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Honeybee vision: analysis of pattern orientation

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Declaration

The work presented in this thesis is entirely my own, with the exception of the electrophysiological recordings discussed in Chapter 9.

Andrew D Giger

Some of the material in this thesis has been published or presented as follows:


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Abstract

Honeybees (*Apis mellifera*) learn the orientation of edges or bars in order to recognise a visual pattern. A number of findings published in recent years suggest that the bee's perception of pattern orientation is independent of other visual cues and is supported by a distinct visual subsystem. The main objective of this thesis is to study the characteristics of this subsystem in detail.

A series of behavioural experiments was carried out to investigate both the chromatic and temporal properties of the bee's orientation analysis. Measurements of orientation discrimination using gratings offering different magnitudes of contrast to the bee's three receptor types revealed that the bee's orientation analysis relies solely on input from the green receptor channel and is therefore "colour-blind" (Chapter 4). Experiments using moving gratings with various velocities and spatial frequencies revealed that the bees discriminate orientation presented at contrast frequencies of up to 50 Hz, irrespective of velocity or spatial frequency. Thus, orientation discrimination appears to be mediated by a neural subsystem that is sensitive to contrast frequency rather than speed (Chapter 5).

Three further studies were carried out to examine the role of orientation analysis in honeybee pattern recognition in different behavioural contexts. It was found that the orientation of a pattern is always learned when the pattern is presented in the frontal visual field, regardless of whether it is useful for the discrimination task at hand or not. Furthermore, in recognising and discriminating oriented patterns, the orientation of the pattern seems to be more important than its intensity distribution, when the two cues are available simultaneously (Chapter 6). When information on pattern orientation is confined to a small portion of the frontal visual field, orientation is learned irrespective of its location. However, if the bees are free to move in front of large training patterns, they tend to learn only the orientation offered in the ventral portion of these patterns, ie. in the area below the bees' goal in the centre of the pattern (Chapter 7). If pattern orientation is presented in the lateral visual field, the bees do not associate it with the food source, but seem to treat it as a type of landmark. Interocular transfer of a laterally learned orientation does not seem to occur. This is also true for laterally learned colours. Orientation is not discriminated if it is presented in the dorsal or ventral visual field, while colour is not discriminated when presented in the dorsal visual field (Chapter 8).

Electrophysiological data was obtained from a number of neurons in the midbrain of the bee, and possible neural substrates for the bee's orientation analysis are discussed. While there are several intriguing parallels between the cellular responses and the behaviour, it is difficult to find a complete explanation for the behaviour in terms of the response characteristics of the neurons studied so far (Chapter 9).

In addition to the studies on orientation analysis, a computer simulation of the bee's optics is described. This simulation is based on anatomical and physiological data from the literature and was developed as a tool for the design of experiments in bee vision (Chapter 2).
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Chapter 1

Introduction

The study of vision in insects reaches back in time as far as the seventeenth century when Hooke (1665), looking through a microscope of his own invention, portrayed for the first time the compound eyes of a male fly (rev. Wehner 1981). Ever since, scientists and philosophers alike have wondered what the world might look like through a compound eye. At the beginning of this century this question started to be addressed using the honeybee (*Apis mellifera*) as an experimental animal. Since then a wide variety of aspects of honeybee vision have been studied, including colour vision, pattern recognition, navigation, phototaxis, motion perception, range estimation and more. In the broader context of these studies, the present thesis concentrates mainly on pattern recognition, particularly on the honeybee's analysis of pattern orientation.

This chapter provides a short introduction to the study of pattern recognition in honeybees. In particular, it summarises what we presently know about the bee's orientation analysis. The last section of this chapter outlines the questions that the present thesis sets out to pursue.

1.1 Visual pattern recognition in honeybees

1.1.1 Early studies

The first behavioural experiments investigating visual pattern recognition in bees were performed by Turner (1911; see 1.2). It is von Frisch, however, who is thought to be the pioneer in the field of bee pattern recognition (apart from other aspects of bee biology), mainly due to his book published in 1914 (which also appeared as a journal article: von Frisch 1915). Some of his findings were that bees can be trained to discriminate between stars and ellipses, but not between squares on the one hand and equilateral triangles, circles and ellipses of the same area on the other hand. Later studies yielded similar results and suggested that bees discriminated shapes only on the basis of their contour length or their contour density² (Hertz 1929a). It was also found that bees show a spontaneous preference for patterns with a high contour density (Hertz 1929b, Zerrahn 1933) as well as for stimuli with a high temporal flicker frequency (Wolf 1933, 1935). The flicker hypothesis of pattern discrimination (Wolf 1933, Zerrahn 1933, Wolf and

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¹ The distinction between the different aspects of vision listed above is probably largely artificial, and considering pattern recognition as an isolated mechanism would be wrong. For the sake of focus, however, I will concentrate on pattern recognition defined as the recognition of visual, two-dimensional, spatial stimuli, excluding colour discrimination.

² The contour density is defined as the ratio of contour length to area of a shape.
Zerrahn-Wolf 1935), derived from these results, posits that bees discriminate between patterns by comparing the temporal frequencies of the intensity modulations experienced by individual ommatidia when the bee is moving above or in front of the pattern.

It was soon realised, however, that contour density alone was not sufficient to explain the bees' discrimination behaviour. For instance, patterns of the same overall contour density were discriminated by bees, if they were of different "type", eg. stars versus concentric circles (eg. Hertz 1933; Figure 1.1a). Therefore, an additional parameter had to be introduced which Hertz termed "figural quality" (as opposed to the "figural intensity" which basically translates to contour density).

At the same time, other researchers were following a somewhat different path. Extending some of von Frisch's (1915) experiments, Baumgärtner (1928) discovered that the effect of pattern differences varies with the position of this difference relative to the entrance to the feeder. With patterns presented in the horizontal plane he found that the bees only pay attention to a narrow annular area bordering the edge of the entrance. In the vertical plane the bees seemed to focus on the lower edge of the entrance hole and base their decision only on pattern features within a short distance from this point. Baumgärtner concluded that the bees did not discriminate shapes as such, but used a "localised colour sense" to learn and recognise patterns.

The importance of the point of focus was further investigated by Friedlaender (1931) and Wiechert (1938). They trained bees on simple dual-colour patterns (such as those shown in Figure 1.1b) and, in subsequent tests, shifted the patterns relative to the entrance hole of the feeder. They found that shifting the patterns in Figure 1.1b along the vertical had little effect, while shifting them horizontally (and thereby changing their appearance around the entrance) substantially altered the bees' choice behaviour. However, the different parts of the pattern could be moved away from the entrance (while preserving their 'left-right' or 'up-down' relationship) without confusing the bees.4

1.1.2 The template hypothesis

These studies, in particular that of Baumgärtner (1928), can be seen as the precursors of what is now known as the template hypothesis (also known as mapping hypothesis or eidetic imagery). According to this hypothesis, bees memorise a pattern (or the visual appearance of a food source in general) in the form of a pictorial representation (the template or "snapshot"). Discriminating between two test patterns then consists of comparing each, point by point, with the memorised template and selecting the one exhibiting the greater overlap.

