue/lavender versus blue/rosemary); two additional bees disappeared after the first test and were never seen again (we still included their data into later data analysis, making n = 18 for the first test).

For the blue and blue-green papers, we also tested 16 bees, four for every possible combination, for the sake of counter-balance.

In experiment c), we tested 16 bees (again, counter-balanced), following this protocol:

- 20 rewards on AX^+ versus BX
- Learning test of 15 decisions, AX versus BX
- 10 rewards on AX^+ versus BX
- Learning test
- 10 rewards on AX^+ versus BX
- Learning test

After making sure (see *Results*) that the two colour sets had the right salience, that the two scents were dosed equally high for the bees, and that all combinations could be learned easily within 20 rewards (60 for the similar papers), we could go on to the exciting part.

Experiment 1: Under What Circumstances Do Bees Decide by Colour, Under What Circumstances Do They Go by Scent?

To follow up to the question which circumstances make bees decide in favour of which stimulus component, we trained bees on bimodal stimuli which differed both in colour and in scent, and later performed conflict tests. As we had no way of determining the subjective similarity of our two scents within the frame of this thesis, we used only one set of scents for all experiments. The factors we could vary (because we knew the subjective similarity of the stimuli) were the colours.

We developed three different versions of the same experiment:

1. one with differential training on the “different” colour set,
2. one with absolute training on the “different” colour set,
3. one with differential training on the “similar” colour set.

We did not train the bees on the similar colour set using absolute conditioning, because experience suggests that absolute conditioning with such similar stimuli would not lead to learning (Dyer and Chittka 2004).

All experiments under E.1.1 (blue and yellow paper) were conducted over the same time frame between July and October of 2012. E.1.2 experiments (blue and blue-green paper) were conducted in August of 2013.

E.1.1.d and E.1.2.b were inspired by Kriston (1973), whose experiments indicated that bees tend to learn first the scent, and later the colour. When we found that some bees needed fewer rewards to learn their task, and some needed more, we made use of this fact and checked if less-rewarded bees decide in favour of scent more frequently.

E 1.1. Dissimilar Colours (Blue and Yellow)

E.1.1.a. Differential Training with Dissimilar Colours

Bees were trained and tested according to the following protocol.

- Initial training: AX^+ versus BY, 15 rewards.
- Learning test: AX versus BY, 15 decisions.

- If the bee passed the learning test, the first conflict test followed.
- If the bee failed the learning test, it was trained for another 5 rewards (making the number of rewards 20 altogether). Then it went through another learning test. If the bee did not pass, it was discarded; if it passed, it was next conflict-tested. Thus, we had two groups, one with 15 initial rewards and one with 20 initial rewards.

- First conflict test T1: AY versus BX, 15 decisions.
- Training: AX^+ versus BY, 10 rewards.
- Second conflict test T2: AY versus BX, 15 decisions.
- Training: AX^+ versus BY, 10 rewards.
- Third conflict test T3: AY versus BX, 15 decisions.

E 1.1.b. Absolute Training with Dissimilar Colours

Bees were trained according to the following protocol:

- Initial training: AX^+, 15 rewards (counting from the first act of reward when the bee was brought to the arena).
- Learning test: AX versus BY, 15 decisions[6].

- If the bee passed the learning test, the first conflict test followed.
- If the bee failed the learning test, it was trained for another 5 rewards (making the number of rewards 20 altogether). Then it went through another learning test. If it did not pass, it was discarded; if it passed, it was next conflict-tested. Thus, we had two groups, one with 15 initial rewards and one with 20 initial rewards.

- First conflict test T1: AY versus BX, 15 decisions.

[6] In this group, we do not have learning test results for all bees. This was because this series of experiments was the first we did, and the importance of a learning test did not occur to us until we had tested several individuals. For a more elaborate explanation, see E.1.0 in *Results*.

- Training: AX^+, 10 rewards.
- Second conflict test T2: AY versus BX, 15 decisions.
- Training: AX^+, 10 rewards.
- Third conflict test T3: AY versus BX, 15 decisions.

We analysed the data using Rv, and also checked for answers to the following questions: Is there a difference in behaviour between absolutely and differentially trained bees? And does the number of initial rewards in both groups have an influence on choice behaviour?

E.1.2. Similar Colours (Blue and Blue-Green)

Bees were trained according to the following protocol.

- Initial training: AX^+ versus BY, 40 rewards (counting from the first act of reward when the bee was brought to the arena).
- Learning test: AX versus BY, 15 decisions.

- If the bee passed the learning test, the first conflict test followed.
- If the bee failed the learning test, it was trained for another 20 rewards (making the number of rewards 60 altogether). Then it went through another learning test. If it did not pass, it was discarded; if it passed, it was next conflict-tested.

- First conflict test T1: AY versus BX, 15 decisions.
- Training: AX^+ versus BY, 10 rewards.
- Second conflict test T2: AY versus BX, 15 decisions.
- Training: AX^+ versus BY, 10 rewards.
- Third conflict test T3: AY versus BX, 15 decisions.

We analysed the data in Rv, and also looked at the number of initial rewards to see if this factor had a significant influence on choice behaviour. For all three groups of animals tested in experiment E1, we also asked if insecure behaviour (aborted landings) is more common in the learning test or in the first conflict test. This could show if conflict situations influence bee behaviour at all, if the test results fail to give us any information about this question.

Experiment 2: Can Bees Which Prefer One Sensory Modality Learn to Use the Other?

In this experiment, we trained bees on stimuli which differed both in scent and colour. After the learning phase, bees were conflict-tested. If they decided clearly in favour of colour, they were then trained on scent-only stimuli. If they decided clearly in favour of scent, they were subsequently trained on colour-only stimuli.

Further conflict tests were made to check if the bees' strategy of using mainly one sensory modality could be changed, or even reversed. Finally, we checked if the bees still remembered the target stimulus component which had been crucial for their decisions during the first conflict test, and the component on which they had received extra training.

As we could only use bees which showed a clear preference for either scent or colour, we had to discard all bees which did not have a clear preference after the first conflict test.

Bees were trained on the blue and yellow paper and lavender and rosemary oil, as follows:

- First training: AX^+ versus BY. When the bee had collected 20 rewards[7] on AX, it was tested:
- Learning test: AX versus BY, 15 choices. To pass this test, a bee had to choose AX with a rate of at least 80% (12 out of 15 landings).
- First conflict test T1: AY versus BX, 15 choices. If a bee chose at least 80% AY, it was treated as a colour preferring bee. If it chose at least 80% BX, it was treated as a scent-preferring bee. If its choice rate was in between, it was discarded.

Following the first conflict test, bees were trained according to this protocol:

Colour-preferring bees:

- Scent-only training: 30 rewards on X^+ versus Y (scented grey stimuli).
- Conflict test T2: AY versus BX.
- Scent-only training: 30 rewards on X^+ versus Y (scented grey stimuli).
- Conflict test T3: AY versus BX.
- Scent-only training: 30 rewards on X^+ versus Y (scented grey stimuli).
- Conflict test T4: AY versus BX.
- Test on colours A versus B, without any scent components.
- Test on scents X versus Y with grey, scented stimuli.

[7] One bee (Nr 12-93) was accidentally only rewarded 15 times.

Scent-preferring bees:

- Colour-only training: 30 rewards on A^+ versus B (blue and yellow stimuli).
- Conflict test T2: AY versus BX.
- Colour-only training: 30 rewards on A^+ versus B (blue and yellow stimuli).
- Conflict test T2: AY versus BX.
- Colour-only training: 30 rewards on A^+ versus B (blue and yellow stimuli).
- Conflict test T2: AY versus BX.
- Test on scents X versus Y with grey, scented stimuli.
- Test on colours A versus B, without any scent components.

We analysed the data using Rv, asking the following questions: How well did colour-preferring bees learn to favour scent over colour? How well did scent-preferring bees learn to favour colour over scent? How well did both groups later remember the stimulus they had previously preferred, and the component they had received extra training on?

Experiment 3: How Well Do Bees Learn and Reverse-Learn the Components of Bimodal Stimuli? How Well Do Bees Reverse-Learn Multimodal Stimuli?

Here we reverse-trained bees on stimuli which differed both in scent and colour. We also checked how well they had learned the components of the stimuli, which they had never been shown alone but only together in a compound.

We trained 16 bees on the blue and yellow papers paired with rosemary and lavender, and 16 bees to the blue and blue-green papers paired with the same scents. Training for the blue and yellow bees went as follows:

- First training: AX^+ versus BY, 20 rewards.
- Learning test: AX versus BY, 10 landings.

If the bee chose less than 90% correctly at this point, it was discarded. Otherwise it was now tested on the components only. Half the bees of each group (considering also the counter-balance of original target stimuli) were tested first for colour, then for scent; the rest, vice versa. We therefore had 4 bees trained to each stimulus combination, out of which 2 were first tested for colour and 2 were first tested for scent. We did this to eliminate any effects of resentment in the bees during the last phase of the unrewarded testing. In the following example, the bee is first tested on scent, then colour.

- Scent test: X versus Y, 10 landings.
- Colour test: A versus B, 10 landings.
- Reverse training: AX versus BY^+, for 30 rewards.

- Learning test: AX versus BY, 10 landings.
- Scent test: X versus Y, 10 landings.
- Colour test: A versus B, 10 landings.
- Re-reverse training: AX^{+} versus BY, for 30 rewards.
- Learning test: AX versus BY, 10 landings.
- Scent test: X versus Y, 10 landings.
- Colour test: A versus B, 10 landings.

Training for the blue-and-blue-green bees followed exactly the same protocol, with the difference that the initial training phase had 60 rewards, not 20. At 40 rewards, bees were given a "sham" learning test of 10 unrewarded landings – this only served the purpose of showing bees that the source could run dry briefly, but not permanently. This, in turn, helped to avoid frustration in the "real" learning test after 60 rewards (personal observation).

With this training and testing protocol we could analyse both reverse-learning of a multimodal stimulus, and how well its components were learned and reverse-learned.

Finally, we also looked at the question: how long did it take bees to switch? Or, more exactly: How long do bees continue to choose the previously-rewarded (now unrewarded) stimulus before deciding to give the previously-unrewarded (now rewarded) one a try? To answer this question, we compared the results of the similar and dissimilar colour groups.

Results

Preliminary Experiments

P.1. Measuring Colour

The results of the measurements are shown in Fig. 7 and 8 in the *Introduction.* The plot shows that the saturated blue and yellow stimuli are more saliently different for bees than the unsaturated blue and blue-green.

P.2. Finding the Detection Threshold for Symrise Lavender and Rosemary Oil

To find the behavioural detection threshold for lavender and rosemary, we trained bees on the rewarded visual stimulus A^+ versus the unrewarded bimodal stimulus AX with logarithmically decreasing concentrations of scent X. Bees were tested up to 5 times on each scent concentration (with 10 rewards between tests, making it up to 50 rewards per scent dose). Each test consisted of 15 landings. To pass, a bee had to choose at least 12 out of 15 landings correctly. If the bees passed, they were then trained and tested on the next lower dose. If they failed five times on a particular scent dose, the experiment was over.

In 2012, we started with 0,01 µl/litre of air in a box, a dose which bees could still clearly perceive; in 2013, however, we started with 0.1 µl/litre, to give bees an easier start.

Tab. 2: Results for the six bees trained on rosemary scent.

Scent conc. > Bee	0.1 µl/l	0.01 µl/l	0.001 µl/l	0.0003 µl/l	0.0001 µl/l
12-B30	Not tested	Test 4 passed	Test 1 passed	Test 1 passed	Not passed
12-B23	Not tested	Test 1 passed	Test 1 passed	Test 2 passed	Not passed
12-B22	Not tested	Test 3 passed	Test 2 passed	Not passed	Not passed
13-B49	Test 2 passed	Test 3 passed	Not passed	Not passed	Not passed
13-B44	Test 1 passed	Test 2 passed	Test 4 passed	Not passed	Not passed
13-B47	Test 1 passed	Test 1 passed	Test 2 passed	Not passed	Not passed

We started out on the hypothesis that rosemary would be much easier to perceive for the bees than lavender (see *Material and Methods*), and so we began this experiment with lavender dosed 2.5 times as high as rosemary for dose compensation. This guess turned out to be quite correct, and no further dose compensation was necessary.

Tab. 3: Results for the six bees trained to lavender scent.

Scent conc. > Bee	0.25 µl/l	0.025 µl/l	0.0025 µl/l	0.00075 µl/l	0.00025 µl/l
12-B25	Not tested	Test 1 passed	Test 1 passed	Not passed	Not passed
12-B24	Not tested	Test 1 passed	Test 5 passed	Not passed	Not passed
12-B31	Not tested	Test 4 passed	Test 2 passed	Not passed	Not passed
13-B43	Test 1 passed	Test 1 passed	Test 3 passed	Not passed	Not passed
13-B45	Test 1 passed	Test 4 passed	Test 1 passed	Not passed	Not passed
13-B48	Test 1 passed	Test 4 passed	Test 4 passed	Test 2 passed	Not passed

The results could not be statistically analysed further due to the irregular schedule of training and testing. However, the results are quite homogenous, lying around 0.001 µl/litre of air (0.0025 for lavender).

In all the following experiments, we dosed lavender 2.5 times as high as rosemary: 20 µl/litre of air for rosemary, and 50 for lavender.

P.3. Learning Rate of Stimulus Components

Here, we checked if the two different brands of paper (Heyda, blue and yellow; and Baehr, blue and blue-green) absorbed the stimulus scents well enough for the purposes of our experiments. We did this by training and testing bees on stimuli which had the same colour, but different scents (AX versus AY). We also checked how quickly a bee could learn to discriminate the Heyda paper colours. To keep this last experiment in line with the first two, we scented both papers with the same scent (AX versus BX). We did not conduct the same test for Baehr paper, because we already had experimental experience in how long it took bees to learn to discriminate the colour (see Reinhardt, diploma thesis, 2010).

We analysed the learning rate and results of the tests. Tests consisted of 15 unrewarded landings. During the training phase, one data point (one box in the boxplots below) was made up of 10 correct rewarded landings, and all the incorrect landings the bee made in between, no matter how many exactly they were.

Experiments with Heyda paper were conducted in June of 2012, experiments involving Baehr paper were conducted in May and June of 2013.

P.3.a. Blue and Yellow Paper, Learning Rate for Different Scents

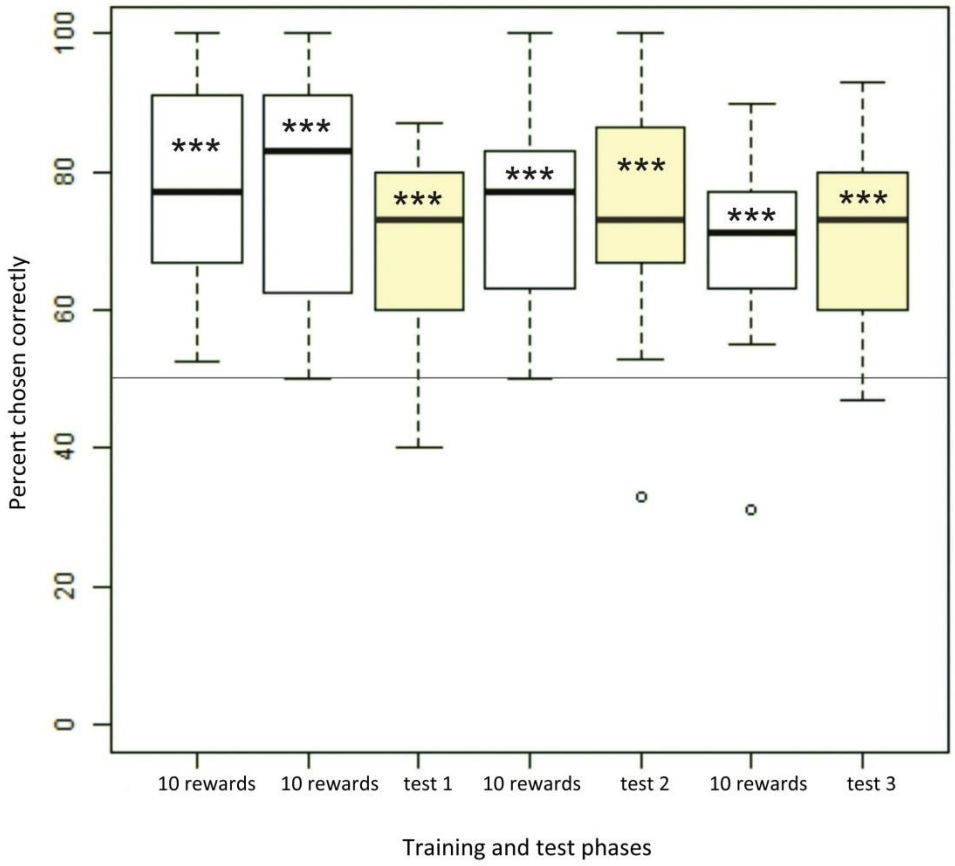

Fig. 10: Results for the AX versus AY experiment with Heyda paper. As all the data groups above were normally distributed, t-tests were used to test against chance level. The bees' performance was highly significantly different from chance level already after 10 rewards to AX (***, $p < 0.001$). Coloured boxes show test results, white boxes show learning during training. n= 18 up to test 1, after that n = 16.

The data recorded and used includes 16 bees (4 trained on each possible scent/colour combination), plus data from two bees which took part in the experiment only until the end of the first test. Since training a single bee was quite a lot of work, we decided to include these two, rather than to exclude existing results.

It is apparent that bees can easily learn to discriminate between the two scents if they are presented to them on Heyda paper (Fig. 10). This result shows not only that this kind of paper absorbs the scent well enough for our experiments, but also that 10 rewards are enough to teach bees reliably which scent is rewarded.

P.3.b. Blue and Blue-Green Paper, Learning Rate for Different Scents

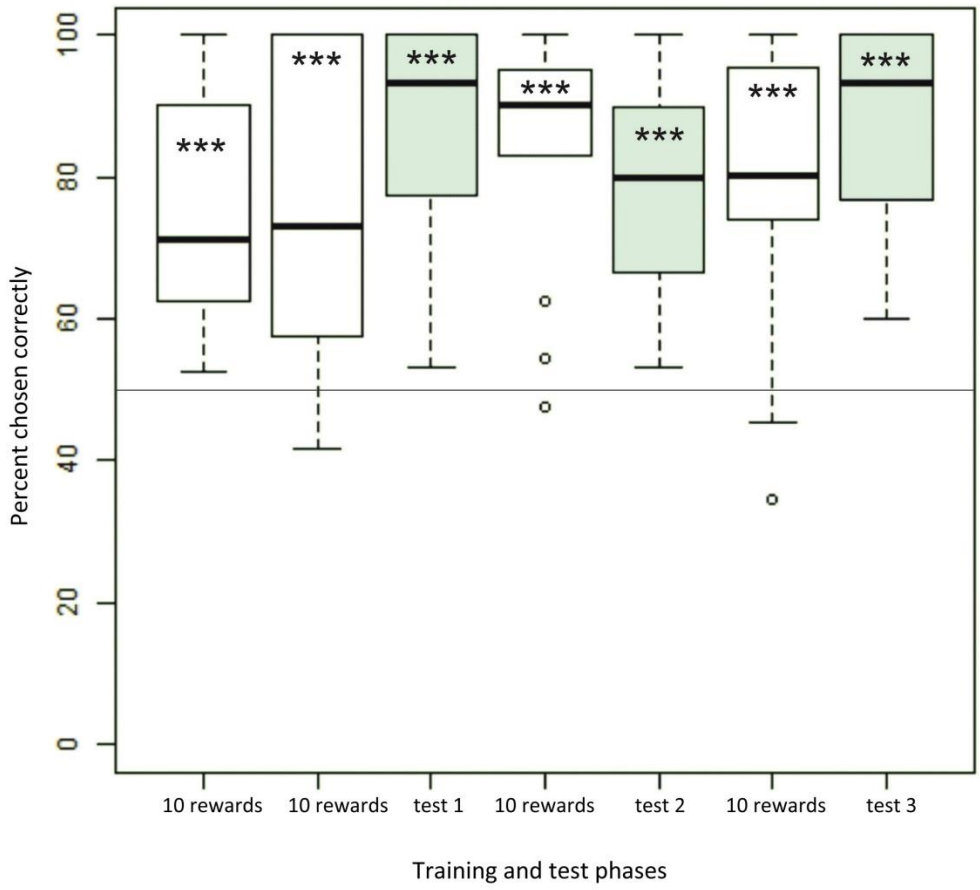

Fig. 11: Results for the AX versus AY experiment with Baehr paper. Normally distributed data was tested against chance level using a t-test, non-normally distributed data was checked using a Wilcoxon test. The bees performed highly significantly different from chance level already after 10 rewards to AX (***, $p < 0.001$). Coloured boxes show test results, white boxes show learning during training. n= 16, counter-balanced.

Similar to the results of P.3.a, the kind of paper used here absorbs the training scents well enough to allow bees to learn to discriminate the two scents within 10 rewards (Fig. 11).

Since the grey “uncoloured” stimulus paper was from the same brand, we decided that would most likely take on the scent equally well as the two coloured papers, and we prepared the grey paper exactly as we would prepare blue and blue-green bimodal stimuli.

P.3.c. Blue and Yellow Paper, Learning Rate for Two Colours

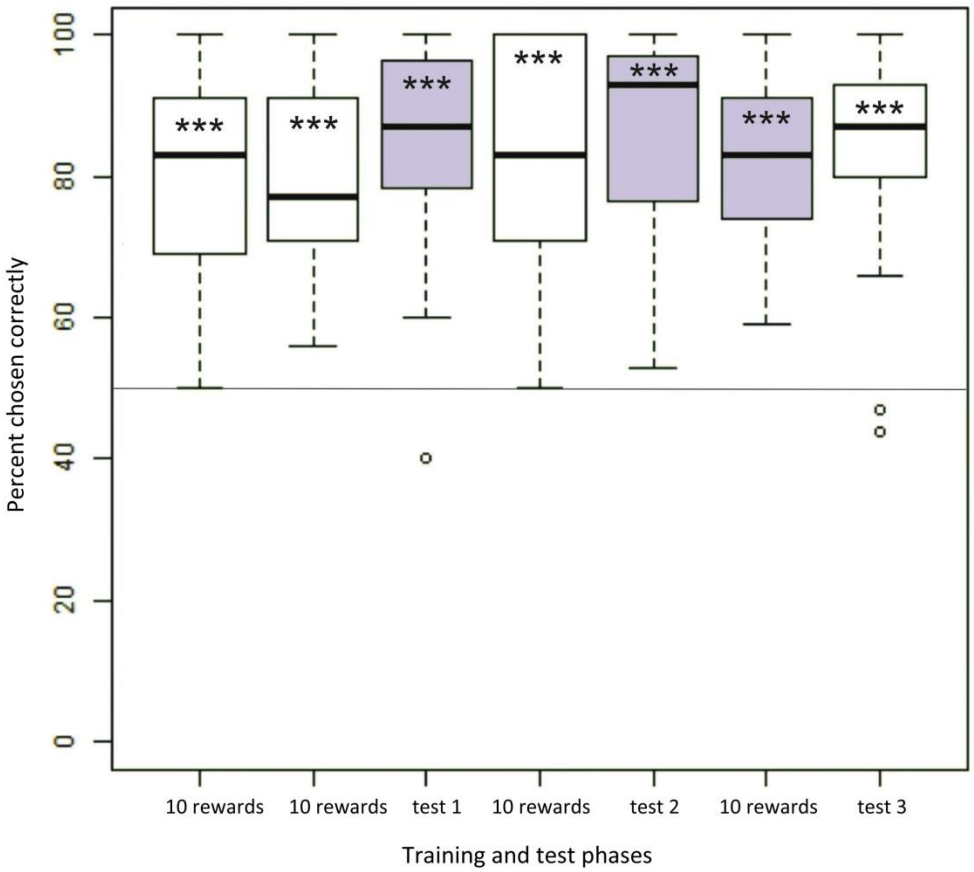

Fig. 12: Results for the AX versus BX experiment with Heyda paper. Normally distributed data was tested against chance level using a t-test, non-normally distributed data was checked using a Wilcoxon test. The bees performed highly significantly different from chance level after only 10 rewards to AX (***, $p < 0.001$). Coloured boxes show test results, white boxes show learning during training. n= 16, counter-balanced, plus data from two bees which disappeared after Test 1, and one bee which disappeared just before Test 2.

The results of this experiment show that bees can discriminate the saturated blue and yellow paper easily after 10 rewards (Fig. 12), if scented with the same scent.

Experiment 1: Under What Circumstances Do Bees Decide by Colour, Under What Circumstances Do They Go by Scent?

In this experiment we tested bees with scent-colour combinations of different salience, and determined with which combination bees chose more by scent, and with which combination they chose more by colour. Our questions were:

E.1.0. Is there a preference for any stimulus combination?

E.1.1.a. When trained differentially to a set of dissimilar colours, do bees choose by colour or by scent?

E.1.1.b. When trained absolutely to a set of dissimilar colours, do bees choose by colour or by scent?

E.1.1.c. Is there a significant difference in behaviour between the above two groups?

E.1.1.d. Does the number of initial rewards influence choice rates?

E.1.2.a. When trained differentially to a set of similar colours, do bees choose by colour or by scent?

E.1.2.b. Does the number of initial rewards influence choice rates?

E.1.3. Do bees react to conflict tests with insecure choice behaviour?

All bees were trained on two bimodal stimuli, AX^+ versus BY, for 15 or 20 rewards (depending on how quickly a bee would learn). They then went through a learning test (AX versus BY), and afterwards through several conflict-tests (AY versus BX) with more training intervals in between.

E.1.0. Is There a Preference for Any Stimulus Combination?

Before answering any of the other questions, we had to check if there was an innate preference for any particular stimulus combination. We did this for the similar and the dissimilar stimuli separately (dissimilar: lavender/blue, rosemary/yellow, rosemary/blue, and lavender/yellow; similar: lavender/blue, rosemary/blue, lavender/blue-green and rosemary/blue-green).

For the blue and yellow stimuli, we checked for normal distribution of the data groups, and found that the “lavender-blue” data were not normally distributed but all others were. Consequently, we used a Kruskal-Wallis test, with which we compared all four data sets to each other, to see if there was a difference between them. We found no significant differences (Fig. 13).

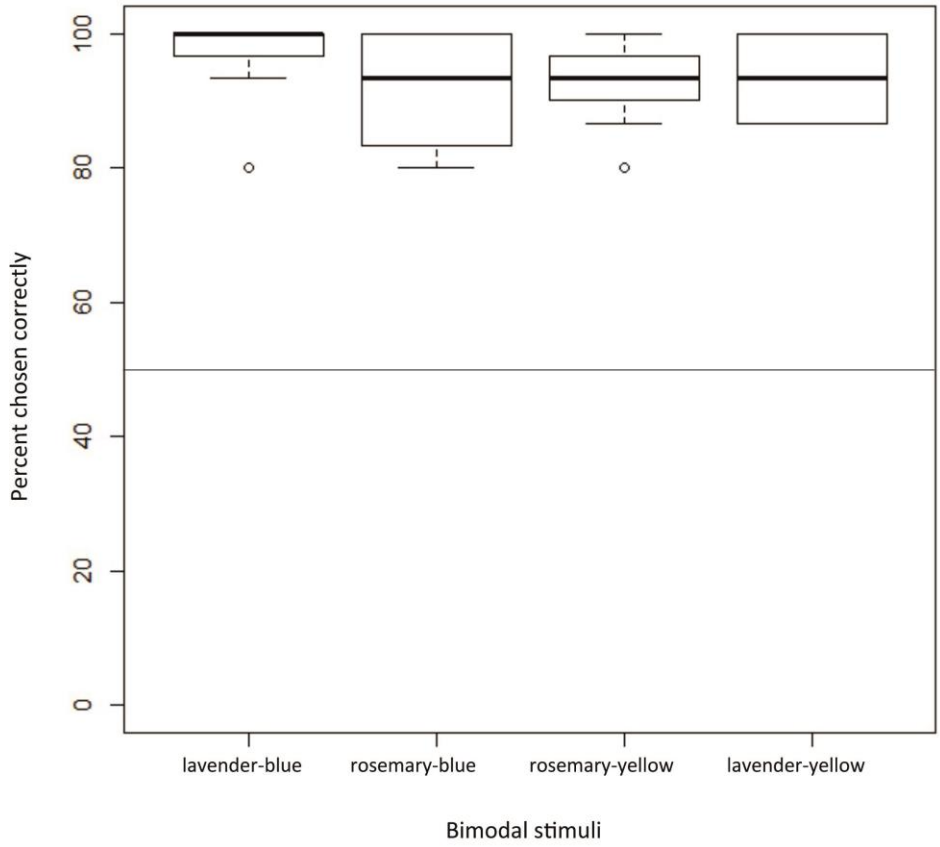

Fig. 13: Results of the learning test for bees trained on combinations of Heyda blue, yellow, lavender and rosemary (E.1.1). We pooled the data of differentially and absolutely trained bees for this test. For lavender blue and rosemary blue, n = 7 each. For rosemary yellow, n = 8, and for lavender yellow, n = 6.

Unfortunately, we only have learning test data for 28 of the 41 bees trained and tested for E.1.1. We therefore pooled the absolutely and differentially trained bees. But we are confident that all bees had learned the combination reliably: of all the individuals which we trained on blue and yellow stimuli over the course of this 3-year work, only one failed the learning test.

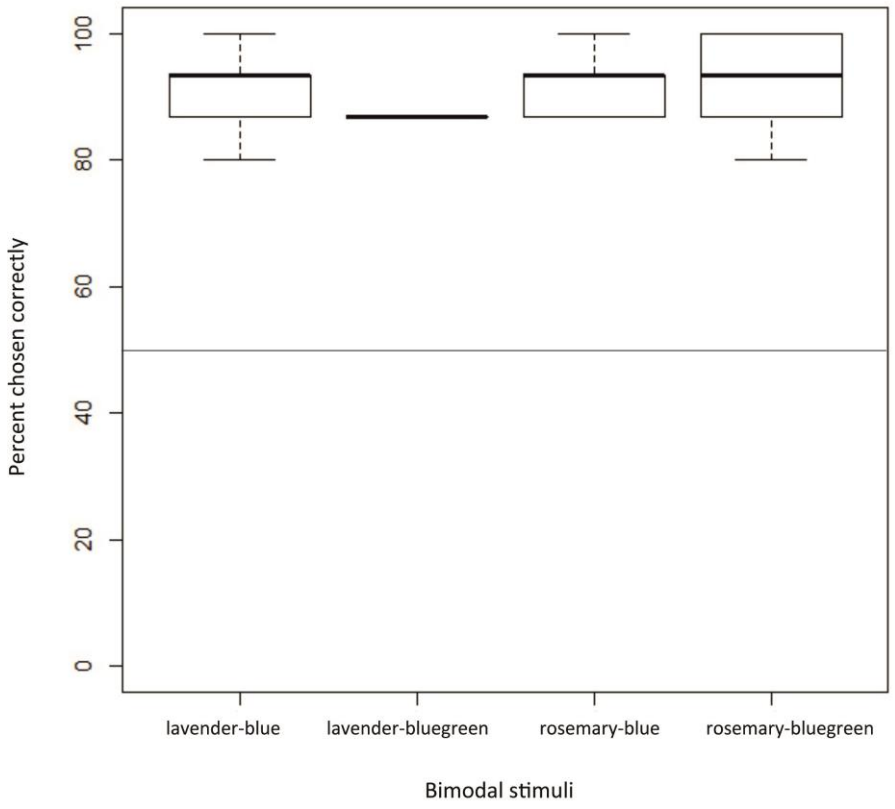

Fig. 14: Results of the learning test for bees trained on combinations of Baehr blue, blue-green, lavender and rosemary (E.1.2). We used a Kruskal-Wallis test and found no significant differences between any of the groups. n= 5 for each box.

For the blue and blue-green stimuli, we again tested for normal distribution and found that all data were normally distributed (Fig. 14). However, we conducted a Bartlett test and found out that the variances of the data sets were inhomogenous, so we conducted a Kruskal-Wallis test.

The tests revealed no differences between the groups of bees trained to different AX^+ combinations (Fig. 13 and 14, respectively). This is not a surprise, since bees with less than 80% correct choices were discarded from the experiment. But it was important to make sure that no stimulus combination was especially favoured by the participating bees by the time the conflict testing started.

E.1.1. Dissimilar Colours (Blue and Yellow)

E 1.1.a. Differential Training with Dissimilar Colours

Bees were trained for 15 or 20 rewards on the stimulus combinations AX^+ versus BY, then tested on AX versus BY (a simple learning test) and finally conflict-tested three times (AY versus BX), with 10 more rewards in between conflict tests. We asked whether bees decide by colour or by scent under this condition, and whether there is a development in one direction or the other (Fig. 15).

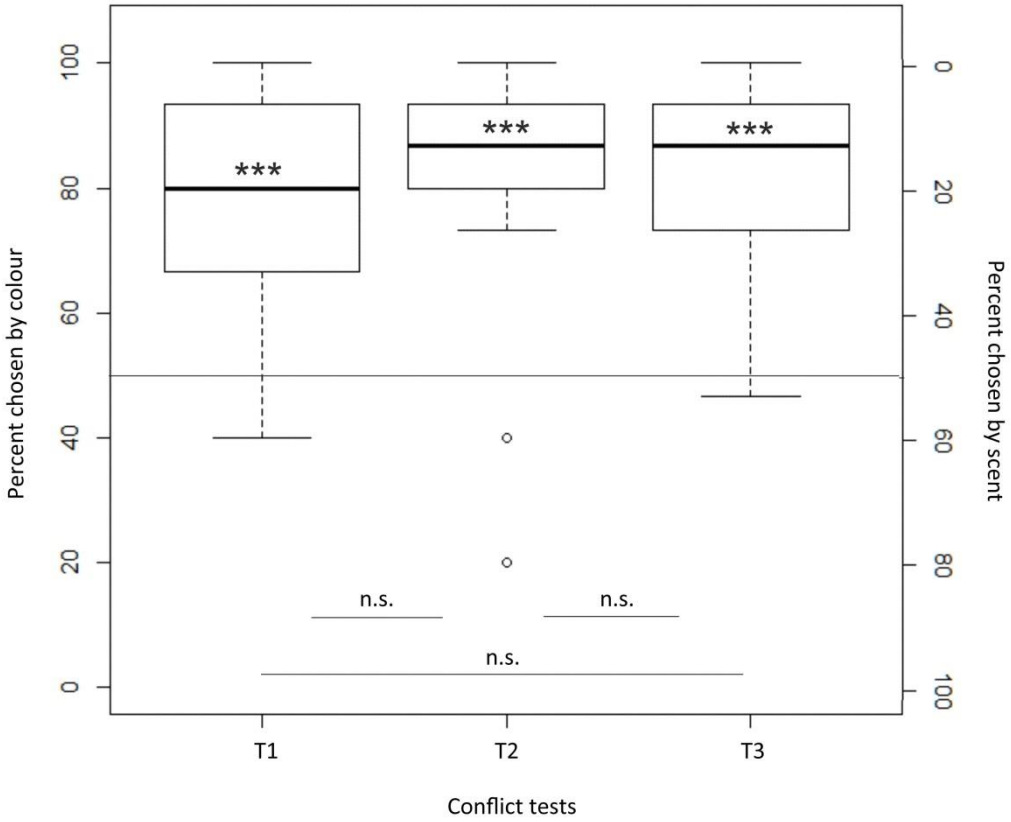

Fig. 15: Results of the conflict tests T1-T3 for bees trained (differentially) and tested on Heyda colours (blue and yellow). Results were highly significantly different from chance (***, $p < 0.001$), but not from each other (n.s., $p \geq 0.05$). n = 21.

T1 data is normally distributed, T2 and T3 are not (Shapiro-Wilk test). We checked for a significant difference from chance level, using a t-test on T1 and a Wilcoxon test on T2 and T3. All three results are highly significantly different from chance level: bees chose highly significantly in favour of colour.

We also compared the results of T1 to T2, T1 to T3, and T2 to T3, using Wilcoxon tests for paired samples, and found no significant differences: there is no change in the bees' behaviour between the 20^{th} and 40^{th} reward.

We accidentally trained and tested one bee too many on the combination lavender/blue, but afterwards decided to keep its data rather than to discard them for the sake of strict counter-balance.

E 1.1.b. Absolute Training with Dissimilar Colours

Bees were trained for 15 or 20 rewards on the stimulus combination AX^+, then tested to AX versus BY (learning test), and conflict-tested three times (AY versus BX), with 10 more rewards in between conflict tests. We asked whether bees decide by colour or by scent in this setup, and whether there is a development in one direction or the other (Fig. 16).

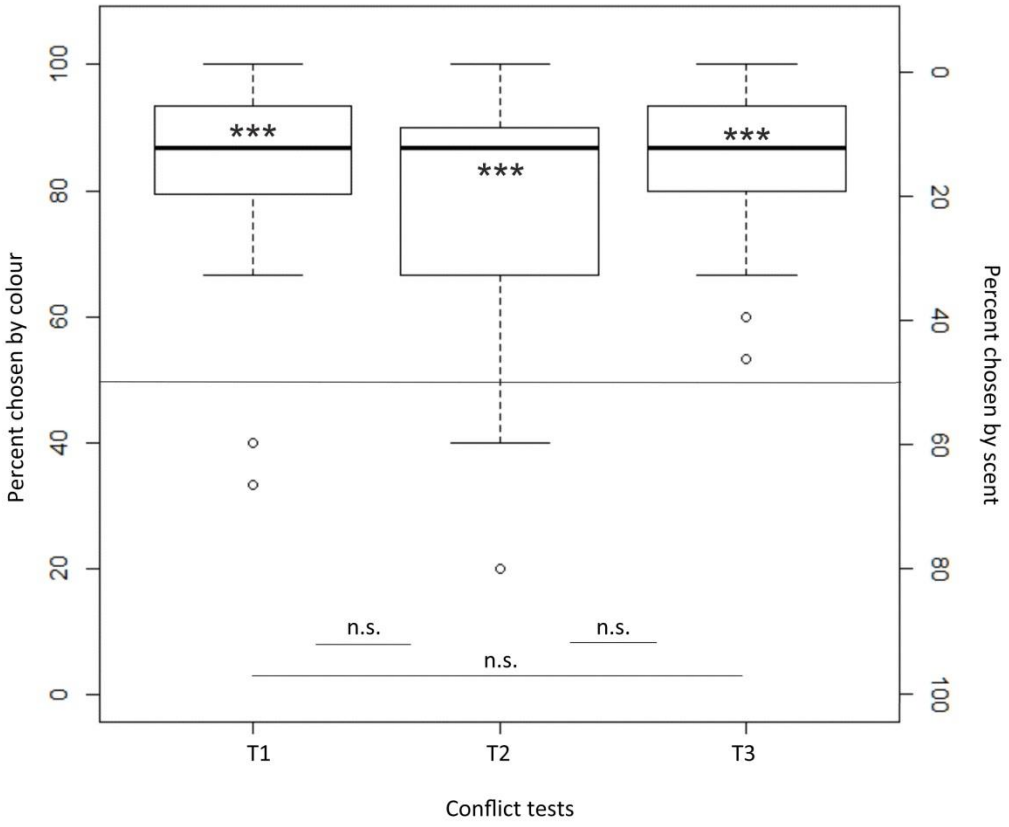

Fig. 16: Results of the conflict tests T1-T3 for bees trained (absolutely) and tested on Heyda colours (blue and yellow). Results were highly significantly different from chance (***, $p < 0.001$), but not from each other (n.s., $p \geq 0.05$). n = 20.

None of the three data sets are normally distributed (Shapiro-Wilk test). We checked for a significant difference from chance level, using a Wilcoxon test. All three results are highly significantly different from chance level, showing that bees chose highly significantly in favour of colour.

We also checked for significant differences between T1 and T2, T1 and T3, and T2 and T3, using Wilcoxon tests for paired samples, and found none: the bees' behaviour between the 20^{th} and 40^{th} reward does not differ significantly.

E.1.1.c. Is There a Significant Difference in Behaviour Between the Above Two Groups?

We asked whether the two groups (absolutely and differentially trained) behaved differently in the conflict tests (Table 4). Tests were conducted using a Wilcoxon test for non-paired samples. There are no significant differences in the choice rates of differentially and absolutely trained bees ($p \geq 0.05$).

Tab.4: results of tests between the differentially and the absolutely trained groups.

T1 absolute versus T1 differential	no significant difference
T2 absolute versus T2 differential	no significant difference
T3 absolute versus T3 differential	no significant difference

E.1.1.d. Does the Number of Initial Rewards Influence Choice Rates?

To see if bees made more choices in favour of colour after longer training, as suggested by Kriston (1973), we pooled the data of the differentially and absolutely trained bee groups. We then compared the results of the first conflict test for the two groups which had been trained for different numbers of rewards (Fig. 17).

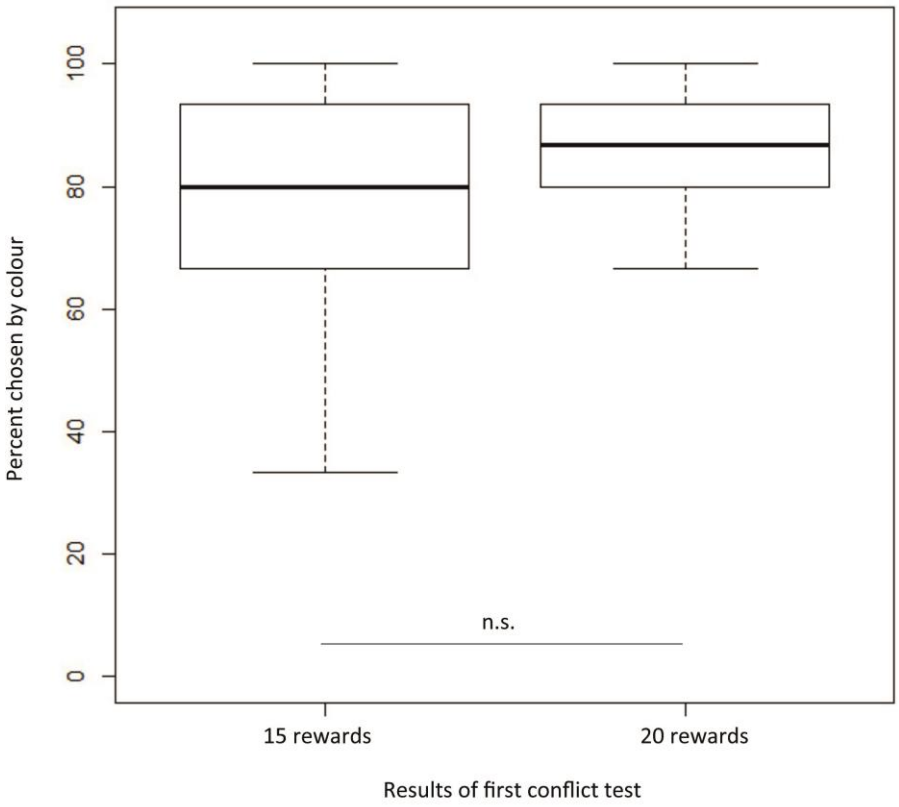

Fig. 17: Comparison of data from bees which had learned the task after 15 rewards (n = 31), and bees which had learned the task after 20 rewards (n = 10; n.s., $p \geq 0.05$).

We tested both groups for normal distribution. The 15-reward group data set is not normally distributed, the 20-reward group data set is. We then checked for a significant difference between the two groups, using a Wilcoxon test for non-paired samples, and found none.

E.1.2. Similar Colours (Blue and Blue-Green)

E 1.2.a. Differential Training with Similar Colours (Blue and Blue-Green)

Bees were trained for 40 or 60 rewards on the stimulus combinations AX^+ versus BY, then tested on AX versus BY (learning test), and finally conflict-tested three times (AY versus BX), with 10 more rewards in between conflict tests. We asked whether bees decide by colour or by scent in this setup, and whether there is a development in one direction or the other (Fig. 18).

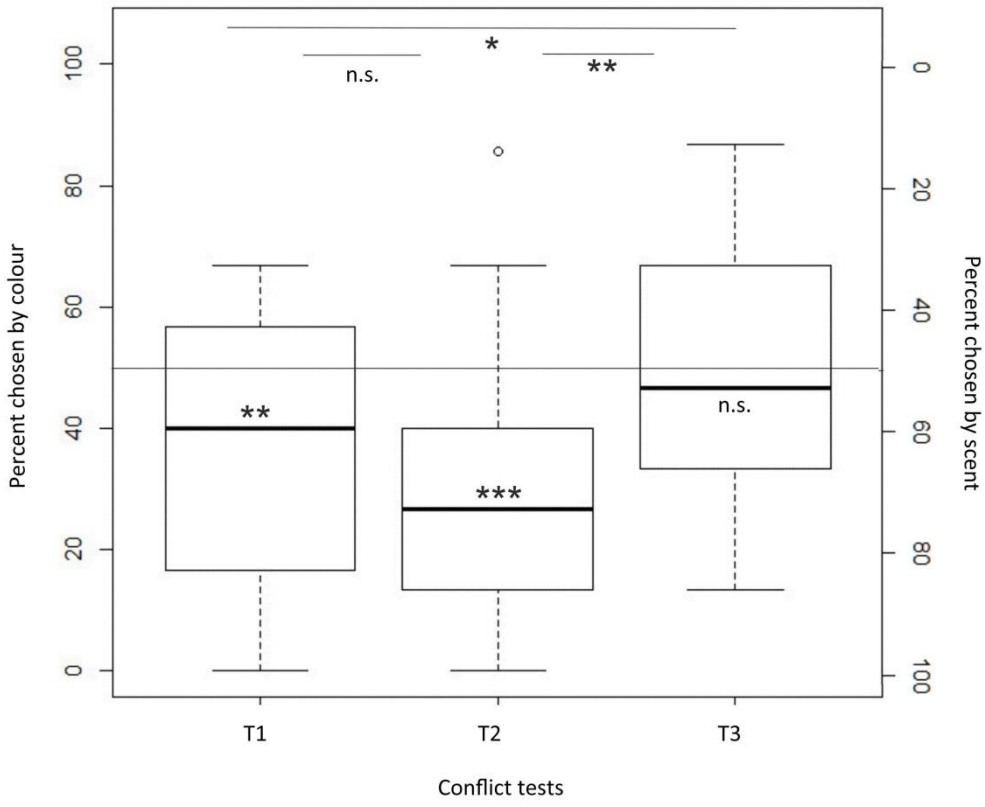

Fig. 18: Results of the conflict tests T1-T3 for bees differentially trained and tested on Baehr colour stimuli, blue and blue-green (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 20.

The results of all three tests are normally distributed (Shapiro-Wilk test). We used a t-test to check for significant differences from chance level, and a t-test for paired samples to determine if there were differences between the results of the three tests. In the first and second test, bees decided clearly in favour of scent, in the third test their choices were random: their behaviour changed significantly in favour of scent between test 1 and 2, and very significantly towards random choices between test 2 and 3.

E.1.2.b. Does the Number of Initial Rewards Influence Choice Rates?

Again, we tested whether we were able to reproduce the effect described by Kriston (1973) by comparing the first conflict test data from bees which had passed the learning test after 40 rewards, and those which had passed it after 60 rewards (Fig. 19).