The first mention of the possibility that bees could remember a visual pattern in point-to-point form can be found in Wehner (1969). His explanation for the bees' discrimination of differently oriented bars was that they compared the black and white

3 When training the bees to discriminate between a bilaterally symmetric arrangement of two 5×5 mm squares, one yellow, the other blue, and the mirror image of this arrangement, Baumgärtner (1928) found that the bees did not learn the task if the squares were positioned farther away than ca. 15 mm from the lowermost point of the entrance hole.

4 Furthermore, the bees could be trained on the same patterns without a conspicuous entrance hole. However, this only relativises the importance of the entrance hole as such, as the bees could have easily defined their own focal point.
Figure 1.1: Training and test patterns used by different authors. a: Patterns of similar contour density, but different type (Hertz 1933). These patterns were presented in the horizontal plane. b: Positive and negative training patterns for the "right-left" experiments of Friedlaender (1931). These blue and yellow patterns (size 3x3 cm) were presented in the vertical plane, with a hole in the centre leading (in the positive pattern) to the reward. c: Two of the training patterns (diameter 24 cm) used by Horridge and Zhang (1996). d: Three examples of random gratings (diameter 24 cm), as used by van Hateren et al. (1990) and in Chapter 4 of this thesis.

areas in the training and testing patterns, respectively. This hypothesis was based on results obtained from experiments in which bees had to discriminate the orientations of black bars on a white background. Three years later Wehner published a detailed study of the effects of changes in the intensity distribution on the bees' recognition of a single edge pattern (Wehner 1972a). He trained bees to collect sugar water from a feeder accessible only through a perspex tube. That tube led through the center of a black and white pattern presented on a disk which, viewed from the entrance to the tube, had an angular diameter of 130°. After training the bees with a particular distribution of black and white areas in the pattern, he confronted them with both the training pattern and a test pattern. Both patterns featured the same tubes in their respective centres but differed in their intensity distribution. The effect of this difference could then be recorded in terms of the bees' choice frequency for the test pattern.

The most intriguing finding of this study was that the effect upon discrimination of intensity differences in two separate regions of the pattern was equal to the sum of the effects produced by the individual differences. Thus, when confronted with a test pattern, the bees behaved as though they compared its spatial intensity distribution in a point-by-point fashion with the intensity distribution of the memorised training pattern.
However, not every area of the pattern has the same weight in this comparison. Firstly, changes in the medial lower part of the pattern seem to be more important than changes in other regions.\(^5\) Secondly, intensity changes are most effective if they occur near the contrast line of the training pattern.

Some of Wehner's (1972a) experiments were repeated by Menzel and Lieke (1983) using chromatic patterns. They showed that the asymmetry effect found by Wehner only emerges with patterns reflecting light exclusively in the long wavelength range, e.g. monochromatic orange bright/dark patterns or, as in Wehner's experiments, black/white patterns lacking reflectance in the UV range. Truly achromatic patterns (i.e., patterns reflecting in UV as well) do not induce any asymmetry effect, while the effect is reversed in monochromatic UV bright/dark patterns. Furthermore, Menzel and Lieke (1983) could demonstrate an asymmetry effect when the bees were trained on a vertical contrast line as well. This result cannot be explained with Wehner's hypothesis of the medial ventral region of the pattern being the most decisive for discrimination. Instead, an "antagonistic asymmetry effect in UV and orange" was proposed (Menzel and Lieke 1983), based on the assumption that bees show a spontaneous preference for patterns with a higher proportion of UV in the upper visual field: the discrimination from the training pattern is better, when the test pattern displays an increased area of bright UV in the upper visual field. Similarly, a decreased area of bright orange in the upper visual field increases discriminability as well.

Applying the same experimental paradigm as Wehner, Anderson (1977a) trained bees to a vertical black stripe on a white background. Subsequent tests with a set of eighty different test stimuli revealed that the similarity between the test stimuli and the training stripe, as perceived by the bees, could be described mathematically as a function of the difference in both the area and the contour density between the two. Unfortunately, this approach neglected Wehner's (1972a) finding that different regions of both the pattern and the bee's visual field have to be weighted differently (see above).

Cruse (1974) described the bees' discrimination of six-pointed stars of different form and contrast with a combination of the two-dimensional cross-correlation coefficient between the two shapes to be discriminated (i.e. their area difference) and their differences both in contour length and contrast. However, because he presented his patterns in the horizontal plane (which renders fixation of the pattern impossible; see below), the use of templates, as we define them here, is ruled out in his experiments.

Both Anderson's (1977a) and Cruse's (1972, 1974) attempts to find a quantitative description of the bee's ability to discriminate patterns by matching templates failed to produce an all-inclusive formula. Anderson found that the bees' discrimination was poorer than expected when the patterns were characterised by high spatial frequencies, while Cruse's formula failed when the training and test patterns were not of equal size.

Putting aside these attempts at a quantitative analysis of the bees' pattern recognition, what do we know about the bees' use of templates? One evident prerequisite is the need to fixate the patterns, at least in the acquisition phase, i.e. during the training. In a typical training on intensity distribution a bee requires a minimum of 10-15 reinforcements (i.e. visits to the experimental setup) before it can significantly discriminate the training

\(^5\) This dorso-ventral asymmetry was documented most clearly by an experiment in which bees trained on a white disc were tested with the training pattern versus a white disc with a black radial sector of 30° width. Testing this pattern in different angular positions revealed that the perceptual difference to the training pattern was largest when the black sector was located below the centre of the pattern.
pattern from, for example, a contrast inverted version of the same pattern (eg. Wehner 1972a). This means that the template is not acquired instantaneously (as the term "snapshot" suggests), but, rather, during a number of visits (ie. like a multiple exposure photograph). This, in turn, requires that the pattern is viewed in the same position on each visit, so that these multiple exposures overlap.

By filming bees approaching a pattern, Wehner and Flatt (1977) could demonstrate that the bees, prior to landing, hovered in front of the entrance of the tube, always adjusting their translational and rotational position to the same values. It is, most likely, during this hovering stage that the bees learn the intensity distribution of the pattern. That study also included experiments in which parts of the bees' eyes were painted over. It could be shown that when, for example, the ventral part of a bee's visual field was obscured, intensity changes were only detected if they occurred above the horizon. Although the bee could freely move in front of the pattern and, in doing so, shift the whole pattern into its dorsal visual field, it paid attention to the intensity distribution only while fixing the pattern. Therefore the pattern's intensity distribution must have been represented retinotopically and acquired in the hovering position only.