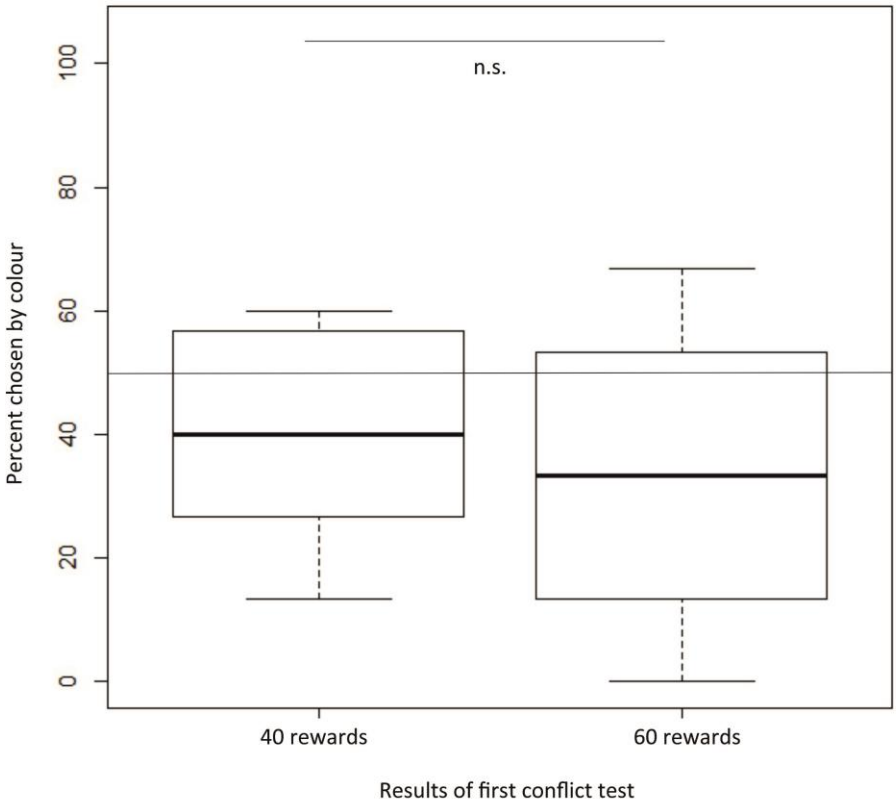

Fig. 19: Results of the first conflict test for bees which had been trained for 40 rewards (n = 8), and bees which had been trained for 60 rewards (n = 12; n.s., $p \geq 0.05$).

Both groups are normally distributed (Shapiro-Wilk test). We checked for a significant difference between the two data sets, using a Welch Two-Sample t-test for unpaired samples, but found no difference.

E.1.3. Do Bees React to Conflict Tests with Insecure Choice Behaviour?

To find out if the unfamiliar bimodal stimuli in conflict tests made bees insecure in their choice behaviour, we compared the abort rates (both target and distractor aborts) between the learning test (during which bees should be quite confident which stimulus was correct) and the results of the first conflict test (where bees might be a lot more confused). We did this with all three groups: differentially or absolutely trained either to blue and yellow paper (Fig. 20 and 21, respectively), and differentially trained to blue and blue-green paper (Fig. 22).

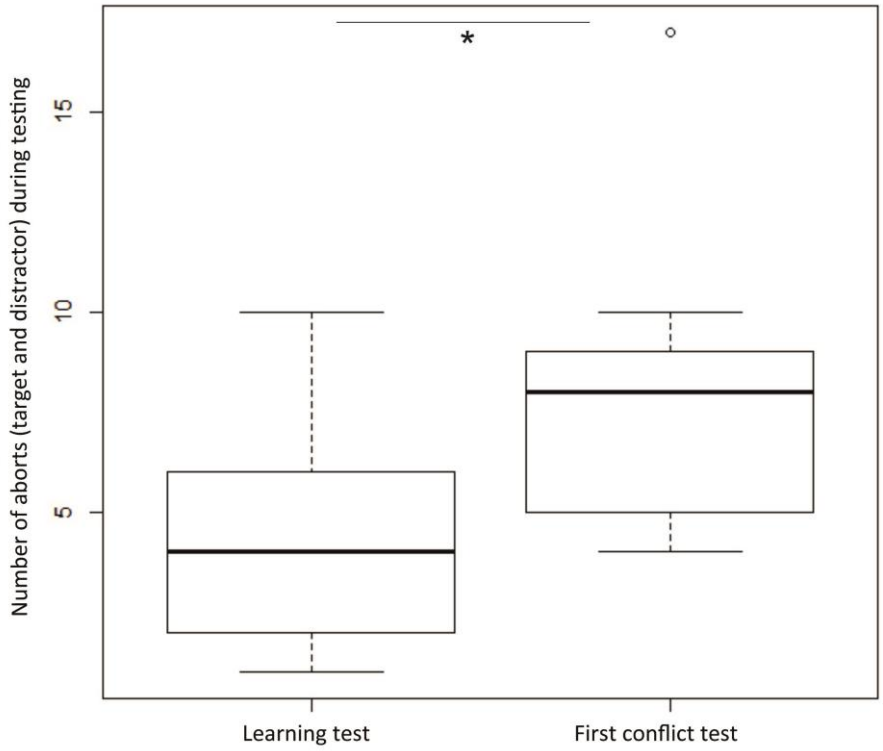

Fig. 20: Comparison of aborts in bees which had been trained differentially to the blue and yellow stimuli. n = 9. We only included bees which had gone through *both* the learning test and the conflict test (*, $p < 0.05$).

We first checked for normal distribution, using the Shapiro-Wilk test, and found that both data sets are normally distributed. So we checked for significant differences using a t-test for paired samples, and found a significant difference: bees had been significantly more certain of their choices during the learning test than during the first conflict test.

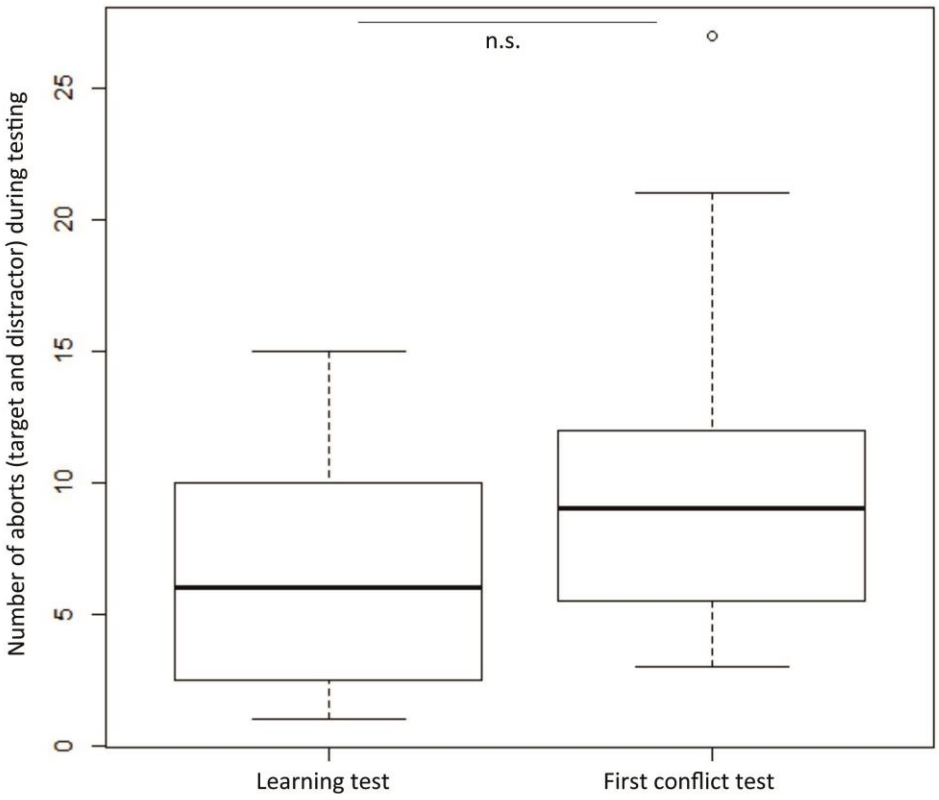

Fig. 21: Comparison of aborts in bees which had been trained absolutely to the blue and blue-green paper stimuli (n.s., $p \geq 0.05$). n = 20.

We first checked for normal distribution, using a Shapiro-Wilk test, and found that only the data set of the learning test is normally distributed. So we checked for significant differences using a Wilcoxon test for paired samples, and found no differences: bees were no more certain of their choices during the learning test than they were during the first conflict test.

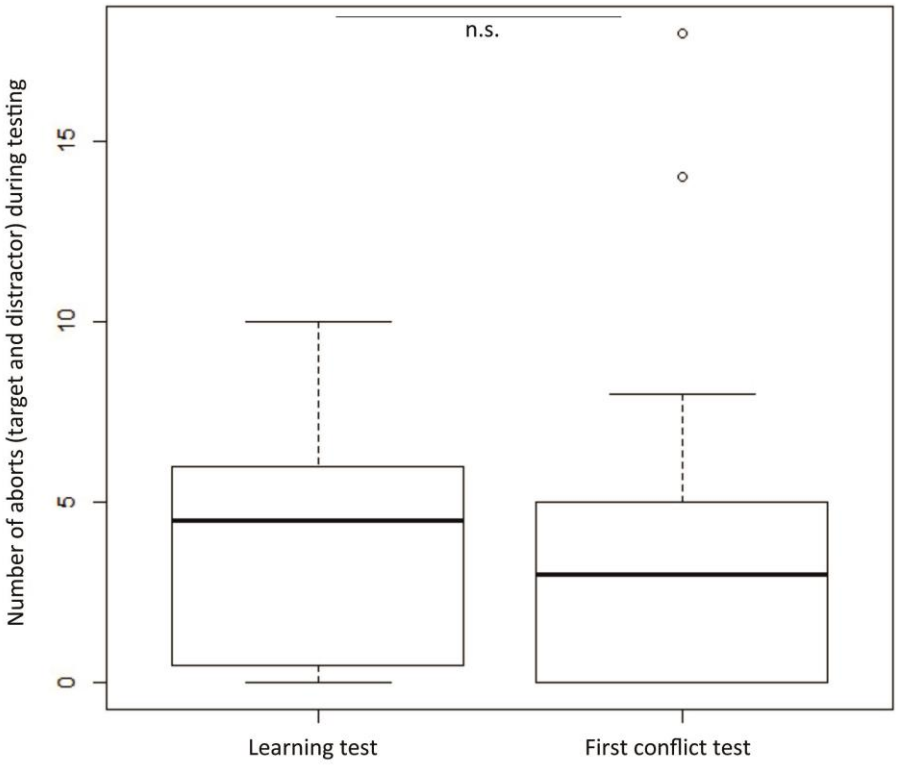

Fig. 22: Comparison of aborts in bees which had been trained differentially to the blue and blue-green paper stimuli (n.s., $p \geq 0.05$). n = 20.

We first checked for normal distribution, using a Shapiro-Wilk test, and found that no data set is normally distributed. So we checked for significant differences using a Wilcoxon test for paired samples, and found no differences. Here, too, bees were equally certain about their choice in the learning test and in the conflict test.

Experiment 2: Can Bees Which Prefer One Sensory Modality Learn to Prefer the Other?

In this experiment, we examined whether bees which use primarily one sensory modality and ignore the other can be trained to do the opposite. We did this by giving the bees extra training on the previously-ignored modality, and conducting several conflict tests to show how their choice behaviour changed.

Our questions were the following:

E.2.0. Is there a preference for any particular stimulus combination?

E.2.1.a. How well do colour-preferring bees learn to favour scent over colour?

E.2.1.b. Do they later remember the colour stimulus they originally used as their main cue?

E.2.2.a. How well do scent-preferring bees learn to favour colour over scent?

E.2.2.b. Do they later remember the scent stimulus they originally used as their main cue?

We had to discard several bees which had no clear preference after the first conflict test, and one bee due to not passing the learning test. All experiments were conducted during August and September of 2012. In this experiment, we used only the very different, highly saturated blue and yellow colour stimuli.

E.2.0. Is There a Preference for Any Stimulus Combination?

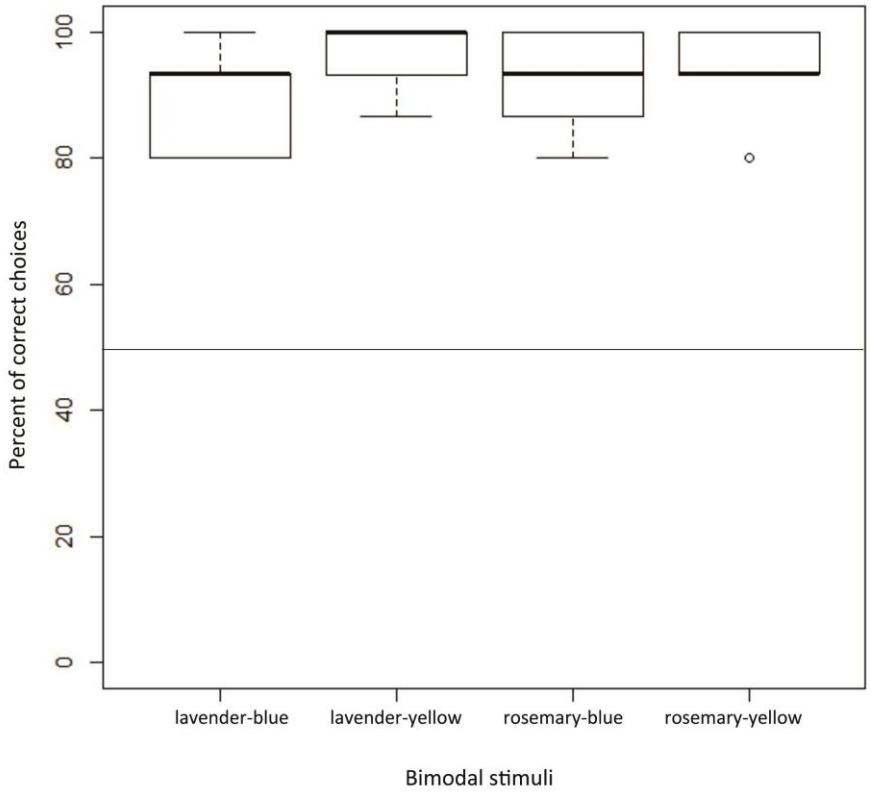

Fig. 23: Boxplots showing the behaviour of bees during the learning test. We found no significant difference between any of the groups. n = 25, as for three bees no initial learning test was made. n for each box is 7, except for rosemary yellow (n = 5) and lavender blue (n = 6).

We compared the results of the learning test to see if there was any preference for a particular stimulus combination. We first checked for normal distribution, using a Shapiro-Wilk test, and found that only two of the four sets are normally distributed. Consequently, we tested for significant differences using a Kruskal-Wallis test (Fig. 23), but found no differences.

E.2.1. Colour-Preferring Bees

E.2.1.a. How Well Do Colour-Preferring Bees Learn to Favour Scent over Colour?

We plotted the conflict test data of all colour-preferring bees into boxplots, and performed a Shapiro-Wilk test. We found out that all data sets are normally distributed except for set T1. We then tested the four results against chance level, using a Wilcoxon test for T1 and the t-test for T2, T3 and T4.

We then compared T1 to T2, using a Wilcoxon test for paired samples, and T2 to T3, and T3 to T4, using a t-test for paired samples.

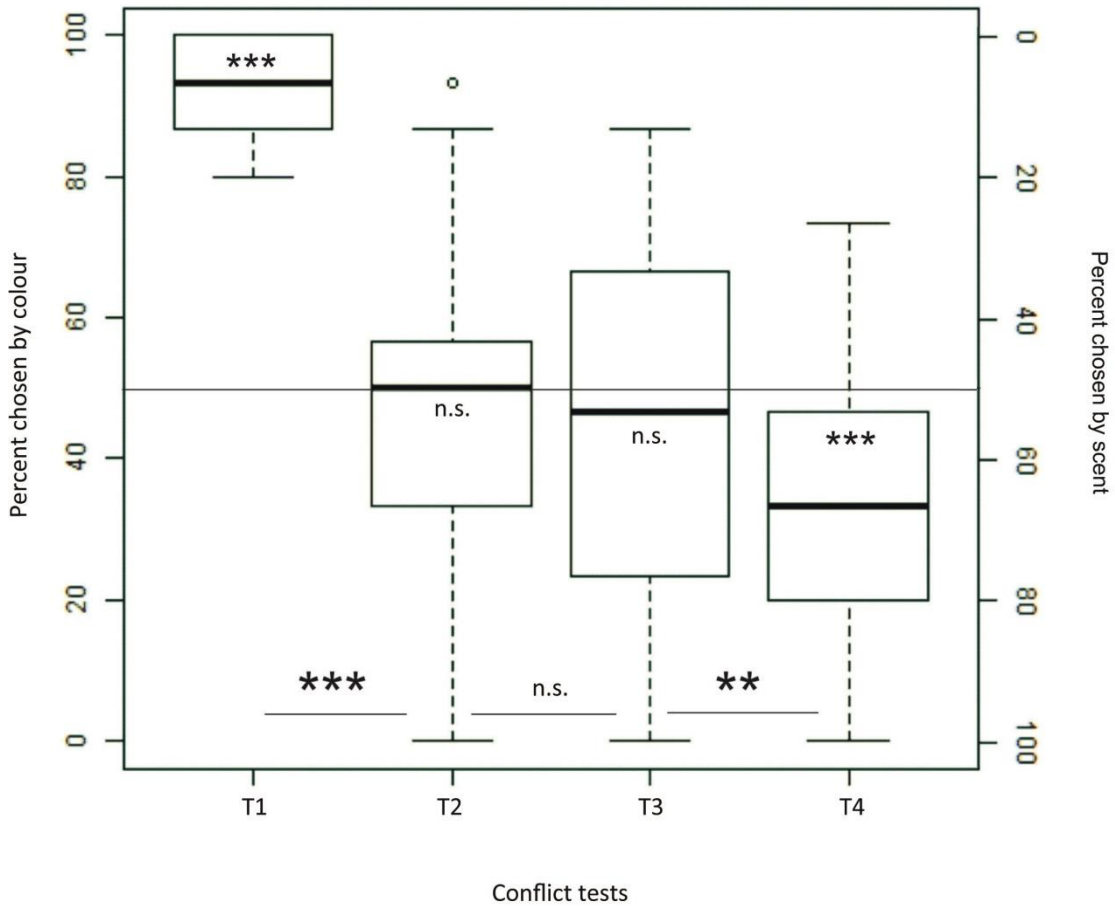

Fig. 24: Results of the conflict tests of 24 colour-preferring bees. T1: after 20 rewards during differential training. T2, T3 and T4: after 30, 60 and 90 rewards in differential training on scent-only stimuli (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 24.

There is a highly significant difference in the behaviour of the bees before training on scent-only stimuli (T1), and after 30, 60 and 90 rewards on scent-only stimuli (T2, T3 and T4, respectively). Between rewards 60 and 90 (T3 and T4), there is even another significant

change in favour of scent. Also, results in T4 are highly significantly different from chance level.

E.2.1.b. Do They Later Remember the Colour Stimulus They Originally Used as Their Main Cue?

At the end of the experiment series, we had tested bees on A versus B (colours only), then X versus Y (scent only). To analyse these data, we first tested for normal distribution (Shapiro-Wilk test) and then, as both sets are normally distributed, checked for a significant difference from chance level, using a t-test (Fig. 25).

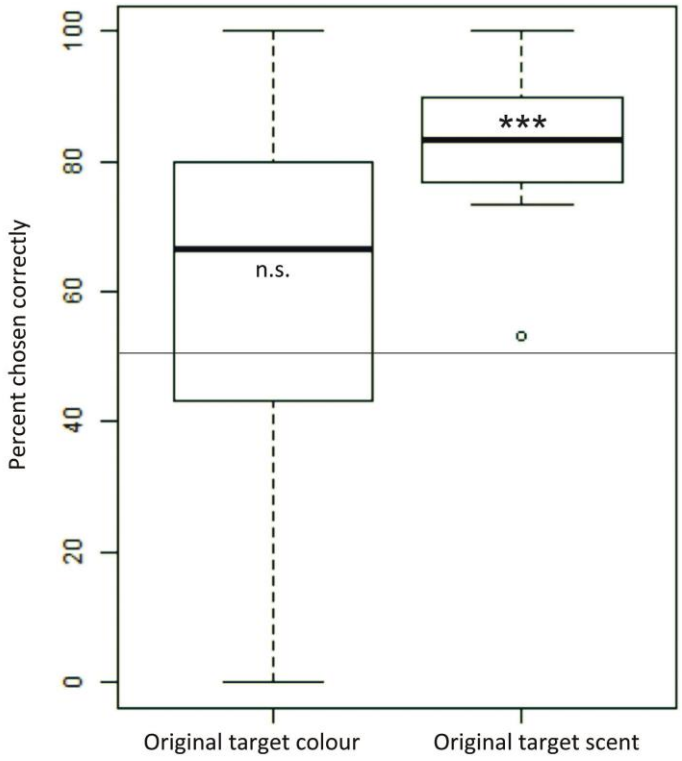

Fig. 25: Preference for original target colour and original target scent were measured after 90 rewards in differential scent-only training, and four conflict tests (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 24.

The data for the original target colour are not significantly different from chance level. The original target scent data, however, are clearly and highly significantly different.

E.2.2. Scent-Preferring Bees

E.2.2.a. How Well Do Scent-Preferring Bees Learn to Favour Colour over Scent?

Due to the small number of animals which preferred scent (n = 4), we could show no significant differences to chance levels, or changes in behaviour, using statistical methods. A common tendency is, however, obvious (Fig. 26).

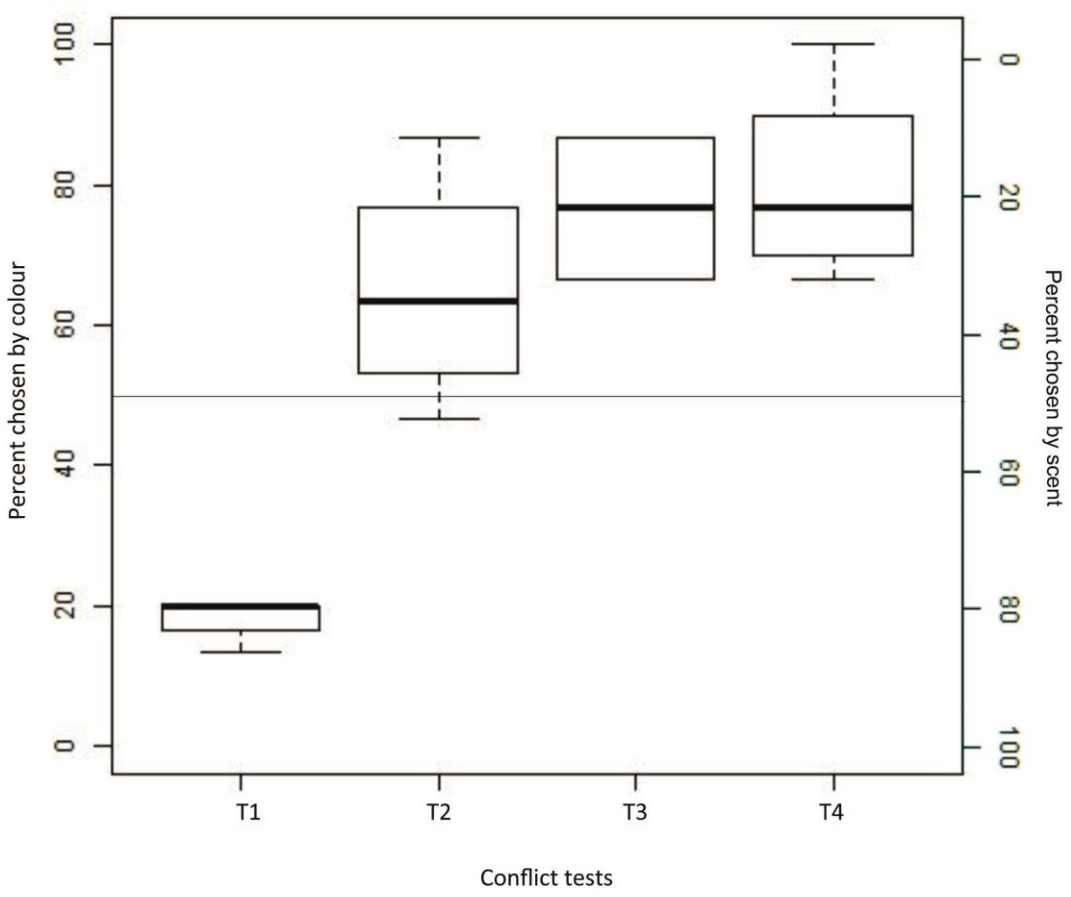

Fig. 26: Results of the conflict tests of 4 scent-preferring bees. T1: after 20 rewards during differential training. T2, T3 and T4: after 30, 60 and 90 rewards in differential training on colour-only stimuli. n = 4.