Several authors have noted that a point-to-point memory representation of a pattern is very uneconomical and therefore unlikely to be implemented in the bee with its necessarily restricted memory capacity (eg. Wehner 1975, Anderson 1977a, Srinivasan 1994). However, there are several ways to reduce the storage space for an eidetic image. The most straightforward option would be to store it at a lower resolution. It has been shown that the resolution of the bees' eidetic images is about 10° (Gould 1986) which is significantly lower than the physical resolution of the bee's eye (see Chapter 2). Another possibility would be to store "part-figures" only, i.e. to concentrate on a particular portion of the pattern and ignore the rest (Gould 1985, but see Gould 1988). A further option is to store the image in terms of more abstract features (eg. edges) but retain their topological arrangement. Whether such a representation would still be called an eidetic image is, however, questionable (see Chapter 10 for discussion). In the case of landmark learning (which appears to be based on eidetic images; Collett and Cartwright 1983) the edges of landmarks alone were shown to be sufficient for the bee's navigation (Cartwright and Collett 1983).

1.1.3 The "perceptual space" hypothesis

Yet another different – but not necessarily incompatible – approach to pattern recognition in bees was taken by Ronacher (1979a, 1979b, 1992). His hypothesis is that bees analyse patterns in terms of abstract parameters such as size or contrast. These parameters can then be treated as coordinates in a "perceptual space" in which the distance between two points, representing two different patterns, corresponds to the perceived difference between these two patterns. Very importantly, the dimensions of this perceptual space depend on the experimental situation. Thus, different trainings produce different perceptual spaces in which the same parameters are assigned different weights. To illustrate the concept of perceptual spaces, it is possible to find, for instance, a size difference between a test and the training pattern that is equivalent to a certain difference in contrast. For example, if a bee is trained to a large, black disc and is then

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6 When referring to this study in later publications, Gould states a resolution of 8°.
presented simultaneously with a small, black disc and a large grey disc, it will choose equally between the two, provided the size and grey level, respectively, are chosen appropriately (trading experiment). Ronacher (1979a) could show that this equivalence fulfils the requirement of transitivity, i.e. if a third test pattern, differing in a third parameter (in Ronacher's experiments it was "annularity"), is perceived as equally different from the training pattern as the small, black disc is, then the same holds for the large grey disc.

Ronacher's perceptual space hypothesis is appealing for several reasons. First of all, it does not try to limit the bee's visual perception of a pattern to one particular mechanism in the way that the flicker and template hypotheses, respectively, do. In fact, it could be seen as a concept that accommodates any number of mechanisms (including template matching, colour vision, range estimation, etc.) in that the output of any such mechanism can be interpreted as a parameter in the perceptual space.  

If Ronacher's hypothesis is seen as a rival to the template hypothesis, however, it has the advantage of being less memory intensive. Storing a template point by point can be very costly in terms of storage space, while the representation of a pattern with a few abstract parameters would be much more economical. Assuming that the bee's small brain is limited in its memory capacity, this is an important argument (see Chapter 6 for discussion). However, Ronacher's work does not specify how these parameters are extracted from the image.

In recent years, several abstract parameters were found to be employed by the bee. One of them is pattern orientation which will be introduced in the next section. Another set of parameters specifies different types of pattern symmetries. In experiments screening for innate preferences, bees showed a slight tendency to prefer bilaterally symmetric patterns over the same patterns rotated by 90°, and similar preferences could be found for radial patterns of bars and sectors (Lehrer et al. 1995). Further experiments involving trainings and tests on radial and concentric patterns (Figure 1.1c) suggest that the bees use the presence or absence of radiating and tangential pattern elements as a cue for pattern discrimination (Horridge and Zhang 1996).

The notion we have today of the bee's visual pattern recognition is one of a variety of interconnected visual subsystems processing different aspects of a pattern in parallel (Zhang and Srinivasan 1993, Horridge 1994). Depending on the situation and the task at hand, the bee might rely more heavily on some of these subsystems than on others. Pattern recognition itself can be seen as a subsystem of the bee's visual processing in general, alongside other subsystems like colour discrimination, optomotor response and navigation, to name only a few.

1.2 Orientation analysis

Turner (1911) was the first to show that bees can discriminate between horizontal and vertical gratings. One of his experiments consisted of training bees to a group of six boxes painted in different colours and patterns (Figure 1.2). Only the box with longitudinal red and green stripes contained a reward. He then performed two different tests, either presenting the bees with a clean set of six boxes exactly as in the training

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7 Some of the earlier workers (rev. Hertz 1935) already showed that the effects of different pattern parameters (eg. figural intensity and figural quality) can cancel each other out.
situation, none of them containing any honey, or testing a (clean) box with longitudinal red and green stripes against each of the other boxes individually. In either case the bees exclusively entered the box that was rewarded during training. Therefore, while discriminating a coloured grating from a black and white grating and other patterns, the bees had also learnt to prefer the vertical grating (surrounding the entrance to the box\textsuperscript{8}) from the horizontal grating of the same colours.

While more interested in positional information of a pattern and the importance of a fixation point (see 1.1.1), Wiechert (1938) trained bees to discriminate a horizontal bar from the same bar rotated by 90°. In subsequent tests she showed that the rewarded bar could be rotated out of the training position by up to 45° and still be preferred over a vertical bar. This discrimination broke down, however, when the bar was rotated by 60°, ie. with an angular difference between the two alternatives of 30°.

The honeybee's ability to discriminate the orientation of bars, gratings and crosses was studied extensively by Wehner and Lindauer (1966). They trained and tested bees on patterns presented in the vertical plane, allowing them to inspect the patterns from close range. In this situation, the bees could detect angular differences in the orientation of bars and gratings as low as 10°. With right-angle crosses, this minimal angular difference was even lower. In discussing possible mechanisms for the bee's orientation discrimination, Wehner and Lindauer (1966) compared these behavioural findings with the responses of the orientation sensitive cells Hubel and Wiesel (1965) found in the cat cortex. They therefore thought of pattern orientation as an abstract parameter. The input to the suggested orientation-sensitive cells was expected to be "the position of black and white areas in the visual field and not the orientation of the contours" (Wehner 1968).

In a later study (Wehner 1969) an attempt was made to explain the above results on the basis of the intensity distribution alone (mapping hypothesis; see Chapter 6 for detailed discussion). Still a few years later, though, further studies seemed to indicate that bees could "generalise" pattern orientation and memorise it as an independent stimulus parameter (Wehner 1971). In the most decisive experiments of this study bees were trained on a white disc containing a black bar of a certain orientation. Wehner

\textsuperscript{8} Turner (1911) observed that the bees, prior to making a decision whether to land or not, "hovered within about a centimetre of the object examined". Assuming this examination took place in front of the entrance to the box, we would expect the pattern on this side of the box to be most important for the discrimination.
could then show that, in the test, a contrast-inverted version of the training pattern (a white bar on a black disc) was preferred over the same pattern rotated by 90°, while it was clearly rejected when presented alongside with the training pattern. However, these results do not prove the generalisation of pattern orientation. The bees could, for instance, have used eidetic representations of parts of the training pattern only (e.g. a single edge).