Of the four bees in this group, one came from each stimulus combination group (one had originally been trained to lavender-blue, one to rosemary-blue, etc).

E.2.2.b. Do Bees Later Remember the Scent Stimulus They Originally Used as Their Main Cue?

At the end of the experimental series, we tested the bees on scents only (X versus Y), and then on colours only (A versus B), to see if they remembered the stimulus components (Fig. 27).

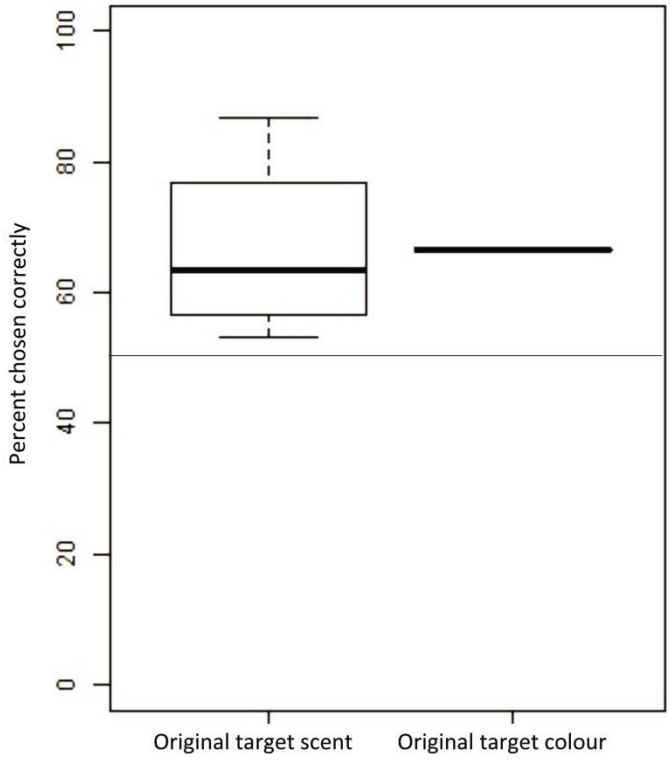

Fig. 27: Preference for original target scent and original target colour were measured after 90 rewards to colour-only stimuli, and four conflict tests. In “original target scent”, n = 4. In “original target colour”, n was only 1, due to bees disappearing towards the end of the experiment.

Due to the low number of bees tested, we cannot prove any statistical significance, and the data do not give an easy answer on how to interpret them.

Experiment 3: How Well Do Bees Learn and Reverse-Learn the Components of Bimodal Stimuli? How Well Do Bees Reverse-Learn Multimodal Stimuli?

In the reverse-learning experiment, we trained bees differentially on two stimuli which differed both in colour and scent. After bees had learned this task, they were reverse-trained, and finally trained back to their old stimulus. At the end of each training or reverse-training phase, the bees were tested on the bimodal stimuli, then on each modality (scent or colour) separately. An example:

Training phase 1: lavender-blue^{+} versus rosemary-yellow

Test 1: lavender-blue versus rosemary-yellow; lavender versus rosemary; blue against yellow.

Training phase 2: lavender-blue versus rosemary-yellow^{+}

Test 2: lavender-blue versus rosemary-yellow; lavender versus rosemary; blue against yellow.

Training phase 3: lavender-blue^{+} versus rosemary-yellow

Test 3: lavender-blue versus rosemary-yellow; lavender versus rosemary; blue against yellow.

With this method, we could not only find out how well bees reverse-learn stimuli that differ on several sensory levels, but also how well the bees learned (and reverse-learned) the components of the compound stimuli.

Finally, we also compared whether bees were more ready to let go of their previous strategy and accept a new potentially rewarding stimulus if the colour set was very different, compared to if it was very similar.

These experiments were conducted during September and early October of 2013.

3.0. Is There a Preference for Any Stimulus Combination?

Before analysing the other data, we first had to make sure whether the results of the learning tests were different between groups. As none of the data groups were normally distributed (Shapiro-Wilk test), we used a Kruskal-Wallis test for both blue and yellow and the blue and blue-green paper data (Fig. 28 and 29, respectively).

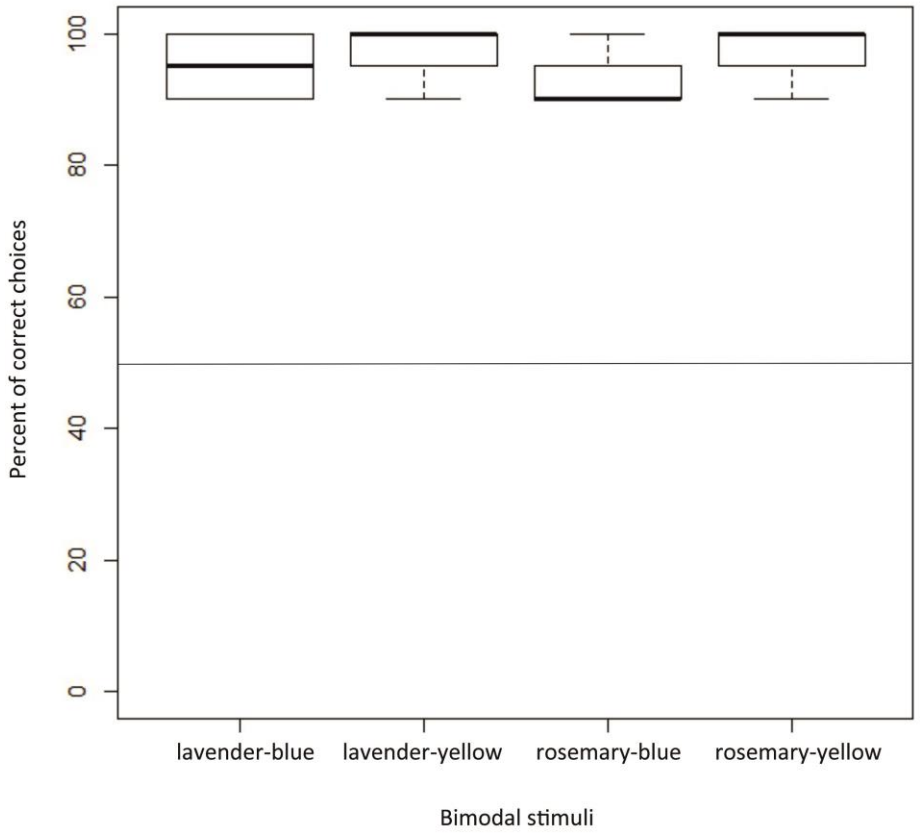

Fig. 28: Results of the learning tests in the blue and yellow colour group. We checked for preferences for any particular compound stimulus in the blue and yellow colours, using a Kruskal-Wallis test. There was no preference for any of the stimuli ($p \geq 0.05$). n for each box = 4.

We compared the results of the learning tests between stimulus combination groups, but there are no significant differences between the groups for the blue and yellow stimuli.

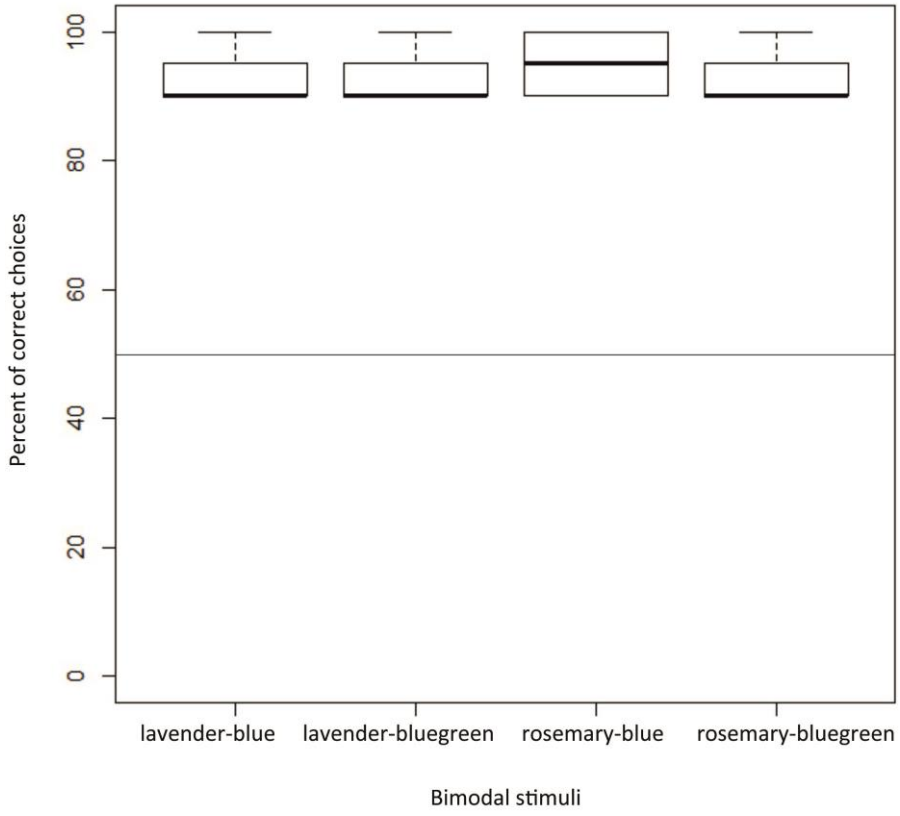

Fig. 29: Results of the learning tests in the blue and blue-green colour group. We checked for preferences for any particular compound stimulus in the blue and blue-green colours, using a Kruskal-Wallis test. There was no preference for any of the stimuli ($p \geq 0.05$). n for each box = 4.

For the similarly-coloured stimuli, just like for the dissimilar ones, there is no significant differences between the different stimulus groups.

3.1. Dissimilar Colour Group (Blue and Yellow)

Here we analysed the data from the bimodal test and the two monomodal tests after 20 rewards, using a Shapiro-Wilk test to check for normal distribution, and subsequently a Wilcoxon test (learning and colour results) or a t-test (scent results, which were normally distributed) to check for significant differences from chance level. We then compared all three using a Wilcoxon test for paired samples (Fig. 30).

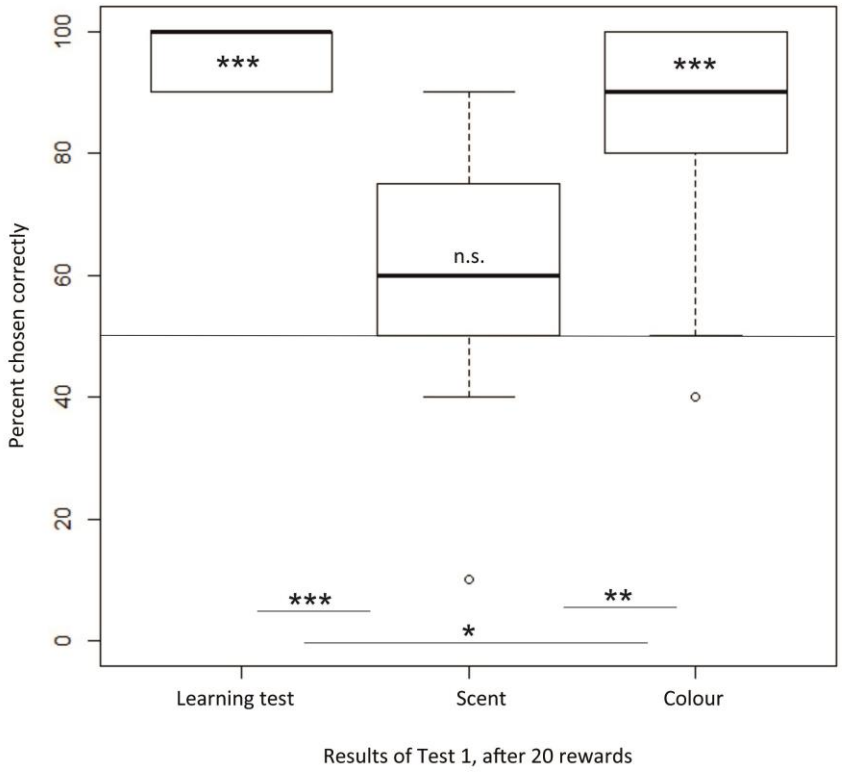

Fig. 30: Results of the first learning test and following scent and colour tests for dissimilar colours (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 16.

The data for the the bimodal stimulus and the visual stimulus have a highly significant difference from chance level, but the scent data show no difference at all.

After another training phase on the original distractor stimulus, we then checked the results of the second learning test and subsequent monomodal tests for normal distribution using a Shapiro-Wilk test, and subsequently a Wilcoxon test (scent and colour results) or a t-test (learning test results, which were normally distributed) to check for significant differences from chance level. We then compared all three using a Wilcoxon test for paired samples (Fig. 31).

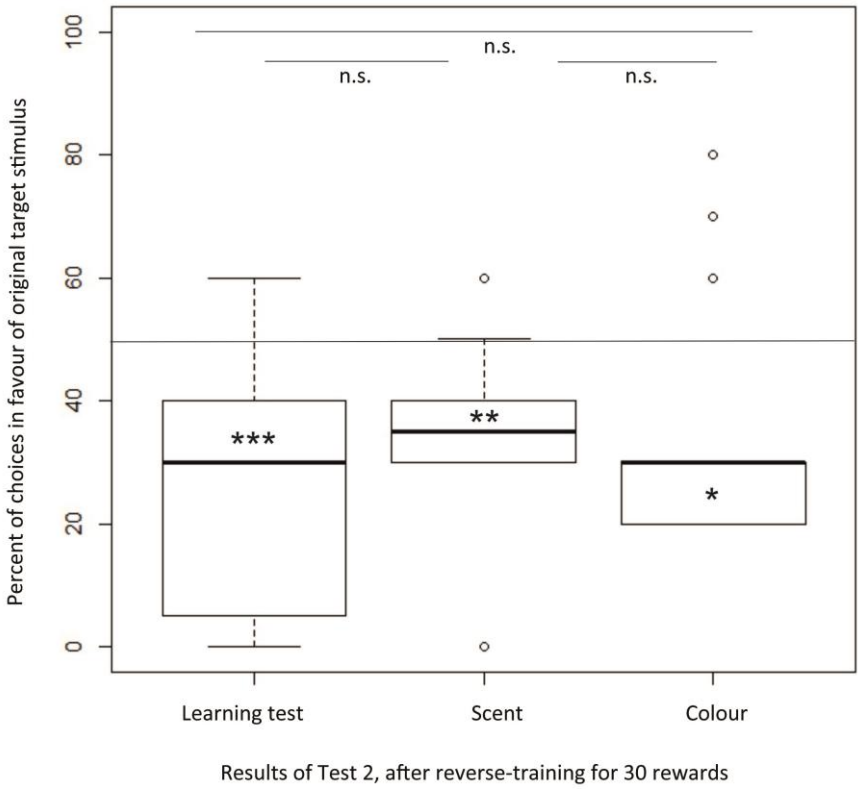

Fig. 31: Results of the second learning test and following scent and colour tests, after reverse-training (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 16.

We found no significant differences between the data of the bimodal and the two monomodal tests. However, all three data groups are significantly different from chance level in favour of the newly-learned, previously unrewarded stimulus and its components.

We then trained bees back on the originally rewarded bimodal stimulus, and looked for significant differences from chance level in test performances. We checked the results of the third learning test and monomodal tests for normal distribution using a Shapiro-Wilk test, and subsequently a t-test (learning and scent results) or a Wilcoxon test (colour results, which were not normally distributed). We then compared all three, using a t-test to compare the learning and scent test, and Wilcoxon tests for colour and scent, and learning and colour (Fig. 32).

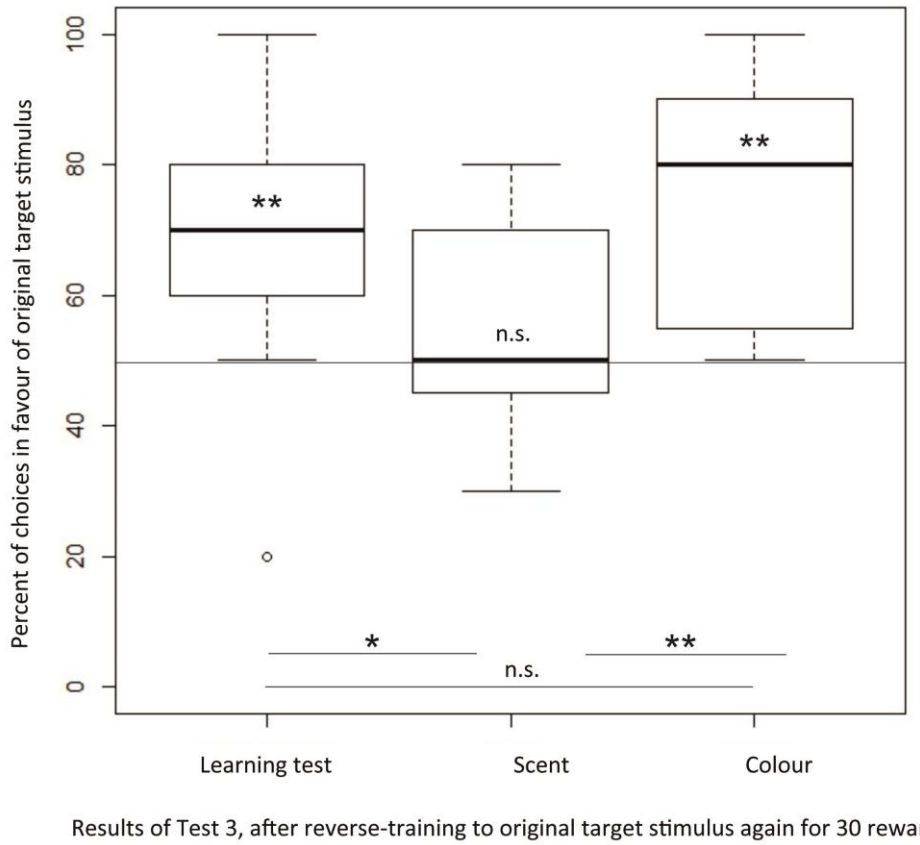

Fig. 32: Results of the third learning test and following scent and colour tests, after reverse-training back to the original target (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 16.

The data for the originally learned target stimulus are highly significantly different from chance level, and so are the data for the originally learned colour. However, the data for the original target scent are no different from chance.

3.2. Similar Colour Group (Blue and Blue-Green)

We repeated experiment E.3.1 with the blue and blue-green paper data. For the first bimodal test, and monomodal tests, we tested the results for normal distribution using a Shapiro-Wilk test, then for differences from chance level, using a Wilcoxon test (learning) or a t-test (scent and colour). We compared the results of the learning test to the scent test, and also to the colour test (using a paired Wilcoxon test in both cases), and scent to colour (with a t-test for paired samples) (Fig. 33).

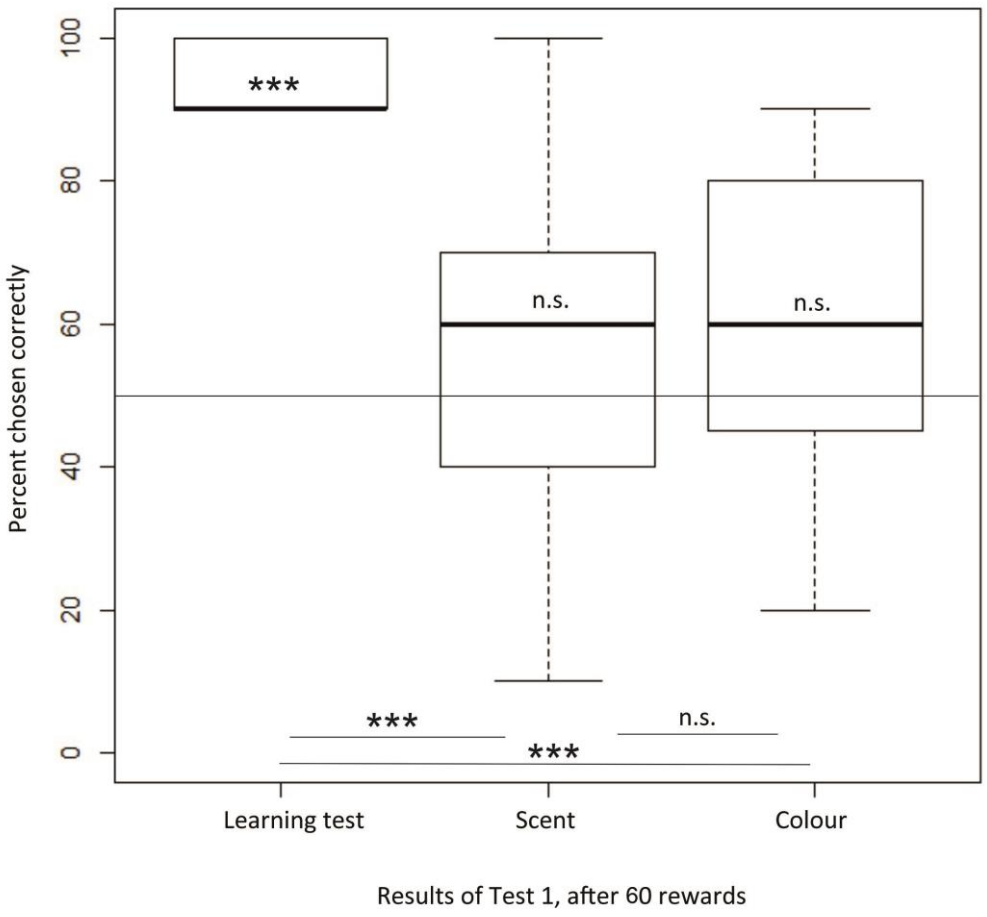

Fig. 33: Results of the first learning test and following colour and scent tests for the similar (Baehr) colour set (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 16.

The bees chose the correct bimodal stimulus with a high significance, but both monomodal stimuli at chance level. The data for both monomodal stimuli re not significantly different from each other, but both are highly significantly different from the data for the bimodal stimulus.

In the second bimodal learning test and monomodal tests, we used a Shapiro-Wilk test, and found that scent and colour were normally distributed. We tested each against chance level, using a t-test. The results of the learning test are not normally distributed, and we tested them against chance level with a Wilcoxon test. Finally, we checked for differences between the groups, using a Wilcoxon test for paired samples (learning test versus scent, and learning test versus colour), or a t-test (scent versus colour) (Fig. 34).