None of the experiments reported up to this point could prove the use of orientation as an abstract pattern parameter. Almost two decades later, this proof was finally produced by van Hateren et al. (1990). The crucial requirement was to prevent the bees from using eidetic images. This was achieved in two ways simultaneously. Firstly, the bees were not trained on a fixed pair of patterns, but rather on a number of irregular gratings that were frequently exchanged. The pairs of gratings used were randomly chosen from a set of 10 random gratings (Figure 1.1d). Each of these consisted of 12 bars (presented on a disc of 24 cm diameter), each having an equal probability of being black or white. This training procedure entails that the intensity distribution is constantly varied, while the pattern orientation is kept constant. Secondly, the bees had to make their decision from a certain distance, preventing them, as it seems, from fixating the patterns. This was achieved by presenting the two patterns at the end walls of the two tunnels of a Y-shaped apparatus (Figure 3.1). In this situation the bees did not discriminate between two fixed random gratings of the same orientation even though they differed in their intensity distributions.

Thus eliminating all cues but the pattern orientation, van Hateren et al. (1990) showed that bees could still be trained to discriminate between two random gratings oriented perpendicularly to each other. Furthermore, after this training the bees could perform the same discrimination on patterns they had never seen before, such as bars, single edges and sinusoidal gratings. These results demonstrated that bees are able to abstract and recognise the orientation of a pattern independently of the pattern itself.

Having found out that bees use pattern orientation as a single parameter, the next question was: how does their visual system extract orientation from the retinal output? One possibility is the use of image motion induced by the bee moving in front of the pattern. A vertical grating would induce only horizontal motion, while perceiving only vertical motion would indicate a horizontal grating. In the case of gratings oriented at 45° and 135°, respectively, the bee's movements would induce specific, unique combinations of directional horizontal and vertical image motions (Figure 1.3).9

This hypothesis was tested by Srinivasan et al. (1993b). Bees trained on horizontal pattern orientation, using the method of van Hateren et al. (1990), were tested on stationary and moving arrangements of black dots on a white background (Figure 1.4). While discriminating horizontal and vertical random gratings very well (Figure 1.4a), the bees could not distinguish between two different patterns of randomly arranged dots (Figure 1.4b). This was to be expected, since these patterns do not contain any global orientational cues.10 However, when the dots were arranged in horizontal and vertical rows, respectively, the bees strongly preferred the pattern with global horizontal orientation (Figure 1.4c), although this pattern induces image motion in all directions.

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9 This effect was first pointed out by Hertz (1935), although more generally and applied to patterns presented in the horizontal plane.

10 The "global orientation" of a pattern (as opposed to its "local orientations") is defined as the predominant orientation of the pattern as a whole.
a third test, an attempt was made to simulate pattern orientation by moving randomly arranged dots (i.e. orientation free patterns) vertically (to simulate horizontal orientation) and horizontally (to simulate vertical orientation), respectively (Figure 1.4d). However, these moving patterns were not discriminated by the bees. Finally, the geometric global orientation was set in conflict with the simulated orientation by presenting the bees with dots arranged in horizontal rows moving horizontally and vertical rows of dots moving vertically (Figure 1.4e). In this situation, the bees strongly preferred the stimulus with the correct geometric orientation.\footnote{This test also serves as a control, demonstrating that bees can perform well with moving stimuli.}

Srinivasan et al. (1993b) also showed that bees can discriminate the orientation of patterns presented electronically in short flashes of only 2 ms every 0.5 s. It is very unlikely that motion cues can be extracted from the patterns under these conditions. These findings, together with the results presented in Figure 1.4, indicate that directionally-selective movement detectors are not involved in the orientation analysis of honeybees.

Srinivasan et al. (1994) then reported an interesting result that provided strong support for a new hypothesis for the mechanism of orientation analysis in the bee. In the Y-maze apparatus described above, bees can discriminate two bars oriented at 45° to each other (Figure 1.5b), but cannot be trained to distinguish between two right-angled crosses oriented at 45° to each other (Figure 1.5c).\footnote{Wehner and Lindauer (1966) were able to train bees to do this discrimination. However, in their tests – as well as their training – the bees were allowed to fly right up to the patterns before making their decision. In this situation the patterns subtend a much wider visual angle (up to ca. 170°) than do the patterns in the Y-maze (less than 50°). For further discussion see Horridge (in press).} This result can be explained by assuming that the analysis of pattern orientation is mediated by a number of orientation-sensitive channels, the orientation tuning curves of which have an angular half-width of
Figure 1.4: Tests on the significance of image motion for orientation analysis after training on horizontal _versus_ vertical random gratings (Srinivasan et al. 1993b). **a:** Learning test. **b:** Test on stationary, randomly arranged dots. **c:** Test on stationary dots arranged in rows. **d:** Test on moving, randomly arranged dots. The direction of motion is indicated by the arrows next to the patterns. **e:** Test on moving dots arranged in rows. Histograms show the choice frequencies with respect to the corresponding patterns. The calibration bar to the left of each histogram equals 1. \( \alpha \): choice frequency in favour of the left pattern \( \pm 1 \) standard deviation. \( n \): total number of choices. \( p \): significance level for \( \alpha \) being different from random choice.

ca. 90°. It turns out that, to allow an unambiguous determination of orientation, a minimum of three orientation channels is required (Srinivasan et al. 1994).

Figure 1.6 illustrates the multichannel model of orientation computation as described by Srinivasan et al. (1994). It features three channels with preferred orientations separated by 60°. The orientation tuning curve of each channel is described by a raised sine and has, therefore, a half-width of 90°. With this model the orientation of a single bar can be unambiguously determined from the ratios between the outputs of the three channels (Figure 1.6a; Figure 1.6b for one channel). However, if the model is presented with a right-angled cross, the output of each channel — and therefore the response of the model — will always be the same, irrespective of the orientation of the cross (Figure 1.6c). It can be shown mathematically and graphically (Figure 1.6c) that the excitations of each channel resulting from the two orientations in the cross always add up to the same output, whatever angle the cross is oriented at. This corresponds very nicely to the bees' behaviour (Figure 1.5c).

Finally, if the model is presented with a cross made up of two bars at an angle of 45°, the output of each channel varies with the orientation of the cross (Figure 1.6d). Thus, a
mechanism based on this model should be able to discriminate between such a cross and the same cross rotated by 90°. As Srinivasan et al. (1994) have found in behavioural experiments, bees are very well able to make this discrimination (Figure 1.5d).

Only two studies of properties of the bee's orientation analysis have been performed so far. Zhang and Srinivasan (1994) found, not surprisingly, that orientation discrimination improves with increasing orientation content in the test patterns. This can be achieved by lengthening the oriented element (e.g., a bar), increasing the number of similarly oriented elements, or aligning a number of elements. Experiments involving white noise textured bars or backgrounds suggest that the orientation information is carried in the spatial intensity distribution of the (low-pass filtered) pattern (Zhang and Srinivasan 1994). However, the bees could also be trained to discriminate the orientations of single edge patterns, suggesting that edges can carry orientational information as well. Zhang and Horridge (1992) tried to assess the extent to which a pattern can be divided into regions carrying different orientations before the bee's discrimination of these local orientations breaks down. This study will be discussed in Chapter 7 of this thesis.