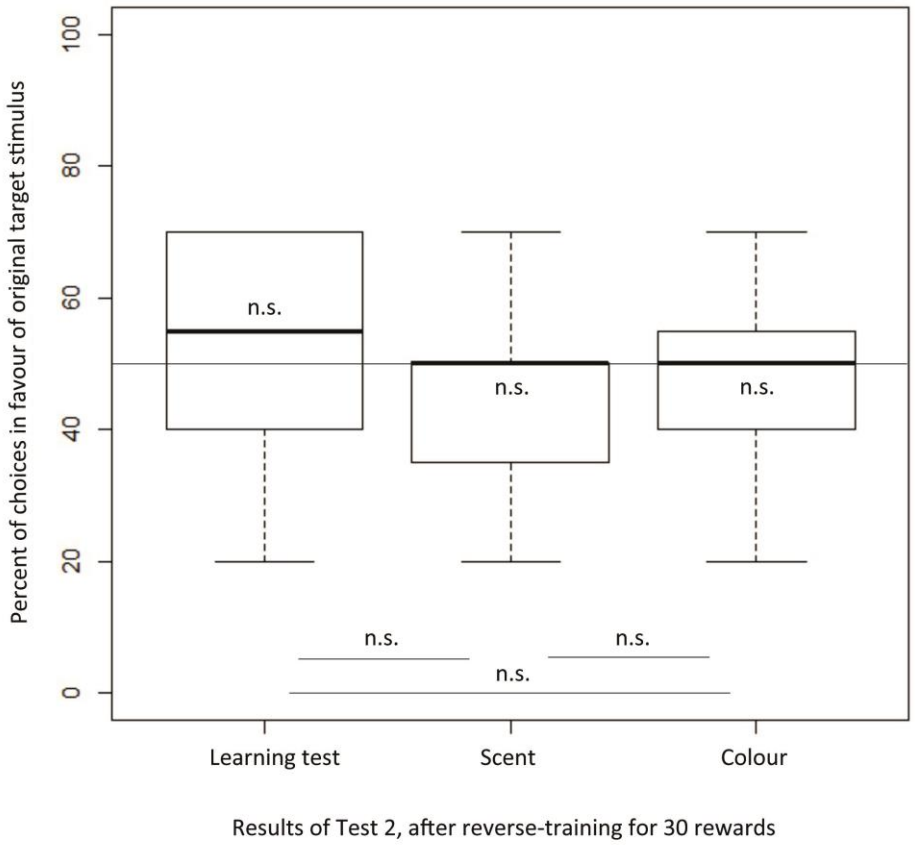

Fig. 34: Results of the second learning test and following scent and colour tests, after reverse-training for 30 rewards (n.s., $p \geq 0.05$). n = 16.

Bees chose the bimodal stimulus and both monomodal stimuli at random. There are no significant differences between the data groups.

In the third learning test and monomodal tests, we again checked for normal distribution, using a Shapiro-Wilk test. Since all three data groups were normally distributed, we checked for differences to chance level using a t-test, and a t-test for paired samples to compare the groups (Fig. 35).

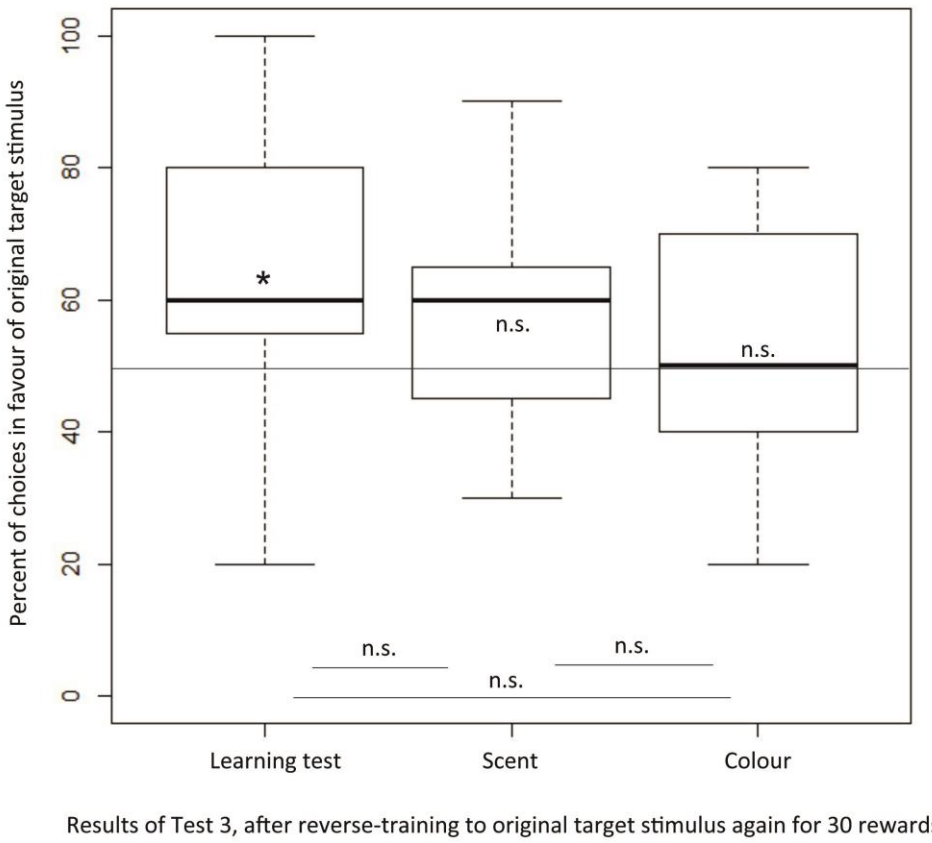

Fig. 35: Results of the third learning test and following scent and colour tests, after re-reverse training on the original target stimulus (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n = 15, as one bee had left the experiment at this point.

We found that the bees chose the correct stimulus with significance. The data for the two monomodal stimuli, however, are at chance level (similar to the results of the initial tests, Fig. 33).

3.3. How Long Does it Take Bees to Switch?

Here we looked at the bees' behaviour right after the reversal: how many times did a bee land on the now-unrewarded (previously rewarded) stimulus, before it made its first landing on the now-rewarded (previously unrewarded) stimulus? In other words: how many times did it insist on its old experiences before it gave the other stimulus a try?

To do this, we compared the abort rates for the first and second reversal, for both the dissimilar (Heyda) paper stimuli and the similar (Baehr) paper stimuli. We checked for normal distribution, using a Shapiro-Wilk test. Only the "Heyda reversal 1" data set is

normally distributed, so we checked for significant differences using a Wilcoxon test for unpaired samples (Fig. 36).

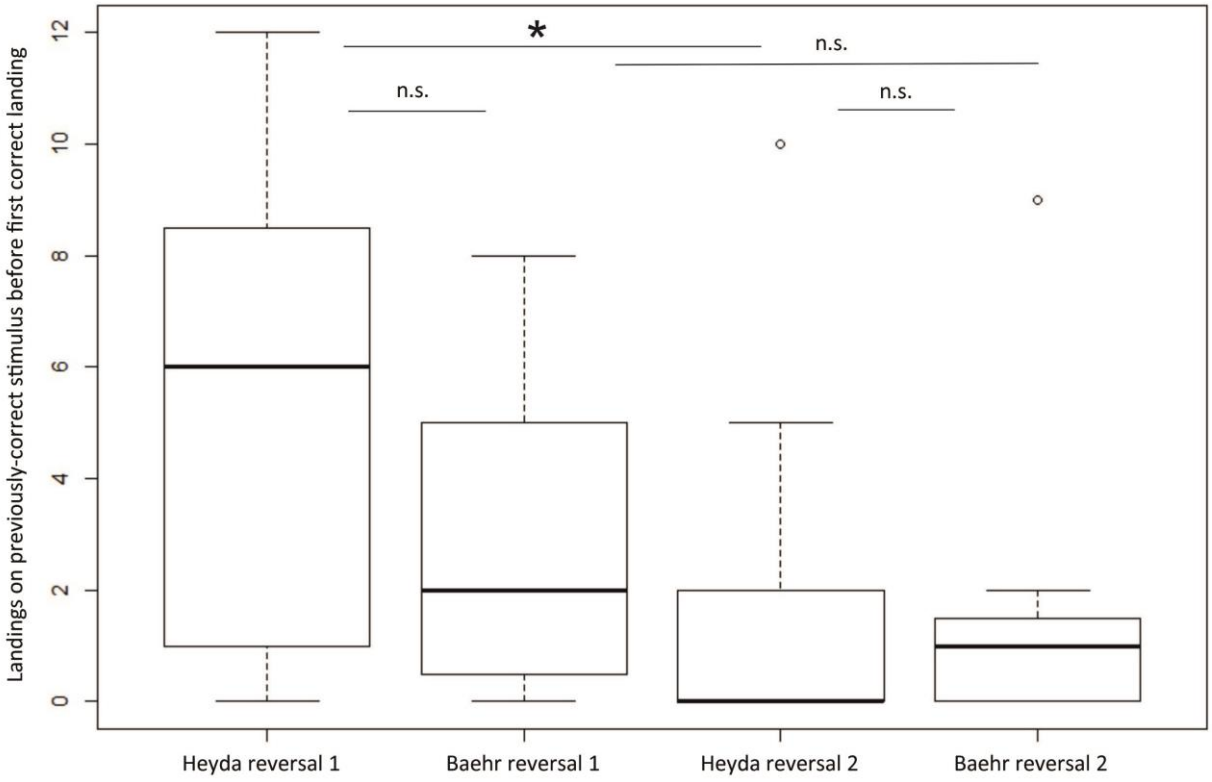

Fig. 36: Absolute number of landings on the new distractor (previous target) before landing on the new target for the first time. n = 15 for each box, because in each group one bee refused to land, and could only be made to visit the now-correct stimulus at all after being brought in front of it, and getting a reward. Since this was not her decision, we did not count these forced landings here (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., $p \geq 0.05$). n for the Heyda boxes= 16, n for "Baehr reversal 1" was 16, but since one bee left the experiment after the second test, n for "Baehr reversal 2" = 15.

There is a significant difference between the reversals for the dissimilar papers, but not for the similar papers. Also, there is no significant difference between the similar and the dissimilar group data.

Discussion

Our results show that the field of bimodal stimuli in foraging is very complex, and that results are highly dependent on the quality of the stimulus components. This became obvious even though we only varied stimulus colours, not scents.

We kept the two scent components constant because we had no way to determine bee-subjective scent similarity within the frame of this study. As bee-subjective colour similarity makes a great difference for the results, bee-subjective scent similarity likely has a great influence on the results as well. We therefore postulate that a fully comprehensive look into colour and scent processing in the honeybee cannot take place until bee-subjective differences between the important chemical components of bee-relevant flower scents, or at least between all bee-relevant natural flower scent mixtures, have been mapped.

Preliminary Experiments

P.1. Properties of the Colour Stimuli

The colour loci of the dissimilar pair (saturated blue and yellow) are very far apart in the bee's colour space, those of the similar pair (unsaturated blue and blue-green) are very close together (Fig. 7). This indicates that saturated blue and yellow are very different, and unsaturated blue and blue-green are indeed very similar, making them two useful stimulus pairs for experiments with varying colour salience. All four of these colours range between 29 and 38.6% brightness, and are therefore much brighter than the background, which has a brightness of 6.3 to 9.5% (Tab. 1). This makes them visually very conspicuous for the bee.

The grey stimulus lies close to the middle of the bee's colour space (Fig. 8). It therefore looks almost grey to bees. Although the hue is noticeably different from the PVC background, the brightness is almost the same (Tab. 1). This makes the grey stimulus much less conspicuous against the background than any of the other stimuli, which are different from the background both in hue *and* brightness. In conclusion, the grey paper is not an ideal, but suitable stimulus for scent-only experiments.

P.2. Finding the Detection Threshold for Symrise Lavender and Rosemary Oil

In this experiment we trained and tested bees on scented versus unscented stimuli of the same colour to find the detection threshold: the point where the bee could not discriminate between the scented and unscented stimuli. Our results showed that the thresholds for Symrise lavender and rosemary oil were around 1/1000 of a µl per litre of air in the scenting boxes for rosemary (Tab. 2), and 2.5 times that dose for lavender (Tab. 3).

Bees had five trials to pass each test, with additional training in between. There are two reasons for this protocol. The first was the observation that when bees are faced with a very

difficult test, followed by a few rewards and then another test, they perform much better in the second test, but their behaviour is also more cooperative (personal observation). Knowing that the rewards will come back seems to keep bees from making choices out of frustration (see also *General Conclusion* below).

The other reason is that bees often take long to learn difficult tasks, followed by a sudden recognition (see also *Material and Methods, P2: Stimulus Scents*). We saw this effect in many instances during the tests, but in many other instances – often within the same bee, but on a different scent concentration – acquisition seemed to be gradual, or completely irregular. As a conclusion to these results, we used 20 µl/l air (rosemary) or 45 µl/l air (lavender) in all the following experiments. These doses were far above the detection threshold but not strong enough to cause the bees discomfort.

Our results resemble those of Fischer (1957). Fischer tried to find the detection threshold for diverse essential oils and pure chemicals, diluted in paraffin oil, using free-flying bees. In his study, bees showed that they could perceive rosemary scent at lower concentrations than lavender. While we used Symrise oils, Fischer used a different brand (Schmoller & Bompard), but the results are similar. It seems to be a general phenomenon that bees can perceive rosemary scent better than lavender.

P.3. Learning Rate of Stimulus Components

P.3.a. Blue and Yellow Paper, Learning Rate for Different Scents

Bees learned the task of discriminating two scents, both paired with the same colour, surprisingly quickly (Fig. 10). We could definitely show that the paper absorbs the scent very well, making it possible for bees to choose correctly with a very high significance even during the first 10 rewards. For conclusions, see P.3.c below.

P.3.b. Unsaturated Blue and Blue-Green Paper, Learning Rate for Different Scents

The blue and blue-green paper, just like the blue and yellow paper, absorbed the scent so well that bees can reach a highly significant rate of correct choices even during the first 10 landings (Fig. 11).

But for future experiments, we had to give bees the opportunity to learn both colour *and* scent of the target stimulus. 10 rewards would have likely been enough to learn the scent only, but it takes bees much longer to learn to discriminate the hues of blue and blue-green (see Reinhardt 2010, and Dyer et al. 2014). Therefore, training with bimodal stimuli had to be much longer, and we decided to use 40 or 60 (experiment E1), and later constantly 60 (E3) rewards before the first conflict test.

P.3.c. Blue and Yellow Paper, Learning Rate for Two Colours

Our results here showed that blue and yellow (blue, and yellow-UV for the bee) can easily be discriminated within the first 10 rewards, even when scented with the same scent (Fig. 12).

Here, as in P.3.a, 15 or 20 rewards were easily enough to make bees learn the correct signal. We therefore decided to use 15 or 20 rewards (E1), or constantly 20 rewards (E2 and E3) for the initial training phase in all experiments where the colours blue and yellow were paired with lavender and rosemary. 15 rewards are the minimum, as it takes roughly 15 rewards for bees to learn how to use the apparatus correctly.

General Conclusions for the Following Experiments

We could not compare the results of P.3.a-c to one another, because a and b had been recorded in 2012 and c in 2013, during different months, and under potentially different climatic and seasonal circumstances (weather conditions were not recorded). Therefore, we cannot show whether the blue and yellow paper took scent on even better than the blue and blue-green paper, or vice versa. Slight differences in scent salience, and therefore quantity of available information, might influence the results of the following experiments – we could not exclude this possibility. But since all colour-scent combinations were learned with a high significance very quickly, it is likely that all bees had very similar conditions at the start of the experiment.

When using the dissimilar colour set, blue and yellow, we always trained bees for a minimum of 15 rewards (15 or 20 in E1, 20 for E2 and E3), which was enough to learn target colour, target scent and the use of the training apparatus. For the blue and blue-green set, we used 40 or 60 (E1) or 60 (E3) rewards for initial training. Since all bees had, theoretically, the chance to learn both signals, any preference of one sensory input over another could not be caused by a lack of opportunity to learn, but had to be caused through processing in the bee's brain.

Also, in all three experiments a-c, bees were so sure of their newly-learned knowledge that they delivered a highly significant result in the first test after 20 rewards – an accomplishment which should not be underrated, as bees easily get frustrated during the first unrewarded test phase, and start to choose at random. Bees will only try to perform well in an unrewarded test phase if they are sure that they are "doing it right". After too few rewards, bees often abandon the apparatus or choose at random when an unrewarded test phase follows. Saliently similar stimuli require a longer training phase than saliently different stimuli before bees are ready for unrewarded tests. This indicates that not only the colours blue and yellow, but also the two scents lavender and rosemary were easy to distinguish for the bees – although we have no way of exactly quantifying *how* easy.

We found that with saliently similar stimuli, it helps to let the bee go through a "sham" test, followed by some more rewards, before the real first test. This makes the bee recognize

that the source of food has not permanently run dry, but will be replenished regularly. With this method, one can avoid random choice behaviour out of frustration in the first "real" test. We did this in experiment E3 with those bees trained to the blue and blue-green papers: all bees were trained for 60 rewards, but after the 40th were given a "sham" test of 10 unrewarded landings, and then trained for another 20 rewards, regardless of the sham test result. As unrewarded tests do not constitute instances of learning, there was no danger of distorting the following results.

Experiment 1: Under What Circumstances Do Bees Decide by Colour, under What Circumstances Do They Go by Scent?

E.1.0. Is There a Preference for Any Stimulus Combination?

A comparison of the results of the learning tests showed that here, as in all other experiments, there was no preference for any of the stimulus combinations by the time the conflict testing began (Fig. 13 and 14). It is possible that there may have been an innate preference (for example for blue tones over yellow and blue-green, see Giurfa et al. 1995, Kriston 1973, or Menzel 1969), but by the time we began to test, there was no preference left. This result is not much of a surprise, considering that only bees which had chosen 80 to 100% correctly were used. Still, we have achieved the ultimate goal of this test: to make sure that our results were not influenced by the bees' individual preferences.

E.1.1: Dissimilar Colours (Blue and Yellow)

E.1.1.a. When Trained Differentially with a Set of Dissimilar Colours, Do Bees Choose by Colour or by Scent?

In this experiment, we could see that bees chose in favour of colour after only 15 or 20 rewards, a behaviour that did not change significantly after more training (Fig. 15). 15-20 rewards were obviously enough for the bees to learn how to use the apparatus and which stimulus was rewarded, but also to form a preference for one cue – in this case, colour.

We did not find the effect which Kriston describes in her 1973 paper: her observation that bees pay more attention to scent during the first few rewards, and later rely more on colour. In her experiments, this change would take place between the 3rd and the 10th reward. It is possible that after the 15th reward it was simply too late to find it, but we could not test earlier. Kriston's training arena was horizontal, with rewards presented directly on top of the stimuli, which is easier for bees to learn to exploit than a vertical arena where they have to land on platforms *in front of* the stimuli, like the one we used. Kriston's training protocol was also different: she offered her bees a bimodal stimulus, and later tested them on scent-only versus colour-only stimuli. Whichever the bee chose, Kriston concluded, had to be less similar to nothing, i.e., more important to the bee.

It is surprising that none of our bees chose clearly in favour of scent. Our result shows that scent is not, as often suspected, necessarily dominant over colour (see Bogdany 1978, and Gould and Gould 1988). Since we can quantify how saliently different the colours are, but we have no way of quantifying the same for the lavender and rosemary scents (*see Material and Methods*), we can only suspect the following: the two colours are much more bee-subjectively different than the two scents. Bees therefore used the easiest cue which demanded the least attention and learning time, especially because the second cue pair only repeated information given by the first – this fits Hebets' and Papaj's "redundant signal" theory (2005).

E.1.1.b. When Trained Absolutely with a Set of Dissimilar Colours, do Bees Choose by Colour or by Scent?

Here, bees chose clearly in favour of colour (Fig. 16), and did not change their preference after further training, just like they did after differential training (above). Apparently the dissimilarity between our blue and yellow is so strong to bees that it does not require differential training to be learned.

Again, we saw no traces of the "Kriston effect". After 15 landings it is probably too late to find it – if it occurs at all in our type of experiment.

E.1.1.c. Is There a Significant Difference in Behaviour Between the Above Two Groups?

We looked for differences between absolutely and differentially trained bees, and found that there were none (Tab. 4). Apparently it does not matter whether bees know the unrewarded stimulus BY before the first conflict test (differentially trained), or not (absolutely trained).

It is possible that this was due to the extreme salience of the blue and yellow colour, and that a repetition of the experiment with slightly more similar (but still easily distinguishable) colours would have shown a difference. Within the framework of this thesis, we could not examine this question further, as our alternative colour pair (unsaturated blue and blue-green) was so similar that absolute training would have been pointless (Dyer and Chittka 2004).

E.1.1.d. Does the Number of Initial Rewards Influence Choice Rates?

Here we looked whether bees rewarded 15 times showed the same choice behaviour as bees rewarded 20 times during the first learning phase (Fig. 17). Kriston's 1973 results suggest that in the initial training phase bees pay more attention to scent, and that after the third reward colour signals become more important and finally dominant after 10-20 rewards. In our study, after 15 rewards there was nothing left of any possible initial scent

preference. Just like in E.1.1.a, where we had performed the same test with blue and blue-green coloured stimuli, the choice rates were the same. After 15 rewards, it was probably too late to find traces of this effect.

E.1.2. Similar Colours (Blue and Blue-Green)

E.1.2.a. When Trained Differentially with a Set of Similar Colours, Do Bees Choose by Colour or by Scent?

In this version of the experiment, where bees were trained and conflict-tested on unsaturated blue and blue-green combined with lavender and rosemary scents, they chose in favour of scent after 40 or 60 rewards, even more in favour of scent after an additional 10 rewards, and finally at random after another 10 (Fig. 18). It is possible that the bees became frustrated with the experiment during the third test, but this seems unlikely, because no evidence for frustration was found in the third test for the blue-and-yellow colour set. Another unlikely possibility is that perhaps here, for the first time, a belated "Kriston effect" took place, and bees chose more in favour of colour than before – after the 60^{th} or 80^{th} reward. We do not have a conclusive explanation.

We know that bees are capable of learning the difference between the colours under these conditions (Reinhardt 2010), but here they largely ignored them. When comparing these results to those of the bees trained differentially on dissimilar colours (E.1.1.a), the logical assumption would be this: while the saturated blue and yellow were probably more saliently different than lavender and rosemary, the unsaturated blue and blue-green were probably *less* saliently different than the scents; and again, bees concentrated on the easiest stimulus property.

E.1.2.b. Does the Number of Initial Rewards Have an Influence on Choice Rates?

Here, as in the group of bees which were trained on blue and yellow stimuli (E.1.1.d), there was no difference in choice rates between the group which had initially been trained for 40 rewards, and the group which had initially been given 60 rewards (Fig. 19). Here, too, the training was apparently too long to see a "Kriston effect".

Comparing the Results of the Dissimilar Colour Groups (Differentially and Absolutely Trained) and Similar Colour Group: How Does the Importance of Scents Vary with the Similarity of Colours?

The results of our experiment show that the importance of scents and colours in bimodal stimuli depends strongly on stimulus quality: scent is not necessarily dominant over colour, or vice versa. Instead, scent becomes increasingly important for the bee if colours are very

similar. If the colours are dissimilar enough, bees can disregard the scent altogether, even if they were only trained absolutely and did not know the unrewarded stimulus before testing.

Our results match those of Giurfa et al. (1994). Their experiment involved scent markings which bees leave on depleted flowers, not the scent of the flowers as such; but the principle also seems to apply with complex flower scents, as our experiments have shown. It appears that it is generally very important to consider the bee-subjective similarity of scent and colour stimuli within and between modalities when designing an experiment[8].

On this background, Gould and Gould's study (1988, 173) and the resulting statement that scent is more important than colour, is put into a new perspective. Gould and Gould trained bees absolutely on a blue peppermint-scented triangle, with an orange, orange-scented disc used as a distractor in tests. Then they varied scent, colour and shape in a series of different experimental designs, and came to the conclusion that scent was more important to bees than colour, and colour in turn more important than shape. We can now suspect that their results would have looked very different if colours had been more similar, or if they had used different scents.

It would now be interesting to repeat our experiment, using two scents whose degree of similarity is known. Conducting a series of experiments to figure out scent similarity within the framework of this thesis was not feasible. Successful attempts to map bee-subjective similarity for scents have been made in the form of generalization experiments (see, for example, Guerrieri et al. 2005), but we still know little. Bees process chemically similar scent signals in neighbouring glomeruli in the antennal lobe, leading to a three-dimensional olfactory perceptual space (Guerrieri et al. 2005, Chittka and Brockmann 2005). Unfortunately, this physiological information does not give us reliable information about the *subjective* similarity scents have for bees (Chittka and Brockmann 2005).

Also, if colour similarity has an influence on how important scent is for a bee's decision, the idea suggests itself that scent similarity in turn influences the relative importance of colour. To show whether this is true, one would have to repeat our experiment with only one set of colours, and two sets of scents with different distances in olfactory space. Here, it would be necessary to have three (or more) scent stimuli with clearly defined differences in olfactory space.