1.3 Thesis outline

The primary objective of this thesis is to deepen our understanding of the honeybee's analysis of pattern orientation and its role in pattern discrimination. From recent studies (see above) we know that bees can learn and apply pattern orientation as an abstract parameter. We also know that this orientation analysis is not dependent on image motion, and that it breaks down when the bee is presented with a right-angled cross. Based on these properties the multichannel model of orientation computation was
Figure 1.6: The multichannel model of orientation computation (Srinivasan et al. 1994).  
(a) Polar (left) and linear (right) plots of responses of three channels with preferred orientations of 30° (solid line), 90° (dashed line) and 150° (dash-dotted line), respectively, as a function of pattern orientation. An example orientation of 50° (230°) is indicated by a bar in the polar plot and a grey, vertical line in the linear plot.  
(b) Response of a single channel (preferred orientation 30°) to a bar at different orientations.  
(c) Response of the same channel to a right-angled cross. The total response (solid line) is the sum of the responses to each of the two bars that make up the cross (dotted lines).  
(d) Response of the same channel to a 45° cross. Same notation as in c.
INTRODUCTION

postulated. One of the intentions of this thesis is to scrutinise the validity of that model. Other questions addressed in this thesis include how pattern orientation is used in different behavioural contexts and what role orientation analysis plays in the bee's pattern recognition, depending on the task at hand. To date, very little is known about these aspects of orientation discrimination.

Chapter 2 contains a detailed description of BEOS, a computer simulation of the optics of the honeybee's eye. This simulation was primarily developed as a tool for the design of visual stimuli to be used in bee experiments. However, it also proved useful in interpreting the results of some of the behavioural experiments presented in later chapters. Chapter 3 describes the methodology and basic experimental setup common to all the behavioural studies featured in this thesis.

The remainder of the thesis – excluding the general discussion at the end – can be grouped into two categories. The first category covers studies of the properties of orientation analysis and its neural substrate, with the aim of finding out more about the neural mechanisms subserving orientation discrimination. The chromatic properties of the bee's orientation analysis are described in Chapter 4, while Chapter 5 presents the results of an experiment measuring the temporal properties of the bee's orientation discrimination behaviour. In Chapter 9 these results are then compared with electrophysiological data from a number of potential candidates for neurons involved in the analysis of pattern orientation.

The second category includes studies investigating how the bees make use of their ability to discriminate pattern orientation under different experimental conditions. Chapter 6 describes experiments in which pattern orientation cues and intensity distribution cues were studied in combination. From Sections 1.1.2 and 1.2 we know that both these aspects of pattern discrimination are more or less well understood in isolation, but their relative importance and their interaction when offered simultaneously has never been examined. The other two studies in this category are related in that both investigate the significance of pattern orientation presented in particular parts of the visual field. The experiments described in Chapter 7 were designed to investigate the bees' use of local orientation in different pattern regions under two different experimental conditions. While earlier studies with patterns of non-uniform orientation tried to identify the minimum size of a pattern region still conveying orientation information, the present investigation focussed primarily on possible asymmetries in the importance of different pattern regions. Finally, the study reported in Chapter 8 was designed to investigate how pattern orientation is used in the peripheral (i.e. lateral, dorsal and ventral) visual field and whether there is interocular transfer of orientation learnt with one eye only.

The last chapter of this thesis, the general discussion, summarises the findings of Chapters 2–9 and points out a number of more general observations that emerge from some of those chapters – individually or in combination – in the broader context of bee vision and experimental biology.
Chapter 2

BEOS: A tool for experimental design

2.1 Introduction

When designing visual experiments, one important aspect to be considered is the visibility of the stimuli to the experimental animal. In our case we have to be sure that the bees in our trainings and tests perceive the patterns we are offering them the way we think they do. As I will demonstrate below, the bee's eye is by no means a simple and uniform structure. This means that, if we want to get a reasonably accurate idea of how an image in front of the bee appears on the bee's retina, we cannot simply filter the input image with an uniform filter. This is where BEOS comes into play.

BEOS (Bee Eye Optics Simulation) is a program I developed as a tool for designing experiments in bee vision. It allows us to view a flat pattern in front of the bee through an approximation of the optics of the bee's eye. By using the simulation, we can check whether a pattern that is planned to be used in an experiment is adequate and appropriate for the question that is being posed.

In this chapter I will first give a brief survey of the theoretical basis of the optics of compound eyes and review the relevant literature, reaching back in time by more than one and a half centuries. I will then describe BEOS in detail and, finally, discuss its application in the last section of this chapter.

2.1.1 The theory of vision in compound eyes

The bee has two different types of eyes. One type, the ocellus, is a small, single lens eye of which the bee has three. The other type, the compound eye, is the one we will be concerned with here, since the ocelli are not involved in the visual tasks we are interested in (Wilson 1978, Stange 1981). A compound eye consists of a number of single units, or ommatidia. Each ommatidium has its own dioptric apparatus and a set of light receptors which receive light from a limited portion of the outside world.

The spatial resolving power of any eye is potentially limited by three factors: (i) the quantity of light available to the eye, (ii) the quality of the eye's optics and (iii) the spatial sampling frequency of the receptor mosaic. Since most of the behavioural experiments reported in this thesis were conducted in broad daylight, the first factor has little relevance here and is therefore not dealt with in detail. Instead – as have most of the authors in this field – I will concentrate on the optical and anatomical limitations.

Optics. A lens can never form a perfect image exactly reproducing the original object. The reasons for this inherent imperfection are spherical aberration, chromatic aberration and diffraction. The effects of the first two factors are relatively small in lenses with
small apertures such as the bee's facets, but with diffraction the opposite is true. Parallel light passing through a lens with small aperture is not focused onto a single point, but is dispersed into the so-called Airy diffraction pattern, in which the intensity distribution shows a central peak (called the Airy disc) surrounded by concentric rings of much smaller amplitude. The halfwidth (width at half height) of the Airy disc is

$$\Delta \rho = \frac{\lambda}{D},$$

(2.1)

where $\lambda$ is the wavelength of light and $D$ is the diameter of the aperture (Hecht 1987, Warrant and McIntyre 1993). This dispersion of light has the effect that the distal end of the rhabdom (positioned on the optical axis) receives some light even if incident rays enter the ommatidium at an angle to the optical axis. If we convolve the Airy disc with the acceptance function of the rhabdom, we get the theoretical angular sensitivity function of the ommatidium (Snyder 1979). The actual sensitivity function, however, does not only depend on the Airy disc and the rhabdome acceptance function, but also on waveguide effects (if the rhabdom is narrow enough to act as a waveguide, which is the case in the bee) and possible light spread in the retina (Warrant and McIlwain 1993). The angular sensitivity function can usually be approximated by a two-dimensional, circularly symmetric Gaussian function of halfwidth $\Delta \varphi$, the acceptance angle of the ommatidium.