In a real foraging situation, bees' decisions are not only influenced by the relative similarity between and within modalities, but also by the order in which they are perceived. This order, in turn, depends on a number of factors, such as wind, weather, temperature, and foliage. Menzel and Müller (1996) still believed von Frisch's hypothesis that colour

[8] On a related note, Tang and Guo (2001) tested *Drosophila* flies for preferences between shape and colour of a stimulus at varying colour intensities. They found that at more than 80% colour intensity, the flies decided by colour; at less than 80%, shape became increasingly important. Also, note Kamin's (1968) experiments where rats were trained on audiovisual stimuli with varying sound intensities, and found that if the sound was low, rats did not learn the sound component well. It seems that stimulus quality is not only important for bee experiments, or for insect experiments in general, but also for experiments with vertebrates.

generally serves as a signal at a distance, and scent primarily close-up (see *Introduction*). They postulated: “Because the bee’s settling on a target is usually taken as the behavioural criterion for learning, there is a strong bias in favour of the odor effects. The influence of colour is hardly quantifiable in color-odor compounds, owing to the uncertainty with which color is perceived, attended to, and chosen”. Our experiments, however, show that bees are perfectly capable of learning the colour compound in a multimodal stimulus and disregarding the scent, providing the colour difference is strong enough. It seems likely that colour perception is not in danger of being “eclipsed” when the bee is close to the flower, but that the two signals are enhancing the processing and the learning of one another.

It would now be interesting to continue our experiment to find at which distance in bee colour space the “turning point” lies: where colours become so similar that it makes more sense for bees to go by the lavender and rosemary scents. To do this, bees would have to be trained and tested on a series of colours more similar than the blue and yellow papers, but less similar than the blue and blue-green papers.

If our experiments were repeated with two scents of a known distance in bees’ olfactory space; and if the “turning point” was found at which bees switch from colour choices to scent choices; and if the distance of the two scents in olfactory space and the distance of the two turning point colours in bees’ colour space were compared – *then* this could serve as an insight into the true relative importance of scent versus colour in a close-up choice situation, and Menzel’s and Müller’s postulation could be checked. As it is, however, stimulus quality takes such a strong influence on the end results that no general statement can be made.

E.1.3. Do Bees React to Conflict Tests with Insecure Choice Behaviour?

We analysed the number of aborts (target and distractor aborts), and used them to detect insecurity in the bees’ choice behaviour. An “abort” is defined as an act of approaching without landing, maybe hovering in front of the stimulus, but then flying away to a different one. In other words: the bee has taken time to discriminate better, and finally decided (rightly or wrongly) that this is not its target stimulus. Since hovering for further inspection is only necessary when the bee is not quite sure, we used aborts as a general indicator for insecurity in the bees.

In the learning test, a bee only had to repeat exactly what it had learned. In the first conflict test, it was faced with a task for which there was no correct solution. We compared the amount of aborts made in both tests, for all three bee groups: trained to blue and yellow paper (differentially and absolutely), and to blue and blue-green paper (differentially) (Fig. 20-22, respectively).

The only group which showed a significant difference in abort numbers was the differentially trained blue-yellow group (Fig. 20). Here, significantly more aborts were made in the conflict test, showing that there is a difference in results between the absolutely

trained (Fig. 21) and the differentially trained blue-yellow group (Fig. 20). From this result, we can conclude three things:

- The training protocol seems to influence not the final results, but the behaviour *before* choices are made. Differentially trained bees showed remarkable insecurity in the first conflict test, probably because they had learned not only what the target was, but also which stimulus components were definitely *not* part of the target.
- The bees' behaviour *was* influenced by scent, even though this does not show up in their choices, only their aborts. All bees chose clearly in favour of colour, but they did not just ignore the scent signals. They were aware that the scent in tests was not as it should be.
- Our results also imply that bees do not necessarily need punishment to learn to avoid a stimulus – depending on stimuli and training procedure, non-rewardedness can be enough (compare also Smith 2012, "learning about the lack of an association of an odor with nectar of pollen is also an important form of learning"). Again, no evidence for this idea can be found in the final choice rate, only in the abort rate. In an ecological background, this makes sense: a landing on an unrewarding (or less-rewarding) stimulus costs the bee energy and time. In a natural situation, landing on the wrong flower is not usually going to result in punishment, but in non-rewardedness, and therefore, a waste of energy and time.

For the bees trained differentially on blue and blue-green (Fig. 22), no significant difference could be shown. Perhaps the salience of blue versus blue-green was too weak to form strong enough associations with the distractor stimulus.

Experiment 2: Can Bees Which Prefer to Use One Sensory Modality Learn to Prefer the Other?

In this experiment, we first sorted bees which decided by scent from bees which decided by colour after 20 rewards, and then gave them extra training on the stimulus component they had previously *not* used. Remarkably, out of the 28 bees we trained here, 24 chose by colour, but 4 chose clearly by scent – unlike in experiment E1 (relative importance of scent and colour in bimodal stimuli). There, 41 bees had been trained with the same colours (saturated blue and blue-yellow), but no bee chose by scent.

Whether this was connected to season (the relevant portion of E1 was conducted in June 2012, the present experiment in July and August 2012), or chance, we do not know. In any case, our result here suggests that individuals can have very different behavioural thresholds for signals from different sensory modalities – a potentially useful way for the hive to adapt to changing environments.

Since the number of scent-preferring bees was only four in this study, a statistical analysis did not make sense. We could not have assembled a greater amount of scent-preferring bees without unreasonably high costs of time and effort, since scent-preferring bees in this

experimental setup are apparently rare and can only be found through several hours of training and testing.

E.2.0. Is There a Preference for Any Stimulus Combination?

Again, there was no preference for any stimulus combination by the time the conflict tests began (Fig. 23), showing that a learning phase of 20 rewards is enough to make bees forget preferences they might have had initially. Also, we can be sure that innate preferences did not influence our other results.

E.2.1. Colour-Preferring Bees

E.2.1.a. How Well Do Colour-Preferring Bees Learn to Favour Scent over Colour?

We defined "colour-preferring bees" as bees which chose with 80% or more in favour of colour during the first conflict test. These bees (n = 24) were then trained on scent-only stimuli. After 30 rewards on scent-only stimuli, their colour preference was gone: the bees chose randomly (Fig. 24). After 60 rewards, the result was still random, but after 90 rewards, bees chose highly significantly in favour of scent. The greatest changes in behaviour took place between scent-only reward 1-30 (colour to random) and 60-90 (random to scent).

This experiment shows that even if bees rely heavily on only one sensory modality, they can switch their behaviour to relying on another as soon as the first stops delivering useful cues. In a changing environment, this flexibility can mean a great advantage – flowers may be hidden in foliage, or the wind may diffuse scents so much that they become useless for the bees. Our result also show bees' great behavioural adaptability. Bees can not only switch targets, they can do so across sensory modalities.

E.2.1.b. Do Bees Later Remember the Colour Stimulus They Originally Used as Their Main Cue?

Having been trained on a colour-scent compound stimulus initially, and later on scent-only stimuli, bees were tested with the two colours alone at the end of the experiment, and afterwards the two scents alone. As it turned out, they now chose their originally most important cue, colour, at a random level. Scent, however, they chose with a very high significance (Fig. 25).

One could argue that the bees had never been shown colour alone before, and maybe did just not recognize it on its own. However, our results from experiment E3 (where we checked if bees trained on bimodal stimuli also recognize the components on their own, see below) refute this idea: with the blue-and-yellow colour set, bees are easily capable of reaching a highly significant result in a colour-only test after 20 rewards on a compound

stimulus. Therefore, if bees do not know the link between target colour and food in our experiment here, the information must have been learned initially but then forgotten.

It is possible that perhaps the original target colour has simply been forgotten because of the time passed since the bimodal training (in the experiment, roughly 3-4 hours) – but this assumption would contradict many older experiments in which bees easily managed to remember rewarded colours for several days or even weeks, after only few rewards (e.g. Menzel 1969, or Bogdany 1878). Eisenhardt (2012) summarizes that "one training trial leads to a transient memory that is stable for a period of minutes to 1 day, whereas three training trials lead to a stable memory lasting for several days" – in our own experiments, the training phase was 20 rewards long, which should be more than enough to settle the information in middle- or long term memory.

The other possibility is that the newly-formed scent memory may have eclipsed the older colour memory. This seems unlikely, considering that visual and olfactory information are processed through different pathways which only converge in the calyces of the mushroom bodies, and the protocerebrum (e.g. Rössler and Groh 2012, Sandoz 2012, Dyer 2010, Paulk et al. 2009). But it cannot be completely ruled out, especially because we do not know much about how intermodal signals interact in the brain (Hebets 2005, Rössler and Groh 2012). If this explanation turned out to be correct, the missing preference for the original target colour would indicate a phenomenon best named "intermodal retroactive inhibition". The term is inspired by Koltermann (1973) and Müller and Pilzecker (1900). Koltermann found the phenomenon of "retroactive inhibition" in bees, but his studies involved only one sensory modality, the olfactory sense: "When bees learn two scents successively through one feeding act each, the second food signal inhibits the remembering performance of the first".

When it comes to inhibition between sensory modalities, we find a similar phenomenon in Kamin's (1968) rat experiments: he trained rats aversively with a light and a sound stimulus. If trained on the compound, and later on only one of the components, the other component was not recognized very well in tests. If trained only on the compound, and then tested on one signal directly, results were far better. Rescorla and Wagner (1972) came to a similar result. They conducted an aversive learning experiment with rabbits: if rabbits were trained on an audio-visual stimulus, and later on the visual component only, they did not remember the audio component in a test.

Unfortunately, neither Kamin nor Rescorla and Wagner conducted learning tests for the stimulus components *alone* before training the animals on one component only, so it is possible that their subjects may never have learned to use the other component in the first place. But the phenomenon is not only found in vertebrates: a similar experiment was performed by Guo and Guo (2005), who trained *Drosophila melanogaster* flies with olfactory and visual cues, using the flies' direction of flight as a visual cue. They then trained one group with the visual cues only (this group did later not recognize the scent cue alone), and one group with scent cues only (this group did not later recognize the visual stimulus alone).

It seems that the overshadowing of one signal over one of a different sensory modality is not only limited to bee brains, or indeed, insect brains.

This finding in bees indicates a limitation to the bee's ability to use multimodal signals in foraging. Multimodal signals enlarge the possibilities of attracting pollinators enormously, especially by functioning on different cognitive channels; but if one characteristic of a species could make a bee forget the other as soon as the other became unavailable, even though the pathways are largely separated, their ability to deal with a multitude of signals in their natural environment could be more limited than previously thought.

E.2.2. Scent-Preferring Bees

E.2.2.a. How Well Do Scent-Preferring Bees Learn to Favour Colour over Scent?

Bees which preferred scent during the first conflict test showed a tendency to switch from scent-based foraging to colour-based foraging after 30, 60 and 90 rewards on colour-only stimuli. Due to the low number of animals (n=4), we can unfortunately not confirm this result statistically (Fig. 26).

A common tendency in all four bees is obvious, and mirrors the result of E.2.1 (how well do colour-preferring bees learn to favour scent over colour?): bees can switch their foraging strategy if their preferred sensory modality does not give any more helpful cues. It seems as if bees can not only learn to disregard colour in favour of scent, but also scent in favour of colour.

E.2.2.b. Do Bees Later Remember the Scent Stimulus They Originally Used as Their Main Clue?

Sadly, the amount of data here is so small that we can only talk about tendencies: all four bees went through the scent-only test and produced an ambiguous result, but three of them did not return for the colour-only test, leaving us with n = 1, which is not even enough for a tendency (Fig. 27).

The idea of repeating the whole experiment with a more similar colour pair, for example our blue and blue-green pair, suggests itself: it would probably produce more scent-preferring bees, and make it easier to study the way they switch to colour. But, as we will see in experiment E3, when bees are trained to a scent-colour stimulus with a weak-salience colour pair, they will not automatically remember the scent component better than with a strong-salience pair.

Experiment 3: How Well Do Bees Learn and Reverse-Learn the Components of Bimodal Stimuli? How Well Do Bees Reverse-Learn Multimodal Stimuli?

Here, we reverse-trained bees and checked how well they learned and reverse-learned the stimuli and their components. To switch preferences, a bee first has to realize that the old target is no longer rewarded. Eisenhardt (2012) defines this as “extinction learning”. She points out that short term memory is used to compare recently visited flowers, and long-term memory serves as a “backup” of older sources the bee can start to use again when needed.

Eisenhardt (2012) suggests that there may be two different kinds of memory for instances of presence and absence of reward, both influenced by the number of rewards before extinction learning. A phenomenon called “spontaneous recovery” inspired this hypothesis: after not being rewarded on a certain stimulus any longer, bees stop responding to it, but then suddenly begin responding again, for a time. We found no effect of this kind in our experiments.

E.3.0. Is There a Preference for Any Stimulus Combination?

As in E1 (relative importance of colour and scent in bimodal stimuli) and E2 (can bees which prefer to use one sensory modality be trained to rely mostly on the other?), there was no preference for any of the four stimulus combinations for either the blue and yellow or the blue and blue-green group, so we could exclude the possibility that the end result might have been influenced by innate preferences (Fig. 28 and 29).

E.3.1. Dissimilar Colour Group (Blue and Yellow)

In the first test, it becomes apparent that the bees learned the combination of scent and colour extremely well, the colour alone very well (but not as well as the compound), and the scent not at all (Fig. 30). The result of the learning test is not a surprise: only bees which passed it with 80% or more correct landings were used in the experiment, all others were discarded. When comparing the learning test to the scent- and colour-only tests, it seems that elemental learning took place here: the two components add up to a compound with the associative strength of both combined. Even though scent alone is not recognized, it seems to contribute to learning in the compound.

As E1 (relative importance of colour and scent in bimodal stimuli) indicated, saturated blue and yellow are more saliently different for the bees than the rosemary and lavender scents, and bees tend to pay more attention to colour than to scent when trained on these stimulus combinations. Kamin (1968) pointed out that in a stimulus combination, the weaker

stimulus is not necessarily learned. He hypothesized that if the weaker signal conveys no information on its own, that it is merely redundant, and animals may not bother to learn it.

At first sight, Kamin's hypothesis seems to apply here. But there was still a significant difference between the results of the bimodal learning test and the colour-only test, indicating that the scent component – although not learned – did still support learning or memory retrieval, or both. This fits several of Hebets' and Papaj's (2005) suggestions (compare *Introduction: Several Signals – What For?*): the "amplifier" hypothesis, the "alerting and attention-altering" hypothesis, or the "context" hypothesis.

In the second test, we see a completely changed situation: the bees had reverse-learned both the compound and the colour – but now they had also learned the scent (Fig. 31). There is only one explanation we can think of: when realizing that the originally learned colour was not an indicator of reward any more, the bees not only turned toward the previous distractor stimulus, but partly away from colour as a signal in general. They began to rely on scent more than previously.

In the third test, compound and colour choices were significantly different from chance rate, scent was chosen at random (Fig. 32). This was probably because now, the originally learned colour had become a reliable signal again, and the "backup" memory of the link between this signal and a reward was still present.

Also, the results of the first and second reversal showed that bees did not "learn to reverse-learn", i.e., expect another switch in the near future and switch as soon as they realized that this point had come. These findings are in line with various results from previous reverse-learning experiments: Dyer et al. (2014), Reinhardt (2010), von Helversen[9] (1974) and Menzel (1969) found similar results in colour-only reverse experiments. Mota and Giurfa (2010) found the same phenomenon in scent-only reverse experiments: bees do not learn to anticipate a switch, the way humans do (for humans, see Reinhardt 2010). Generally, bees are capable of rule-learning (Menzel and Giurfa 2006), and we had initially hypothesized that if monomodal stimuli did not make it possible for bees to learn the switching rules, perhaps bimodal stimuli would offer enough salience, or enough context for more difficult memory functions. But this was not the case.

Menzel (1969) found an effect he called the "overlearning-reversal effect": the more frequently a colour is rewarded, the easier it is for the bee to switch (he rewarded bees between 5 and 30 times on colour-only stimuli). We found no such effect here. The bees which had been trained to blue and blue-green stimuli for 40 or 60 rewards did not switch at all (see below), while the bees which had been trained for only 20 rewards to the blue and yellow stimuli did switch easily. This shows that stimulus quality is, again, tremendously important for the honeybees' learning and choice processes. Under certain circumstances, it might even be the most important factor in pollination.

[9] Von Helversen, using a very different training protocol from ours, trained bees on saturated blue and yellow paper in a colour-only reverse experiment. His bees could learn to switch several times, provided the reward phases were long enough; but even under these circumstances, bees did not learn-to-reverse-learn.

It should be noted that Komischke et al. (2002) successfully trained bees to switch their scent preference four times, but these bees were not *reverse*-trained but trained to a novel target stimulus every time. It seems that bees are capable of learning new stimuli several times a day, but not the same stimulus as rewarded and non-rewarded alternately.

E.3.2. Similar Colour Group (Blue and Blue-Green)

In the first test, bees had learned the bimodal stimulus very well – but the components alone were both chosen at chance level (Fig. 33). This shows that in the case of a compound stimulus, A and X do not necessarily add up to AX, but can add up to a completely new stimulus, a case of configural learning.

Unlike in the blue and yellow group, here Hebets' and Papaj's "emergence hypothesis" (2005) describes the result more accurately. Another possibility is that by providing context for each other, A and X enhance each other's salience, or formation or retrieval of memory, but here, this mutual enhancement would have to be extremely strong.

From E1.2.a (relative importance of saliently similar colour and scent in bimodal stimuli) we know that bees choose mostly by scent in a conflict test with the similar colour set. Bees could use scent as a cue *even if it was paired with the "wrong" colour*, but here, without colour, memory of the rewarded scent was virtually non-existent, although they had gone through the same training as the group from E.1.2. There, the protocol had been:

Training AX^+ versus BY, testing AY versus BX

Here, the test in question went like this:

Training AX^+ versus BY, testing X versus Y

It seems that just the presence or absence of a colour makes a difference for a bee's scent memory. Even the wrong colour seems to be a better reminder for scent than no colour.

In the second test, the bees chose the compound and both components at random (Fig. 34). Apparently, they could master the task of learning a compound with such stimulus properties (test 1, Fig. 33), but not the task of reverse-learning it. There are two possible explanations. One is connected to salience: if one signal is very strong, it might help with learning the other (see E.3.1 blue and yellow colour set), but if none of them is very strong, mutual support might be very weak. The other explanation is that maybe the relative weakness of the colour signals (and the consequent effort of identifying them correctly) made the bees unwilling to try very hard.

In the third test (Fig. 35), the choice rates were similar to the first test: AX was rewarded again, and bees quickly went back to choosing their original target stimulus. But they still had not learned to link any of the stimulus components to a reward.

In Reinhardt 2010 (see also Dyer 2014), bees were trained on the same paper stimuli, but without a scent component. In that study, a portion of the bees could very clearly switch

between the colours. Here, despite using similar reward phases, bees did not even reverse-learn the colours. Not only did the bimodality of the compounds do nothing to help bees "learn to learn", it seemed to impair even one reversal.

General Comparison of the Results of Similar and Dissimilar Colour Groups: Initial Learning of Stimulus Components

In colour-scent experiments with bumblebees, Katzenberger et al. (2014) found that "the sum of saliences[10] of individual components predicted the salience of the compound stimuli", but with notable exceptions if stimulus salience was particularly strong or particularly weak. With very salient stimuli, the compound was often less salient than it should be if signals were simply added up. But two very weak stimuli often led to a much more salient combination than expected. In our blue-and-yellow group, there seems to be no discrepancy between the expected outcome of the "equation" and the actual outcome:

Very strong colours + rather weak scents = extremely strong compound

In the blue-and-blue-green group, however, we found that

Very weak colours + rather weak scents = extremely strong compound

This is an extreme example of the same phenomena Katzenberger et al. found.

But while in the blue-and-yellow group, the colour component was learned, in the blue-and-blue-green group, neither scent nor colour were recognized alone. The first therefore constitutes a case of elemental learning (Giurfa et al. 2003: "[p]rocessing of the compound as the sum of its elements"), the second a case of configural learning (Giurfa et al. 2003: the compound stimulus being "different from the sum of its elements"). Here, our results again emphasize the importance of stimulus quality when planning a bee experiment, as bees' strategies of dealing with information depend strongly on the relationship of information fragments to one another.

The physiological machinery behind these strategies is not yet well understood (see also *Introduction*). Gerber and Smith (1998) suggest that honeybee vision and olfaction reinforcement might work separately: VUMmx1 innervates only the olfactory regions of the mushroom body, but not the visual collar. "Thus, within the mushroom body, visual processing cannot rely on the same internal reinforcement signal as olfactory processing" (Gerber and Smith 1998). A common reinforcement path would suggest that blocking should occur, but this is not the case – at least not in the input region of the mushroom body. Still, there is clearly interaction between the two signals in the brain. Gerber and Smith suggest the protocerebrum and the output regions of the mushroom bodies as possible candidates. If their assumptions are correct, this would explain why bees can easily skip the learning of one factor in a bimodal stimulus, but learn the other.

[10] Please note: Katzenberger et al. checked individual salience of each stimulus against a neutral background, not similarity of stimulus pairs. Presumably, like us, they had no reliable way of quantifying scent similarity.

It seems to make sense that changing one of the two factors would confuse bees, e.g. that they might treat different colour morphs with the same scent as if they were different species. But there is conflicting evidence for this idea (summarized in Kunze and Gumbert 2001), and perhaps this conflicting evidence is again an effect of stimulus quality.

General Comparison of the Results of Similar and Dissimilar Colour Groups: Reverse-Learning of Stimuli and Their Components

Why did bee trained on blue and yellow paper reverse-learn stimuli and their components, but bees trained on blue and blue-green did not? Perhaps in a situation where both stimulus components are relatively weak and bees are unsure about recognizing them, it makes more sense to learn only the compound but not the components: when faced with a flower that displays only one of the components, and the bee is not sure whether it is the right species, it might easily land on the wrong type. In this hypothetical scenario, only the combined input from olfaction and vision would make a bee sure enough for landing. It might make more sense to cling to well-learned information about the more salient combination, rather than learn new and more difficult information about the components. (Our bees landed anyway; but then, we displayed our stimuli not in a changing environment but a stable setup where bees had come to expect regular, reliable rewards.)

For Dyer et al.'s paper (2014), bees were tested in colour-only reverse experiments with the same blue and blue-green papers we used in our study, and the effectiveness of the bees' behaviour was analysed with computer-generated environment models. They sorted bees into behavioural groups, according to their loyalty to the original target stimulus; we have no reason to do this here, since our bees do not show several different group tendencies. Dyer et al's results indicated that stubborn bees which do not switch may be useful for the hive: some bees make the repeated switch easily, some never do, but most bees show a behaviour which lies in between. Dyer et al. suggest that "a Stay bee loyal to one flower type might act as a 'watch-bee' consistently monitoring flowers that have been previously found rewarding, ready to inform the hive once they become viable targets". Beekman et al. (2003) pointed out why this might be an advantage: if all available foragers went to one good source but a better one became available, the colony would never know about it. By making sure that some bees stray, possibly better sources can be found more easily. Beekman et al. also report that scouting for new food sources takes a lot of time and energy, while sources which may be replenished at a later point would be forgotten – therefore, "stubborn" stay bees could serve to alert others when food from these sources became available again.

Similar results to those of Dyer et al. (2014) already showed up in Opfinger's scent reverse-training experiments (1949): a ratio of some extremely faithful (or stubborn) bees, some easy switchers, and a majority of bees which were faithful to a source only until the sucrose flow stopped. Mota and Giurfa (2010) reverse-trained bees to scents and found that some bees do not learn at all, others learn but do not reverse. An important difference

between Mota and Giurfa's experiment and ours is that we (and also Dyer and Opfinger) used free-flying bees, which were clearly in the foraging stage of life. Also, if you train bees to come to an experiment table, those bees which fail to learn *at all* will not even learn to visit the table regularly, and therefore never be trained in the first place. Giurfa and Mota, on the other hand, used harnessed bees and tested them by means of the proboscis extension reflex (PER). They recruited them by capturing them at the entrance of the hive, harnessed them and fed them sucrose paired with scent stimuli, so that bees learned to extend their proboscis for drinking whenever perceiving the rewarded stimulus. This method is very reliable to check learning progress, but it also means that Giurfa and Mota got all ages and kinds of bees, even the ones which did not learn, but they ran a risk of using bees in the guard stage of their lives, which had no foraging experience.

Bodgany (1978) points out the importance of multimodal signals (which he calls "specific units") when studying bee pollination: he trained bees to combinations of colour, scent and time (we could not include the time component because an experiment took place over the course of a whole day, and homogenous and exact timing was impossible to produce). His results revealed that "[w]hen either one of the combined signals is varied, orientation by the other signal deteriorates". After the results of our experiment, this seems even more plausible especially with the similar colour set. Bogdany also points out that bees always face several signals at the same time in natural foraging conditions, so the full range of natural bee behaviour can only be shown in multimodal experiments – having seen the results of our experiments, especially E3 (How well do bees learn and reverse-learn the components of bimodal stimuli? How well do bees reverse-learn multimodal stimuli?), we strongly agree.

3.3. How Long Does It Take Bees to Switch?

Our bees did not unlearn to fly to the previously correct stimulus easily – compare, for example, Greggers and Menzel (1993). They called the non-rewarded experience "inhibitory learning", and pointed out that the number of trials before the honeybee gives up on one stimulus depends on the quality of reward it received on this stimulus before. In our experiments, all rewards had the same sucrose concentration (doses were not measured accurately).

In the blue-and-yellow group (Fig. 36), bees showed a slight but significant difference between the first and second reversal. The second switch took place faster, either because bees were ready to use their "backup" memory of the correct stimulus, or because they were confused. The significant result in the third learning test in this group (the bees easily learned to fly back to the originally rewarded stimulus), however, makes the first possibility more likely.

In the blue-and-blue-green group, there was no significant difference, only a tendency to switch faster the second time. Considering that the result of the third learning test was only

just significant, confusion seems to play a greater role here than in the blue-and-yellow-group, and possibly lead to an early landing on the newly-rewarded stimulus.

Interestingly, there were no significant differences between the two bee groups. Especially during the first switch, one would expect bees to switch similar stimuli faster (because the certainty should be lower), and to switch dissimilar stimuli more slowly, because bees should be more certain that they were choosing the right one. But our result tells us that bees trained on blue and yellow stimuli were not significantly surer than bees trained on blue and blue-green.

Another interesting point is that in the similar colour bee group, there was no significant difference between the first and the second reversal, even though the difference looks dramatic in Fig. 36. This evokes the impression that during the second switch, bees insisted on landing on the original distractor stimulus *even though* they had not learned to link it to a reward after the first switch. Perhaps this ratio is caused by the number of bees tested (n = 15), and a larger number would produce clear results.

Outlook

The material and methods we used for this study show that olfactory experiments with free-flying bees in an outdoors setting can produce valid results, and that our methods can be used for multimodal experiments. For multimodal experiments which aim to understand pollination strategies and their ecological implications, such a method can even be more meaningful than multimodal laboratory experiments with harnessed bees.

Our results also show that bee behaviour is strongly dependent on stimulus quality, a factor that has so far rarely been standardized between and within research teams and experimental series. Bee-subjective salience and bee-subjective similarity between stimuli influence the choices a bee makes, leading to

1. Choices by the most conspicuous and saliently different components
2. Elemental or configural learning of different stimuli, when tested in the same experiment
3. More or less successful reverse-learning of compounds and their components

It would now be advantageous for future studies to measure stimulus quality and stimulus similarity whenever possible. This would, of course, require time-consuming behavioural tests before the actual experiments; but it would also make it possible for future researchers to compare their own stimuli to those used by other teams. For colours, this would be relatively easy, but the biggest obstacle is the construction of the bee's olfactory space, which is only in a fledgling state at the moment. The full understanding of the bee's olfactory space would open up a completely new range of possibilities for multimodal experiments, whether they deal with pollination ecology or brain processes.

We have also shown that a bee's strategy to use mainly one sensory modality is not genetically fixed, but reversible. This surprising finding raises the question what would happen if bees were reverse-trained between sensory modalities: e.g., first on colour, then on scent, then on colour, then back to scent. Would bees be able to reverse-learn better than within one sensory modality? Or would between-modality blocking make switching even harder? If the first possibility turned out to be true, it would be highly interesting to find out if bees in the field tend to switch between modalities, rather than within them, to make their work easier.

Our results also show the importance of multimodal stimuli in pollination experiments. Since our bees could, for example, recognize a scent if connected to a saliently similar but wrong colour, but not without a colour attached, presence or absence of an unrelated second component alone can make a difference in choice behaviour. Using monomodal stimuli in tests could distort the results of an experiment strongly, if one tries to draw conclusions about the bees' behaviour in a natural foraging situation where scent and colour usually occur together.

Finally, by analysing abort behaviour during landing, we could show differences in bees' choice behaviour that actual landings alone did not show. It would therefore be advantageous (and very easy to do) to record and analyse not only landings, but also abort behaviour in free-flying bees experiments in the future to see the full scope of choice behaviour.

Appendix A: Data

Bees are numbered by year, e.g.: 13-B90 is the 90th bee trained and tested in the year 2013. If two bees were given the same number by accident, they were renamed 13-B90a and 13-B90b.

Preliminary Experiments

P.1. Stimulus Colours (Fig. 7 and 8)

Reflection in stimuli and hangers was the following:

nm	Yellow	Sat. blue	Blue-green	Unsat. blue	Grey paper	Hangers, light	Hangers, dark, and disc
300	0,20699221	0,10863124	0,22287664	0,22406501	0,10450495	0,01932899	0,01669213
310	0,22588188	0,10489436	0,21451417	0,2176672	0,10916939	0,02022576	0,01766547
320	0,23959014	0,09795703	0,19546125	0,20586515	0,11749953	0,01973548	0,01678831
330	0,24490113	0,09316012	0,18210293	0,20287661	0,13667008	0,02161584	0,01605528
340	0,24656333	0,09014084	0,18236715	0,20983129	0,14834052	0,0222282	0,01614455
350	0,23246641	0,09308623	0,19221639	0,2237978	0,16206374	0,02549357	0,01753741
360	0,2064395	0,10159166	0,22047838	0,25321936	0,16744966	0,03223517	0,02046308
370	0,18253027	0,11651726	0,26526613	0,29657168	0,17257062	0,04412161	0,02912121
380	0,15675962	0,13739251	0,31630873	0,34822567	0,17164285	0,06328964	0,04096283
390	0,1369979	0,17612396	0,37617086	0,39676803	0,16541718	0,09785254	0,06953657
400	0,13131472	0,24312857	0,43381639	0,45545551	0,14979304	0,13635142	0,09323874
410	0,13093056	0,37918441	0,47140188	0,49724318	0,13607776	0,14817366	0,10202998
420	0,12923412	0,47454224	0,49387437	0,51696299	0,12597849	0,14567481	0,10141998
430	0,13351903	0,52646558	0,51551679	0,53225107	0,11740554	0,14580913	0,10108535
440	0,14666428	0,61163635	0,54971434	0,5413466	0,11204986	0,14707665	0,10041392
450	0,16527535	0,62476093	0,57026868	0,54004381	0,10590457	0,14746002	0,10143414
460	0,20044959	0,63469283	0,59773355	0,5325882	0,10597303	0,14758609	0,09514613
470	0,26525207	0,64104905	0,63119787	0,51389988	0,10251156	0,14841734	0,09697874
480	0,35599715	0,62109113	0,66180618	0,49384738	0,09841719	0,14524478	0,09723371
490	0,46951546	0,58970561	0,67542882	0,46720039	0,09742477	0,14429258	0,09498066
500	0,59166936	0,54933877	0,66523103	0,44310703	0,0974914	0,14453818	0,09411332
510	0,70508208	0,5013758	0,64360125	0,4199761	0,09542651	0,14391759	0,09242836
520	0,77069754	0,43508364	0,6029183	0,38889368	0,096979	0,14186016	0,09143445
530	0,80449529	0,37634879	0,55379925	0,36041628	0,09770458	0,14414497	0,0897246
540	0,82067983	0,31291701	0,49755803	0,33285036	0,09476393	0,14320969	0,08999472
550	0,8318736	0,25479073	0,43506648	0,30814768	0,0972891	0,14054633	0,08666746
560	0,84420103	0,2016404	0,36594405	0,27675249	0,09697085	0,13801705	0,08521068
570	0,84171671	0,16313744	0,30820378	0,24451014	0,09865382	0,13888136	0,08364593
580	0,856555	0,13443529	0,26320532	0,21924765	0,10089277	0,1368735	0,08348114
590	0,86084039	0,11663531	0,23187007	0,20057565	0,09876387	0,13537909	0,08215486

600	0,85352995	0,10771126	0,20460799	0,19178469	0,09319686	0,13272591	0,07897045
610	0,84877629	0,10284531	0,19240774	0,18808966	0,09420895	0,12984215	0,07764205
620	0,83974183	0,10199681	0,18975908	0,19390169	0,09458519	0,12741813	0,0770157
630	0,85342781	0,10345085	0,19557507	0,2117217	0,09559002	0,12630643	0,07650823
640	0,85638153	0,11065121	0,20697489	0,23094682	0,0978712	0,12517357	0,07432518
650	0,85877359	0,11597343	0,2162659	0,25009206	0,101257	0,1225152	0,07474257

To turn the data into ternary plots, the data were charged against the sensitivity in the bee's receptors (data by Peitsch et al. 1992):

nm	UV sensitivity	Blue sensitivity	Green sensitivity
300	0,6052	0,3272	0,2001
310	0,9834	0,3403	0,2201
320	1,3616	0,3533	0,2302
330	1,7399	0,3795	0,2402
340	1,876	0,4057	0,2502
350	2,0172	0,445	0,2502
360	1,876	0,4973	0,2502
370	1,4121	0,5627	0,2302
380	0,9683	0,6413	0,2201
390	0,7262	0,7721	0,2101
400	0,5245	0,903	0,1901
410	0,3833	1,0339	0,1801
420	0,2824	1,1647	0,1801
430	0,2219	1,2825	0,1801
440	1,41E-01	1,3087	0,2201
450	8,07E-02	1,2563	0,3102
460	2,02E-02	1,0862	0,3903
470	0	0,8768	0,4803
480	0	0,6543	0,6004
490	0	0,458	0,7005
500	0	0,2487	0,8005
510	0	1,31E-01	0,9006
520	0	6,54E-02	0,9706
530	0	2,62E-02	1,0007
540	0	0	1,0007
550	0	0	0,9806
560	0	0	0,9006
570	0	0	0,7805
580	0	0	0,6404
590	0	0	0,5003
600	0	0	0,3602
610	0	0	0,2602
620	0	0	0,1901
630	0	0	1,20E-01

640	0	0	6,00E-02
650	0	0	2,00E-02

Preliminary Experiments

P.2. Stimulus Scents

For results, see Tab. 2 and 3 in *Results*.

P.3. Learning Rate of Stimulus Components

AX versus AX, blue and yellow (Fig. 10). Table shows percentage of correct choices.

Bee	Training 1	Training 2	Test 3	Training 3	Test2	Training 4	Test 3
P-B42	63	71	40	91	33	83	73
P-B12	67	91	80	71	73	67	93
P-B14	91	59	80	55	73	83	67
P-B13	52,6	83,3	46	59	86	31	60
P-B26	100	100	60	77	87	90	80
P-B8	77	59	87	50	60	71,4	50
P-B41	91	100	83	83	67	77	73
P-B9	63	83	73	83	87	55	60
P-B27	91	91	80	67	53	58	80
P-B34	83	53	71	67	100	74	87
P-B40	83	83	73	83	67	59	47
P-B10	69	100	60	100	80	67	80
P-B6	67	83	73	91	80	77	87
P-B31	71	50	53	58	67	71	73
P-B32	100	67	73	77	87	67	47
P-B33	56	91	47	77	73	77	80
P-B7	83	62,5	67	N.A.	N.A.	N.A.	N.A.
P-B5	77	71,4	77,3	N.A.	N.A.	N.A.	N.A.

AX versus AY, blue and blue-green (Fig. 11). Table shows percentage of correct choices.

Bee	Training 1	Training 2	Test 1	Training 3	Test 2	Training 4	Test3
P-B77	71	77	93	83	100	91	100
P-B67	66,7	55,6	71,4	54,5	73,3	45,5	60
P-B60	100	100	86,7	83,3	100	100	100
P-B63	90,1	71,4	93,3	100	80	71,4	93,3
P-B70	90	100	100	100	73,3	83,3	100
P-B76	71,4	41,7	66,7	100	66,7	76,9	86,7
P-B58	58,8	62,5	86,7	62,5	80	76,9	93,3
P-B69	62,5	100	93,3	90,1	53,3	100	66,7
P-B59	52,6	52,6	80	83,3	66,7	77	86,7
P-B65	58,8	71,4	53,3	100	60	90,9	66,7
P-B62	71,4	100	100	90,1	100	100	100
P-B73	68,8	100	75	90,1	86,7	90,1	100
P-B75	100	100	100	83,3	86,7	34,5	66,7

P-B72	62,5	58,8	100	90,1	93,3	100	93,3
P-B66	100	56,3	100	90,1	86,7	62,5	93,3
P-B68	83,3	75	93,3	47,6	66,7	76,9	86,7

AX versus BX, blue and yellow (Fig. 12). Table shows percentage of correct choices.

Bee	Training 1	Training 2	Test 1	Training 3	Test 2	Training4	Test 3
P-B21	0,91	0,91	0,77	0,5	0,6	0,65	0,66
P-B23	0,83	0,91	0,93	1	1	1	0,93
P-B4	0,58	0,77	0,87	0,67	0,67	0,77	0,8
P-B29	0,83	0,56	0,4	0,77	0,87	0,82	0,47
P-B28	0,83	0,71	0,6	0,83	0,94	0,83	0,86
P-B18	0,63	0,71	0,93	0,71	0,86	0,71	0,86
P-B30	0,5	0,71	0,87	1	1	0,71	0,93
P-B25	0,71	1	1	0,8	0,93	0,77	1
P-B61	0,5	0,71	0,73	0,59	0,87	0,83	0,93
P-B37b	0,67	0,63	0,6	0,56	0,67	0,59	0,44
P-B37a	0,91	0,71	0,87	0,83	1	0,91	1
P-B38	0,77	1	1	1	0,53	0,91	0,87
P-B39a	1	0,91	0,93	1	1	1	1
P-B35	0,83	0,77	0,93	0,83	0,93	0,83	0,87
P-B36	0,91	1	1	1	0,93	0,91	0,93
P-B16	0,97	0,71	0,87	0,91	0,93	0,91	0,8

Experiment 1

E.0. Results of Learning Tests (Fig. 13): Dissimilar (blue and yellow) colour set, differentially and absolutely trained bees.

Diff.	**Bee**	% correct	**Abs.**	**Bee**	% correct
	Rosemary Yellow				
	12-B51	NA		12-B65	93,33333
	12-B49	NA		12-B66	86,66667
	12-B76	100		12-B67	93,33333
	12-B42	93,33333		12-B71	80
	12-B43	93,33333		12-B74	100
	Lavender Yellow				
	12-B40	NA		12-B60	86,66667
	12-B48	NA		12-B58	93,33333
	12-B75	93,33333		12-B56	86,66667
	12-B80	NA		12-B72	100
	12-B50	NA		12-B81	100
	Lavender Blue				
	12-B32	NA		12-B55	80
	12-B41	NA		12-B59	100
	12-B45	NA		12-B61	100
	12-B53	NA		12-B63	93,33333
	12-B77	100		12-B79	100
	12-B78	100			
	Rosemary Blue				

12-B44	NA	12-B73	100
12-B46	100	12-B69	80
12-B47	NA	12-B70	80
12-B54	93,33333	12-B64	100
12-B32a	NA	12-B148	86,66667

E.0. Results of Learning Tests (Fig. 14): Similar (blue and blue-green) colour set, differentially trained bees.

Bee	% correct
Lavender Blue	
13-B90	80
13-B94	93,3
13-B96	93,3
13-B98	86,7
13-B79	100
Lavender Blue-Green	
13-B83	86,7
13-B81	86,7
13-B92	86,7
13-B101	86,7
13-B85	86,7
Rosemary Blue	
13-B82	100
13-B80	93,3
13-B95	86,7
13-B99	86,7
13-B97	93,3
Rosemary Blue-Green	
13-B87	100
13-B93	93,3
13-B91	86,7
13-B86	80
13-B88	100

E.1.1.a. Dissimilar Colours (Fig. 15): Differentially trained bees, data of the conflict tests T1-T3.

	% of choices in favour of colour		
Bee	T1	T2	T3
Rosemary Yellow			
12-B51	73,33333	86,66667	86,66667
12-B49	40	80	73,33333
12-B76	53,33333	73,33333	93,33333
12-B42	93,33333	80	80
12-B43	80	80	53,33333
Lavender Yellow			

12-B40	80	100	100
12-B48	93,33333	86,66667	86,66667
12-B75	66,66667	40	80
12-B80	93,33333	100	80
12-B50	80	73,33333	46,66667
Lavender Blue			
12-B32	60	86,66667	60
12-B41	66,66667	100	93,33333
12-B45	80	80	73,33333
12-B53	93,33333	100	93,33333
12-B77	73,33333	100	60
12-B78	100	93,33333	100
Rosemary Blue			
12-B44	93,33333	93,33333	86,66667
12-B46	66,66667	80	86,66667
12-B47	66,66667	80	86,66667
12-B54	80	20	100
12-B32a	93,33333	93,33333	86,66667

E.1.1.b. Dissimilar Colours (Fig. 16): Absolutely trained bees, data of the conflict tests T1-T3.

	% of choices in favour of colour		
Bee	T1	T2	T3
Lavender Blue			
12-B55	86,6666667	93,3333333	86,6666667
12-B59	100	86,6666667	93,3333333
12-B61	93,3333333	80	100
12-B63	40	73,3333333	93,3333333
12-B79	93,3333333	100	93,3333333
Rosemary Yellow			
12-B65	86,6666667	86,6666667	93,3333333
12-B66	85,714286	86,6666667	86,6666667
12-B67	66,6666667	53,3333333	53,3333333
12-B71	100	57,1428571	86,6666667
12-B74	93,3333333	86,6666667	86,6666667
Lavender Yellow			
12-B60	93,3333333	40	66,6666667
12-B58	86,6666667	86,6666667	93,3333333
12-B56	66,6666667	86,6666667	60
12-B72	86,6666667	66,6666667	100
12-B81	86,6666667	80	80
Rosemary Blue			
12-B73	93,3333333	93,3333333	93,3333333
12-B69	33,3333333	20	73,3333333
12-B70	73,3333333	66,6666667	80
12-B64	86,6666667	100	86,6666667

12-B148	86,6666667	100	100

E.1.1.c. Is There a Significant Difference Between the Above Two groups? (Tab. 4)

For data, see E.1.1.a and E.1.1.b. For statistical calculations, see Appendix B.

E.1.1.d. Number of Initial Rewards (Fig. 17)

For choice rates, see T1 of E.1.1.a, and E.1.1.b.

Diff.	**Bee**	Rewards	**Abs.**	**Bee**	Rewards
	Rosemary Yellow				
	12-B51	15		12-B65	15
	12-B49	15		12-B66	15
	12-B76	15		12-B67	15
	12-B42	15		12-B71	15
	12-B43	15		12-B74	15
	Lavender Yellow				
	12-B40	20		12-B60	15
	12-B48	20		12-B58	15
	12-B75	20		12-B56	15
	12-B80	15		12-B72	15
	12-B50	15		12-B81	20
	Lavender Blue				
	12-B32	15		12-B55	15
	12-B41	15		12-B59	15
	12-B45	15		12-B61	15
	12-B53	15		12-B63	15
	12-B77	15		12-B79	15
	12-B78	20			
	Rosemary Blue				
	12-B44	15		12-B73	20
	12-B46	15		12-B69	15
	12-B47	15		12-B70	14
	12-B54	20		12-B64	20
	12-B32a	21		12-B148	20

E.1.2.a. Similar Colours: Differential Training (Fig. 18): Differentially trained bees, data of the conflict tests T1-T3.

	% of choices in favour of colour		
Bee	T1	T2	T3
Lavender Blue			
13-B90	60	46,7	86,7

13-B94	40	26,7	40
13-B96	13,3	33,3	46,7
13-B98	53,3	60	66,7
13-B79	46,7	0	53,3
Lavender Blue-Green			
13-B83	6,7	0	20
13-B81	66,7	13,3	73,3
13-B92	13,3	26,7	46,7
13-B101	26,7	33,3	46,7
13-B85	60	0	26,7
Rosemary Blue			
13-B82	60	20	73,3
13-B80	40	85,7	40
13-B95	60	33,3	53,3
13-B99	40	46,7	66,7
13-B97	0	13,3	53,3
Rosemary Blue-Green			
13-B87	20	66,7	66,7
13-B93	0	6,7	20
13-B91	20	26,7	14,3
13-B86	40	20	13,3
13-B88	40	26,7	40

E.1.2.b. Number of Initial Rewards (Fig. 19)

For choice rates, see E.1.2.a, T1.

Lavender Blue	
13-B90	60
13-B94	40
13-B96	40
13-B98	40
13-B79	60
Lavender Blue-Green	
13-B83	60
13-B81	60
13-B92	40
13-B101	60
13-B85	40
Rosemary Blue	
13-B82	40
13-B80	60
13-B95	60
13-B99	40
13-B97	60
Rosemary Blue-Green	

13-B87	60
13-B93	60
13-B91	60
13-B86	60
13-B88	40

E.1.3. Number of Aborts

Dissimilar Colours: Differentially Trained (Fig. 20)

	Absolute number of aborts	
Bee	Learning T.	T1
12-B-78	4	9
12-B-77	2	5
12-B-75	4	5
12-B-48	2	8
12-B-54	1	4
12-B-46	6	7
12-B-43	10	9
12-B-42	5	17
12-B-76	9	10

Dissimilar Colours: Absolutely Trained (Fig. 21)

	Absolute number of aborts	
Bee	Learning T.	T1
12-B148	2	8
12-B73	10	8
12-B69	5	5
12-B70	6	3
12-B64	2	9
12-B60	2	6
12-B58	10	3
12-B56	7	18
12-B72	5	12
12-B81	4	21
12-B65	13	13
12-B66	15	12
12-B67	11	5
12-B71	9	4
12-B74	11	27
12-B55	2	9
12-B59	3	9
12-B61	1	6
12-B63	6	9

12-B79	10	10

Similar Colours: Differentially Trained (Fig. 22)

	Absolute number of aborts	
Bee	Learning T.	T1
13-B90	4	3
13-B94	0	0
13-B96	4	0
13-B98	0	0
13-B79	1	0
13-B83	5	1
13-B81	6	0
13-B92	6	18
13-B101	10	4
13-B85	0	1
13-B82	1	0
13-B80	8	5
13-B95	6	7
13-B99	6	14
13-B97	6	5
13-B87	6	8
13-B93	0	4
13-B91	2	3
13-B86	8	5
13-B88	0	2

Experiment 2

E.0. Results of Learning Tests (Fig. 23)

Bee	% correct
Lavender Blue	
12-B105	93,33333
12-B141	80
12-B91	N.A.
12-B132	93,33333
12-B97	80
12-B96	100
12-B145	93,33333
Rosemary Blue	
12-B99	93,33333
12-B100	100
12-B104	80
12-B113	93,33333

12-B126	100
12-B139	80
12-B138	100
Rosemary Yellow	
12-B90	
12-B130	93,33333
12-B93	
12-B112	100
12-B131	80
12-B98	100
12-B109	93,33333
Lavender Yellow	
12-B106	92,85714
12-B103	86,66667
12-B94	100
12-B113b	100
12-B116	100
12-B133	100
12-B146	93,33333

E.2.1.a and E.2.2.a. Choice Rates in Conflict Tests (Fig. 24 and 26)

	% chosen in favour of colour			
Bee	T1	T2	T3	T4
Lavender Blue				
12-B105	86,66667	46,66667	20	13,33333
12-B141	13,33333	60	86,66667	80
12-B91	100	53,33333	86,66667	66,66667
12-B132	93,33333	93,33333	86,66667	53,33333
12-B97	100	86,66667	73,33333	73,33333
12-B96	93,33333	13,33333	66,66667	20
12-B145	86,66667	40	46,66667	53,33333
Rosemary Blue				
12-B99	93,33333	53,33333	46,66667	46,66667
12-B100	100	53,33333	33,33333	3
12-B104	20	86,66667	86,66667	73,33333
12-B113	93,33333	66,66667	73,33333	73,33333
12-B126	100	40	33,33333	26,66667
12-B139	80	53,33333	80	46,66667
12-B138	80	53,33333	53,33333	46,66667
Rosemary Yellow				
12-B90	100	46,66667	60	13,33333
12-B130	80	33,33333	0	0
12-B93	80	13,33333	20	35,71429
12-B112	100	26,66667	60	33,33333
12-B131	100	33,33333	26,66667	6,666667

12-B98	20	46,66667	66,66667	100
12-B109	100	53,33333	46,66667	33,33333
Lavender Yellow				
12-B106	20	66,66667	66,66667	66,66667
12-B103	93,33333	26,66667	6,666667	13,33333
12-B94	86,66667	33,33333	20	20
12-B113b	86,66667	73,33333	26,66667	26,66667
12-B116	86,66667	60	13,33333	33,33333
12-B133	100	73,33333	66,66667	40
12-B146	93,33333	0	40	20

Bees 12-B141, 12-B104, 12-B98, and 12-B106 were scent-preferring bees (Fig. 26), all others preferred colour (Fig. 24).