The Fourier transform of the angular sensitivity function, the modulation transfer function (MTF), tells us what range of spatial frequencies the dioptric apparatus of the ommatidium can transmit. The frequency at which this function reaches zero is called the cut-off frequency ($v_{co}$). This frequency is (Land 1989)

$$v_{co} = \frac{D}{\lambda}.$$

(2.2)

Any intensity modulations at spatial frequencies above $v_{co}$ are not perceived by the retina. This is the optical limitation to the spatial resolving power of the compound eye.

**Anatomy.** The spatial sampling frequency ($v_s$) of a compound eye is a function of the interommatidal angle $\Delta \varphi$ (i.e., the angle between the optical axes of neighbouring ommatidia). According to the Whittaker-Shannon sampling theorem, the highest spatial frequency an array can resolve is

$$v_s = \frac{1}{2\Delta \varphi}$$

(2.3)

for a square array (Snyder 1977) or a hexagonal array along one of its axes (i.e., parallel to one of the diagonals of an individual hexagon). If the grating is oriented perpendicularly to an axis of a hexagonal array, then (Snyder 1977)

$$v_s = \frac{1}{\sqrt{3}\Delta \varphi}.$$

(2.4)

In these situations each period of the grating would be sampled by two receptors. In other words, the receptors could be aligned with the extrema of the grating, such that a sequence of neighbouring receptors would view alternately dark and light areas.\(^\text{1}\)

\(^{1}\) On the other hand, if the receptors are aligned to view the edges of the gratings, the whole array of receptors would perceive a uniform grey.
**Eye parameter.** In an ideal eye, the sampling frequency of the receptor mosaic should be lower than or equal to the cut-off frequency of the optics. A higher sampling frequency would be useless, since higher frequencies never reach the retina. If we combine equations (2.2) and (2.3) we get (Land 1989)

\[ v_{\infty} \geq v_s, \text{ or } \frac{D}{\lambda} \geq \frac{1}{2\Delta\phi}, \text{ or } D\Delta\phi \geq \frac{\lambda}{2}. \quad (2.5, 2.6, 2.7) \]

The eye parameter \( p = D\Delta\phi \) (Snyder et al. 1977) is a measure of how close the eye comes to the diffraction limit. With 500 nm light its minimal value (when \( v_{\infty} = v_s \)) is 0.25 \( \mu \)m, but real \( p \) values range from about 0.3 \( \mu \)m in the fovea of some insects (e.g. the sand wasp *Bembix*; Horridge 1977) to 31 \( \mu \)m in the king crab *Limulus* (Land 1989). In other words, most compound eyes undersample their environment. One reason for this is that, if the amount of information in the image is to be maximised, the eye parameter is a function of the light level at which the eye operates (Snyder et al. 1977). Accordingly, animals active in bright daylight often feature low values of \( p \), while the highest \( p \) values are found in nocturnal and marine animals (Horridge 1977).

In this section we have only covered the resolving power of the lenses and the spatial resolution of the retina. The visual acuity, i.e. what the animal perceives and acts upon in a behavioural experiment, depends on many more factors such as the angular velocity of the pattern, its contrast and colour, as well as on the mechanisms underlying subsequent processing. Neural and behavioural mechanisms that might improve or impair visual acuity include lateral inhibition, neural pooling and temporal scanning. These aspects will not be covered here, since BEOS is a simulation of the bee’s optics only.

### 2.1.2 Bee vision

**The interommatidial angle \( \Delta\phi \).** Götze (1927) and Baumgärtner (1928) were the first to make quantitative measurements on the compound eyes of the bee. Götze determined the extent of the bee’s visual field by assuming that the hairs on the cornea of the compound eye are parallel to the adjacent ommatidial axes (which led to an underestimate of the visual field and binocular field). He also made some fairly crude measurements of the interommatidial angles, using histological sections in various planes. Baumgärtner’s measurements of \( \Delta\phi \), although based on the same technique, are much finer. Figure 2.1 (broken lines) shows Baumgärtner’s mean \( \Delta\phi \) as a function of the ommatidium number.

It is obvious that the interommatidial angles are not uniform. The angles vary between 1.7–7.5° in the vertical section\(^2\) and between 2.7–4.0° in the horizontal section. The minima correspond to ca. 8° down from the horizontal and 60° to the side from the frontal direction, respectively (Baumgärtner 1928; not directly inferable from Figure 2.1.). However, since the sections were made relative to the eye’s shape, and not relative to its natural position in space, we cannot directly apply these resolution optima to the bee’s behaviour. Baumgärtner had some indications from behavioural experiments (see

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\(^2\) Baumgärtner’s definition of the vertical interommatidial angle – as well as del Portillo’s later on – corresponds to half of \( \Delta\phi \), as it is used here. Their data have been converted to match the definition of \( \Delta\phi \), given in Figure 2.3b.
Figure 2.1: Baumgärtner's and del Portillo's measurements of $\Delta \varphi$ in the bee's eye. Abscissa: interommatidial angle $\Delta \varphi$ in degrees. Broken lines: Baumgärtner's data. Solid lines: del Portillo's data. a: Vertical $\Delta \varphi$. Vertical section containing ca. 144 ommatidia. (1 unit = 5 ommatidia) The lower end of the scale corresponds to the ventral rim of the eye. b: Horizontal $\Delta \varphi$. Horizontal section containing ca. 48 ommatidia. The lower end of the ordinate corresponds to the posterior rim of the eye. (Redrawn from del Portillo 1935).

Chapter 1) of where in the bee's visual field these optima should lie and suggested that the bee might align them by tilting its body.\(^3\)

The main difficulty in applying Baumgärtner's measurements to the bee's visual acuity is that the ommatidium's anatomical axis does not necessarily correspond to its optical axis, i.e. the ommatidium is often looking in a different direction from the one to which it is geometrically aligned. Baumgärtner (1928) measured differences of up to 40° (at the ventral rim of the eye).

Del Portillo (1935) repeated Baumgärtner's measurements of $\Delta \varphi$ (Figure 2.1, solid lines). In the vertical sections his results are slightly different, as he was cutting in a different plane (through the middle of the eye rather than along a plane through both eyes as Baumgärtner did). He reports the vertical $\Delta \varphi$ to range between 2.2° and 5.2°. In the horizontal plane his measurements basically match Baumgärtner's, except for the border regions, where he found smaller values. He attributes this phenomenon to "developmental deficiencies".

It was not until almost fifty years later that more accurate (i.e. non-histological) measurements of the bee's interommatidial angles were performed. Seidl (1982; Seidl and Kaiser 1981) mapped out the bee's visual field by studying the pseudopupil under

\(^3\) He filmed bees approaching a vertical pattern and found that the angle between the longitudinal axis of the bees and the horizontal varies between 15–30°.
antidromic illumination. This optical technique permitted the determination of the optical axis of each ommatidium in a coordinate system centred on the bee's head which can be related to the external world. Since the measurements were done on whole heads, they can be expected to be much more accurate (less artefact prone) than measurements on histological preparations. Seeidl's results are depicted in Figure 2.2.