E.2.1.b. Choices of Original Target Colour and Scent, Colour-Preferring Bees (Fig. 25)

Bee	Colour	Scent	
Lavender Blue			
12-B105	73,33333	N.A.	
12-B91	100	N.A.	
12-B132	64,28571		53,33333
12-B97	93,33333	N.A.	
12-B96	80	N.A.	
12-B145	66,66667		100
Rosemary Blue			
12-B99	80	N.A.	
12-B100		N.A.	
12-B113	86,66667	N.A.	
12-B126	80		73,33333
12-B139	73,33333		73,33333
12-B138	80		80
Rosemary Yellow			
12-B90	53,33333	N.A.	
12-B130	53,33333		86,66667
12-B93	40	N.A.	
12-B112	40		100
12-B131	33,33333		86,66667
12-B109	26,66667	N.A.	
Lavender Yellow			
12-B103	80	N.A.	
12-B94	0	N.A.	
12-B113b	46,66667		80
12-B116	60		93,33333

12-B133	86,66667	86,66667
12-B146	26,66667	80

E.2.2.b. Choices of Original Target Colour and Scent, Scent-Preferring Bees (Fig. 27)

Bee	Scent	Colour	
12-B141	60		66,66667
12-B104	86,66667	N.A.	
12-B98	66,66667	N.A.	
12-B106	53,33333	N.A.	

Experiment 3

E.3.0. Choice Rates in the Learning Test (Fig. 28 and 29)

See E.3.1., T1 in stimulus compound chart, for Fig. 28. For Fig. 29, see E.3.2., T1 in stimulus compound chart.

E.3.1. Dissimilar Colours: Reverse-Learning Test Results (Fig. 30-32).

Stimulus compounds

	% of choices in favour of original target stimulus		
Bee	T1	T2	T3
13-B105	90	0	90
13-B115	100	0	60
13-B123	100	60	60
13-B110	100	30	70
13-B113	100	30	100
13-B117	90	0	60
13-B122	90	10	70
13-B111	100	30	50
13-B114	90	40	80
13-B116	100	60	70
13-B104	100	0	70
13-B121	100	50	80
13-B106	100	30	70
13-B119	90	40	60
13-B112	90	40	20
13-B118	90	33,3	90

Scent only

	% of choices in favour of original target

	stimulus		
Bee	T1	T2	T3
13-B105	90	0	60
13-B115	40	30	40
13-B123	50	40	50
13-B110	60	50	50
13-B113	60	50	80
13-B117	60	40	40
13-B122	10	30	70
13-B111	90	30	80
13-B114	60	30	70
13-B116	40	40	50
13-B104	80	0	40
13-B121	70	40	70
13-B106	50	60	60
13-B119	60	30	30
13-B112	70	40	50
13-B118	80	30	50

Colour only

	% of choices in favour of original target stimulus		
Bee	T1	T2	T3
13-B105	90	30	100
13-B115	100	30	90
13-B123	100	80	90
13-B110	100	30	80
13-B113	100	30	90
13-B117	50	30	50
13-B122	80	30	80
13-B111	90	20	50
13-B114	40	30	77,78
13-B116	60	20	80
13-B104	100	20	90
13-B121	90	70	80
13-B106	100	20	90
13-B119	80	20	50
13-B112	90	20	50
13-B118	80	60	60

E.3.1. Dissimilar Colours: Reverse-Learning Test Results (Fig. 33-35).

Stimulus compounds

	% of choices in favour of original target stimulus		
Bee	T1	T2	T3
13-B136	0,9	0,4	0,6
13-B134	1	0,4	0,8
13-B133	0,9	0,2	0,6
13-B132	1	0,5	N.A.

13-B131	0,9	0,6	0,8
13-B130	0,9	0,6	0,5
13-B129	0,9	0,6	0,8
13-B127	0,9	0,7	0,5
13-B128	0,9	0,5	0,8
13-B126	1	0,7	0,5
13-B125	0,9	0,4	0,6
13-B124	0,9	0,5	0,8
13-B141	0,9	0,7	1
13-B138	0,9	0,2	0,2
13-B139	1	0,7	0,6
13-B140	1	0,7	0,6

Scent only

	% of choices in favour of original target stimulus		
Bee	T1	T2	T3
13-B136	0,7	0,5	0,3
13-B134	0,9	0,2	0,7
13-B133	0,5	0,2	0,6
13-B132	0,7	0,5	N.A.
13-B131	0,6	0,5	0,5
13-B130	1	0,4	0,8
13-B129	0,4	0,5	0,5
13-B127	0,6	0,3	0,6
13-B128	0,6	0,4	0,9
13-B126	0,4	0,7	0,4
13-B125	0,8	0,5	0,7
13-B124	0,7	0,3	0,3
13-B141	0,3	0,7	0,4
13-B138	0,3	0,4	0,6
13-B139	0,1	0,6	0,6
13-B140	0,5	0,5	0,6

Colour only

	% of choices in favour of original target stimulus		
Bee	T1	T2	T3
13-B136	0,5	0,2	0,3
13-B134	0,3	0,5	0,7
13-B133	0,2	0,4	0,2
13-B132	0,6	0,5	N.A.
13-B131	0,8	0,3	0,4
13-B130	0,8	0,4	0,4

13-B129	0,8	0,4	0,5
13-B127	0,9	0,5	0,8
13-B128	0,6	0,6	0,5
13-B126	0,7	0,7	0,3
13-B125	0,5	0,4	0,6
13-B124	0,8	0,5	0,4
13-B141	0,7	0,6	0,7
13-B138	0,2	0,6	0,7
13-B139	0,5	0,2	0,8
13-B140	0,4	0,5	0,5

E.3.3. Landings on incorrect (previously correct) stimulus before trying correct stimulus (Fig. 36)

Dissimilar colours

Bee	Reversal 1	Reversal 2
13-B118	9	0
13-B106	10	5
13-B119	1	3
13-B112	1	0
13-B121	7	0
13-B104	7	N.A.
13-B116	0	0
13-B114	2	1
13-B111	8	0
13-B122	1	0
13-B113	N.A.	0
13-B117	12	1
13-B123	6	0
13-B110	3	4
13-B115	1	1
13-B105	9	10

Similar colours

Bee	Reversal 1	Reversal 2
13-B136	8	2
13-B134	1	0
13-B133	0	0
13-B132	2	N.A.
13-B131	8	1
13-B130	N.A.	1
13-B129	7	0
13-B127	0	1
13-B128	1	2
13-B126	2	0
13-B125	0	2

13-B124	0	1
13-B141	2	9
13-B138	8	0
13-B139	3	0
13-B140	1	0

Appendix B: Statistical Tests and Results

P.3. Learning Rate of Stimulus Components

AX versus AX, blue and yellow (Fig. 10)

Shapiro-Wilk test for normal distribution:

Training 1	p-value = 0.5844	normally distributed
Training 2	p-value = 0.1819	normally distributed
Test 1	p-value = 0.1032	normally distributed
Training 3	p-value = 0.842	normally distributed
Test 2	p-value = 0.3441	normally distributed
Training 4	p-value = 0.125	normally distributed
Test 3	p-value = 0.1972	normally distributed

Test for significant differences from chance level (here, since all data are normally distributed, all t-tests):

Training 1	t-test	p-value = 3.772e-07	highly significant
Training 2	t-test	p-value = 1.768e-06	highly significant
Test 1	t-test	p-value = 3.992e-05	highly significant
Training 3	t-test	p-value = 5.765e-06	highly significant
Test 2	t-test	p-value = 3.416e-05	highly significant
Training 4	t-test	p-value = 6.143e-05	highly significant
Test 3	t-test	p-value = 3.607e-05	highly significant

AX versus AY, blue and blue-green (Fig. 11)

Shapiro-Wilk test for normal distribution:

Training 1	p-value = 0.077	normally distributed
Training 2	p-value = 0.02051	-
Test 1	p-value = 0.01743	-
Training 3	p-value = 0.004881	-
Test 2	p-value = 0.4192	normally distributed
Training 4	p-value = 0.03928	-
Test 3	p-value = 0.003578	-

Test for significant differences from chance level (t-tests for normally distributed data, otherwise Wilcoxon test):

Training 1	t	p-value = 1.328e-05	highly significant
Training 2	wilcox	p-value = 0.0009551	highly significant
Test 1	wilcox	p-value = 0.0004566	highly significant
Training 3	wilcox	p-value = 0.0005435	highly significant
Test 2	t	p-value = 7.974e-07	highly significant
Training 4	wilcox	p-value = 0.0009976	highly significant
Test 3	wilcox	p-value = 0.0004435	highly significant

AX versus BX, blue and yellow (Fig. 12)

Shapiro-Wilk test for normal distribution:

Training 1	p-value = 0.1661	normally distributed
Training 2	p-value = 0.06554	normally distributed
Test 1	p-value = 0.004303	-
Training 3	p-value = 0.03702	-
Test 2	p-value = 0.004775	-
Training 4	p-value = 0.6077	normally distributed
Test 3	p-value = 0.003385	-

Test for significant differences from chance level (t-tests for normally distributed data, otherwise Wilcoxon test):

Training 1	t	p-value = 2.542e-07	highly significant
Training 2	t	p-value = 8.299e-09	highly significant
Test 1	wilcox	p-value = 0.0001847	highly significant
Training 3	wilcox	p-value = 0.0004426	highly significant
Test 2	wilcox	p-value = 0.0004596	highly significant
Training 4	t	p-value = 1.547e-08	highly significant
Test 3	wilcox	p-value = 0.0008236	highly significant

Experiment 1

E.0. Differences between stimulus combination groups

Blue and yellow stimuli (Fig. 13)

Shapiro-Wilk test for normal distribution:

Lavender blue	p-value = 0.000373	-
Rosemary blue	p-value = 0.06373	normally distributed
Rosemary yellow	p-value = 0.156	normally distributed
Lavender yellow	p-value = 0.167	normally distributed

Kruskal-Wallis test between the four groups: p-value = 0.5367, therefore no difference between groups (H0 cannot be rejected).

Blue and blue-green stimuli (Fig. 14)

Lavender blue-green	p-value = 0.3254	normally distributed
Lavender blue	p-value = 0.8174	normally distributed
Rosemary blue	p-value = 0.3133	normally distributed
Rosemary blue-green	p-value = 0.423	normally distributed

Bartlett test for homogeneity of variances: p-value < 2.2e-16, consequently not ANOVA but Kruskal-Wallis test: p-value = 0.433 – no difference between groups (H0 cannot be rejected).

E.1.1. Dissimilar Colours

Differentially trained bees (Fig. 15):

Shapiro-Wilk test for normal distribution:

T1	p-value = 0.1176	normally distributed
T2	p-value = 0.0001714	-
T3	p-value = 0.03442	-

Test for significant differences from chance level: (t-tests for normally distributed data, otherwise Wilcoxon test):

T1	t	p-value = 9.853e-08	highly significant
T2	wilcox	p-value = 0.0001817	highly significant
T3	wilcox	p-value = 7.496e-05	highly significant

Test for differences between test results showed no significant differences:

T1 vs T2	wilcox	p-value = 0.1412	not significant
T1 vs T3	wilcox	p-value = 0.2945	not significant
T2 vs T3	wilcox	p-value = 0.4739	not significant

Absolutely trained bees (Fig. 16):

Shapiro-Wilk test for normal distribution:

T1	p-value = 0.0002357	-
T2	p-value = 0.01118	-
T3	p-value = 0.01044	-

No group was normally distributed, therefore we checked for differences from chance level using Wilcoxon tests:

T1	wilcox	p-value = 0.0001577	highly significant
T2	wilcox	p-value = 0.0004577	highly significant
T3	wilcox	p-value = 8.788e-05	highly significant

Test for differences between test results, using Wilcoxon tests for paired samples:

T1 vs T2	wilcox	p-value = 0.342	not significant
T1 vs T3	wilcox	p-value = 0.7751	not significant
T2 vs T3	wilcox	p-value = 0.08329	not significant

The tests showed no significant differences between the two groups.

Comparison between conflict tests of absolutely and differentially trained bees (Tab. 4):

We used a Wilcoxon test for non-paired samples. There were no significant differences:

Absolute T1 vs Differential T1	p-value = 0.2232	not significant
Absolute T2 vs Differential T2	p-value = 0.4431	not significant
Absolute T3 vs Differential T3	p-value = 0.3459	not significant

Comparison between bees which had been trained for 15 rewards initially, and those which had been trained for 20 rewards (Fig. 17):

Shapiro-Wilk test for normal distribution:

15	p-value = 0.004862	-
20	p-value = 0.3906	normally distributed

As only one data set was normally distributed, we compared them using a Wilcoxon test for non-paired samples, which showed that there was no difference (p-value = 0.1851).

E.1.2. Similar Colours (Fig. 18)

Similar colours, differentially trained bees:

Shapiro-Wilk test for normal distribution:

T1	p-value = 0.1167	normally distributed
T2	p-value = 0.1532	normally distributed
T3	p-value = 0.4784	normally distributed

Test for significant differences from chance level (all data were normally distributed, so we used t-tests):

T1	t	p-value = 0.006655	highly significant
T2	t	p-value = 0.0006955	significant
T3	t	p-value = 0.5859	not significant

Test for differences between the results of the conflict tests, using Wilcoxon tests for paired samples:

T1 vs T2	wilcox	p-value = 0.4219	not significant
T1 vs T3	wilcox	p-value = 0.02302	significant
T2 vs T3	wilcox	p-value = 0.002688	highly significant

Comparison between bees which had been trained for 40 rewards initially, and those which had been trained for 60 rewards (Fig. 19):

Shapiro-Wilk test for normal distribution:

40	p-value = 0.1101	normally distributed
60	p-value = 0.386	normally distributed

As both data sets were normally distributed, we then used a Welch Two-Sample t-test for unpaired samples, but with p-value = 0.4229, we found no significant difference between the groups.

E.1.3. Aborted Landings

Dissimilar Colours: Aborts made by differentially trained bees (Fig. 20):

Shapiro-Wilk test for normal distribution:

Learning test	p-value = 0.4083	normally distributed
T1	p-value = 0.1099	normally distributed

Consequently, we used a t-test for paired samples, and found a significant difference between the groups: p-value = 0.02856.

Dissimilar Colours: Aborts made by absolutely trained bees (Fig. 21):

Shapiro-Wilk test for normal distribution:

Learning test	p-value = 0.1761	normally distributed
T1	p-value = 0.008808	-

Consequently, we used a Wilcoxon test for paired samples, and found no significant difference between the groups: p-value = 0.05165.

Similar Colours: Aborts made by differentially trained bees (Fig. 22):

Shapiro-Wilk test for normal distribution:

Learning test	p-value = 0.02713	-
T1	p-value = 0.0006426	-

As groups were not normally distributed, we used a Wilcoxon test for paired samples, and found no significant difference between the groups: p-value = 0.7256.

Experiment 2

E.0. Differences between stimulus combination groups (Fig. 23):

Shapiro-Wilk test for normal distribution:

Lavender blue	p-value = 0.1012	normally distributed
Lavender yellow	p-value = 0.0232	-
Rosemary blue	p-value = 0.02906	-
Rosemary yellow	p-value = 0.1458	normally distributed

Only two groups were normally distributed, so we checked for differences between groups using a Kruskal-Wallis test, which showed that there were none (p-value = 0.6334).

E.2.1. Colour-Preferring Bees

Learning to prefer scent (Fig. 24):

Shapiro-Wilk test for normal distribution:

T1	p-value = 0.001252	-
T2	p-value = 0.8805	normally distributed
T3	p-value = 0.4844	normally distributed
T4	p-value = 0.3598	normally distributed

Tests for significant differences from chance level (t-tests for normally distributed data, otherwise Wilcoxon test):

T1	wilcox	p-value = 1.615e-05	significant
T2	t	p-value = 0.5164	not significant
T3	t	p-value = 0.3761	not significant
T4	t	p-value = 0.0007992	significant

Differences existed only in T1 and T4.

Test for differences between test results (Wilcoxon tests for paired samples for non-normally distributed data, otherwise t-tests for paired samples):

T1 vs T2	wilcox	p-value = 2.792e-05	highly significant
T2 vs T3	t	p-value = 0.7538	not significant
T3 vs T4	t	p-value = 0.005161	highly significant

Tests for memory of previously rewarded stimulus components (Fig. 25):

Shapiro-Wilk test for normal distribution:

Colour	p-value = 0.2262	normally distributed
Scent	p-value = 0.3177	normally distributed

T-test for significant differences from chance level:

Colour	t	p-value = 0.3761	not significant
Scent	t	p-value = 2.522e-06	highly significant

E.2.2. Scent-Preferring Bees (Fig. 26 and 27)

There were no statistical tests, because n was only 4.

Experiment 3

E.0. Differences between stimulus combination groups

Blue and yellow group (Fig. 28):

Shapiro-Wilk test for normal distribution:

Lavender blue	p-value = 0.02386	-
Lavender gelb	p-value = 0.001241	-
Rosemary blue	p-value = 0.001241	-
Rosemary yellow	p-value = 0.001241	-

None of the data sets were normally distributed. The following Kruskal-Wallis test showed that there were no significant differences between groups (p-value = 0.4542).

Blue and blue-green (Fig. 29):

Shapiro-Wilk test for normal distribution:

Lavender blue	p-value = 0.001241	-
Lavender blue-green	p-value = 0.001241	-
Rosemary blue	p-value = 0.02386	-
Rosemary blue-green	p-value = 0.001241	-

None of the data sets were normally distributed. The following Kruskal-Wallis test showed that there were no significant differences between groups (p-value = 0.8451).

E.3.1. Dissimilar Colour Group (Fig. 30-32)

Shapiro-Wilk test for normal distribution:

T1	Compound	p-value = 3.805e-05	-
	Scent	p-value = 0.2885	normally distributed
	Colour	p-value = 0.002654	-
T2	Compound	p-value = 0.0615	normally distributed
	Scent	p-value = 0.02008	-
	Colour	p-value = 0.0001612	-
T3	Compound	p-value = 0.1605	normally distributed
	Scent	p-value = 0.2866	normally distributed
	Colour	p-value = 0.008346	normally distributed

Test for significant differences from chance level (t-tests for normally distributed data, otherwise Wilcoxon test):

T1	Compound	wilcox	p-value = 0.000321	highly significant
	Scent	t	p-value = 0.05562	-
	Colour	wilcox	p-value = 0.000875	highly significant
T2	Compound	t	p-value = 0.0008137	highly significant
	Scent	wilcox	p-value = 0.00194	highly significant
	Colour	wilcox	p-value = 0.01102	significant
T3	Compound	t	p-value = 0.001073	highly significant
	Scent	t	p-value = 0.1554	-
	Colour	wilcox	p-value = 0.002246	highly significant

Tests for significant differences between data (t-tests for paired samples for two normally distributed data sets, Wilcoxon tests for paired samples if at least one data set was not normally distributed):

T1	Compound vs scent	wilcox	p-value = 0.0006998	highly significant
	Compound versus colour	wilcox	p-value = 0.01187	significant
	Colour versus scent	wilcox	p-value = 0.005235	highly significant
T2	Compound vs scent	wilcox	p-value = 0.2507	not significant
	Compound versus colour	wilcox	p-value = 0.228	not significant
	Colour versus scent	wilcox	p-value = 0.8601	not significant
T3	Compound vs scent	t	p-value = 0.01703	highly significant
	Compound versus colour	wilcox	p-value = 0.1336	not significant
	Colour versus scent	wilcox	p-value = 0.002246	highly significant

E.3.2. Similar Colour Group (Fig. 33-35)

Shapiro-Wilk test for normal distribution:

T1	Compound	p-value = 1.33e-05	-
	Scent	p-value = 0.9875	normally distributed
	Colour	p-value = 0.2121	normally distributed
T2	Compound	p-value = 0.02978	-
	Scent	p-value = 0.2411	normally distributed
	Colour	p-value = 0.3092	normally distributed
T3	Compound	p-value = 0.1474	normally distributed
	Scent	p-value = 0.5847	normally distributed
	Colour	p-value = 0.3953	normally distributed

Test for significant differences from chance level (t-tests for normally distributed data, otherwise Wilcoxon test):

T1	Compound	wilcox	p-value = 0.0002733	highly significant
	Scent	t	p-value = 0.2618	not significant
	Colour	t	p-value = 0.1652	not significant
T2	Compound	wilcox	p-value = 0.4993	not significant
	Scent	t	p-value = 0.2039	not significant
	Colour	t	p-value = 0.3332	not significant
T3	Compound	t	p-value = 0.01044	significant
	Scent	t	p-value = 0.1551	not significant

Colour	t	p-value = 0.6893	not significant

Tests for significant differences between data (t-tests for paired samples for two normally distributed data sets, Wilcoxon tests for paired samples if at least one data set was not normally distributed):

T1	Compound vs scent	wilcox	p-value = 0.0006781	highly significant
	Compound versus colour	wilcox	p-value = 0.0006928	highly significant
	Colour versus scent	t	p-value = 0.8702	not significant
T2	Compound vs scent	wilcox	p-value = 0.07129	not significant
	Compound versus colour	wilcox	p-value = 0.1757	not significant
	Colour versus scent	t	p-value = 0.9029	not significant
T3	Compound vs scent	t	p-value = 0.2843	not significant
	Compound versus colour	t	p-value = 0.09478	not significant
	Colour versus scent	t	p-value = 0.425	not significant

3.3. How Long Does It Take Bees to Switch? (Fig. 36)

Shapiro-Wilk test for normal distribution:

Heyda 1	p-value = 0.08057	normally distributed
Baehr 1	p-value = 0.001757	-
Heyda 2	p-value = 0.0001183	-
Baehr 2	p-value = 1.319e-05	-

Test for differences between performance in reversal 1 (Baehr vs Heyda) and reversal 2 (Baehr vs Heyda)

Reversal 1	wilcox	p-value = 0.1156	not significant
Reversal 2	wilcox	p-value = 1	not significant

Tests within colour groups: Heyda reversal 1 vs Heyda reversal 2, and Baehr reversal 1 vs Baehr reversal 2.

Heyda	wilcox	p-value = 0.005928	significant
Baehr	wilcox	p-value = 0.1268	not significant

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