Seeidl's $\Delta \phi_h$, i.e. the interommatidial angle in the horizontal plane, shows little variation along the vertical. It ranges from 2.1° to 4.6° (Seeidl 1982) and its minimum along a given latitude is mostly located between ca. 30° and 45° to the vertical midline. $\Delta \rho_v$ ranges from 1.2° to 4.9°. It shows a minimum at the equator, increasing towards both the dorsal and the ventral rim of the eye. It is quite uniform along a given latitude, except at the equator, where it decreases towards the anterior and the posterior rim of the eye, and the dorsal rim, where the opposite is the case.

These optical measurements (Figure 2.2) compare quite well with the older histological data (Figure 2.1). The overall pattern in all of them is the same: both horizontal and vertical interommatidial angles are minimal somewhere in the centre of the eye and increase towards the borders.

The acceptance angle $\Delta \rho$. The visual field of individual *Apis mellifera* ommatidia have been measured optically and electrophysiologicaly and calculated using ray-tracing techniques. The results of these studies can be grouped into two distinct classes with $\Delta \rho$ values differing by a factor of two. The first class includes Kuiper's (1962) and Varela and Wiitanen's (1970) studies. Kuiper measured the acceptance angle optically on "scaps" cut from the eye while frozen in liquid air. He found a bell shaped acceptance function with a halfwidth (width at half height) of about 6.5° and a total width of about 20°. Varela and Wiitanen measured the refractive indices of the different parts in the ommatidium, as well as their dimensions and relative position, and used this data to calculate an admittance function using both ray-tracing techniques and the Gaussian thick lens formula. The resulting function had the shape of a Gaussian with halfwidth $\Delta \rho = 5.55°$.

The second class of studies found acceptance angles of only half this width. The acceptance functions measured using optical techniques by Wiedemann (1965, see also Autrum and Wiedemann 1962) and Eheim (1972, see also Eheim and Wehner 1972) had a halfwidth of 2.60° (Eheim 1972) and a total width of ca. 7° (Wiedemann 1965, Eheim 1972). Laughlin and Horridge (1971) measured $\Delta \rho$ electrophysiologically and found it to be 2.6° as well. Ohly (1968, cited in Eheim and Wehner 1972) used the pseudopupil under antidromic illumination to determine the total width of the acceptance function to be about 8°. The exact results of all these studies are summarised in Table 2.1.

It is not clear what caused the difference between the first two studies listed in Table 2.1 and the remaining studies in the list. Kuiper's results might contain an artefact caused by the freezing of the preparation. It has been pointed out (Laughlin and Horridge 1971) that Varela and Wiitanen failed to take diffraction effects within the

---

4 Antidromic illumination reverses the path of the light through the eye's optics. The insect's head is illuminated from inside, so that the light follows the rhabdom to its distal tip and leaves the ommatidium through the dioptric apparatus.

5 Varela and Wiitanen’s (1970) "admittance function" is defined as the percentage of rays reaching the rhabdom of those falling onto the ommatidium as a function of angle of incident.
Figure 2.2: Seidl’s measurements of $\Delta \varphi$ in a bee’s right eye. The angular space around the bee is represented by the posterior (left) and anterior (right) hemisphere. Each dot represents a measurement. Upper half: horizontal interommatidial angle ($\Delta \varphi_h$). Lower half: vertical interommatidial angle ($\Delta \varphi_v$). (After Seidl 1982).

cone tip into consideration, which might account for the difference between their admittance function and the intensity distributions measured in the last four studies listed in Table 2.1. All in all I prefer to rely on the second class of results with a $\Delta \varphi$ of 2.6°.

Some of the studies summarised in Table 2.1 differentiated between acceptance angles in the horizontal and vertical direction, respectively. None of them found a significant difference between the two directions. Furthermore, Wiedemann (1965) and Eheim (1972) report slightly larger angles in the horizontal, while in Laughlin and
Horridge's (1971) results the opposite is true. It can therefore be assumed that the acceptance function of the bee's eye is radially symmetric.

Finally, one of the studies (Eheim 1972) took the adaptation state of the eye into account as well. The results show that $\Delta \rho$ in the dark adapted state (marked as (d) in Table 2.1) is slightly larger than in the light adapted state (l). However, this difference was not statistically significant.

All the measurements listed above were done on ommatidia in the central eye region, i.e. the region looking sideways. We do not know how $\Delta \rho$ varies across the eye. From the theoretical point of view of an ideal match between interommatidial angle and ommatidial acceptance angle one might expect $\Delta \rho$ to vary with $\Delta \phi$, but so far there is no experimental evidence for that.

**Table 2.1:** Summary of measurements of the acceptance function of honeybee ommatidia. h: horizontal halfwidth. v: vertical halfwidth. l: light adapted. d: dark adapted.

<table>
<thead>
<tr>
<th>Study</th>
<th>Technique</th>
<th>Halfwidth ($\Delta \rho$)</th>
<th>Total width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuiper (1965)</td>
<td>optical on frozen prep.</td>
<td>ca. 6.5°</td>
<td>ca. 20°</td>
</tr>
<tr>
<td>Varela and Wiitanen</td>
<td>ray-tracing</td>
<td>5.55°</td>
<td>ca. 15°</td>
</tr>
<tr>
<td>(1970)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiedemann (1965)</td>
<td>optical on frozen prep.</td>
<td>—</td>
<td>h: 7.2° ± 1.4°</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>v: 6.7° ± 1.1°</td>
</tr>
<tr>
<td>Eheim (1972)</td>
<td>optical on fresh prep.</td>
<td>h: 2.56° (l); 2.78° (d)</td>
<td>h: 7.42° ± 0.24°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>v: 2.53° (l); 2.75° (d)</td>
<td>v: 6.68° ± 0.14°</td>
</tr>
<tr>
<td>Ohly (1968)</td>
<td>pseudopupil</td>
<td>—</td>
<td>ca. 8°</td>
</tr>
<tr>
<td>Laughlin and Horridge</td>
<td>electrophysiology</td>
<td>h: 2.5° ± 0.4°</td>
<td>ca. 7°</td>
</tr>
<tr>
<td>(1971)</td>
<td></td>
<td>v: 2.7° ± 0.8°</td>
<td></td>
</tr>
</tbody>
</table>

**Acuity measured in behavioural experiments.** The first behavioural measurements of visual acuity in honeybees were performed by Baumgärtner (1928). He trained bees to distinguish between squares of different colours in the vertical plane and then moved the colour patches further into tunnels, away from the bees. He then recorded at what distance the bees were not able to discriminate the colours from outside the tunnels any more, i.e. when the bees entered the wrong tunnel equally often as the right one.

This approach is likely not to measure the bees' acuity, but rather their intensity discrimination ability, since an object could give rise to a mean intensity difference in a single ommatidium even if it is much smaller than $\Delta \phi$. It is well known (Hertz 1934, Palka and Pinter 1975) that the ability to resolve a periodic grating (which is the usually accepted measure of visual acuity) is not directly related to the ability to detect a single spot. Baumgärtner's experiments will be considered further in Section 2.3.2.

The same shortcoming applies to the experiments of Wolf (1931). He changed the interval length of a sequence of rectangular landmarks along the flight path of the bees, thus varying the angular size of each landmark seen from the previous one in the
sequence. The time it took the bees to find their way along this succession of landmarks was then interpreted as an indication of whether the bees could see a landmark when they passed the previous one.

A better approach to behavioural acuity measurements is to use extended sources such as gratings. A point source (such as a single rectangle) has a broad spatial frequency spectrum with no lower limit. Therefore it is impossible to determine the cut-off frequency of an optical system with a single spot. A perfect grating, on the other hand, does not contain any frequencies lower than its fundamental frequency, or only the fundamental frequency in the case of a sinusoidal grating.

Most studies of visual acuity in insects and crustaceans using gratings rely on the optomotor reflex. This reflex causes the animal to follow any wide field movement the eyes perceive, i.e. to stabilise the image of its surroundings on the retina. In the natural situation this helps a moving animal to stabilise its path in a stationary environment. Under experimental conditions the optomotor response can be utilised to determine whether a pattern (usually a grating) on the inside of a rotating cylinder can be resolved by the animal located at the centre. If the animal attempts to follow the movement of the cylinder, it perceives the pattern; if it does not move or moves in the wrong direction, it is not able to resolve the pattern and sees either a uniform mean luminance or some geometric interference pattern.

The first researcher (and to my knowledge the only one) to use optomotor experiments to measure visual acuity of honeybees was Hertz (1934). She placed her bees in the centre of a rotating, striped cylinder under a glass dome just big enough for the bee to turn in place. Using rather subjective criteria to interpret the ambiguous response of the bees she determined the minimum resolvable angular wavelength of the grating to be 4°–5°. Kunze (1961) performed an extensive study of the optomotor response in bees. However, when testing gratings of different wavelengths he limited the bees’ sampling interval artificially to 5°. His results are therefore not useful in determining the anatomical sampling frequency of the bee’s eye.

It can be argued that measuring the limits of the optomotor response only reveals the acuity of this visual subsystem and not necessarily the optical acuity of the bee’s eye. To overcome this problem Srinivasan and Lehrer (1988) trained bees to discriminate between two patterns in a Y-shaped testing apparatus similar to the one shown in Figure 3.1. Entering the Y at the "trunk", the bees had to decide between a black and white grating at the end of one of the branches of the Y and a uniformly grey area at the end of the other branch. By making the branches progressively longer and thereby moving the two patterns farther and farther away from the bees, the angular wavelength of the gratings as seen from the "decision chamber" (the branching point of the Y) could be reduced. The minimum spatial frequency at which the bees could just perceive the modulation of the grating was determined to be 0.27 c/deg (which corresponds to a

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6 Hecht and Wolf (1929) performed similar experiments, but these seem to have measured a different behaviour. Their bees crawled on an "inclined transparent surface" below which a luminous grating was moved. The response of the bees was "a sudden change in the direction of [their] progression, which [was] opposite in sign to the movement of the pattern." The minimal spatial wavelength of the grating to elicit this behavioural response at the maximal level of illumination was about 2°.

7 They also trained bees to discriminate between horizontal and vertical gratings, but the acuity measured that way, of course, potentially only reflects that of a subsystem as well, namely orientation discrimination.
grating wavelength of 3.7°). A detailed account of this study will be given in the discussion of this chapter.

**The Wiitanen-Varela model.** Computer simulation of the optics of the bee eye has been attempted previously by Wiitanen and Varela (1971). However, their matrix based simulation has two major shortcomings. Firstly, it assumes the ommatidia to be arranged in a regular hexagonal array with a constant interommatidial angle of Δφ = 1.5° (which we now know to be the absolute minimum Δφ in the bee's eye, applying only to Δφv along the equator). And secondly, the acceptance function it uses has a halfwidth of Δp = 5.55° (based on the results of Varela and Wiitanen (1970) obtained by ray tracing analysis).

Apart from these weaknesses the program might have been suitable for the tasks to which it was applied (but see Discussion). However, it is not appropriate for more general use, that is to simulate the bee's view of any arbitrary pattern. For example, the projection of the pattern to be viewed onto the surface of the model eye has to be done manually. It is apparent from the results presented in Wiitanen and Varela (1971) that this is a considerable source of error.

**2.2 Description of BEOS program**

BEOS (Bee Eye Optics Simulation) is a computer program that simulates the view through the optics of a honeybee's eyes. It was developed as a tool to aid the design of experiments on bee vision.

**2.2.1 The model eye**

BEOS is based on a single model eye with an array of sampling stations (the model ommatidia) approximating the bee's array of ommatidia as described by Seidl (1982). The visual field of the single eye covers the frontal hemisphere of the model bee. The left half of this visual field corresponds to the portion of the visual field between the frontal and the lateral median of the left eye, while the right half corresponds to the equivalent portion of the visual field of the right eye. The array of sampling stations is created by the following procedure. Starting with a frontal ommatidium, ommatidia are progressively added to the left and to the right, spaced by the interommatidial angle Δφh, which is a function of the azimuth (α, see Figure 2.3a). At α = 0 (frontal) Δφh is 3.7°. With increasing α, Δφh decreases linearly down to 2.8° at α = 45°, after which it increases, again linearly, back to Δφh = 3.7° at α = 90°. When α reaches 90° (the limit of the frontal hemisphere) it is set back to 0 and ε, the elevation (Figure 2.3a), is set to ε = Δφ/2 to commence the creation of the next row (see Figure 2.3b for explanation). To create a hexagonal array α is now set to Δφh / 2 before the first two (almost frontal) ommatidia of this row are added. The row can then be completed in the same way as the first one. The rows at ±ε and at −ε are created simultaneously. After completion of the second row α is set back to 0 again, ε is set to Δφv and the process starts over. In this

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8 The definition of this Δφ corresponds to Δφv in BEOS (see Figure 2.3). Due to the regularity of their hexagonal array Wiitanen and Varela's Δφv can be calculated to be Δφv = √3 ⋅ Δφh = 2.6°.
Figure 2.3: Coordinate system of the model eye of BEOS. a: View of the bee's visual field represented by a sphere. The bee is thought to be positioned at the centre of the sphere, facing out of the page, towards the lower left corner (along the axis where $\alpha = 0, \varepsilon = 0$). $\alpha$: azimuth. $\varepsilon$: elevation. b: Definition of horizontal and vertical interommatidial angles. The hexagonal array represents the surface of the eye (not drawn to scale). All the optical axes intersect in the same point, the centre of the eye.

manner row after row is filled up with ommatidia. Each time before starting a new row the elevation is increased by $\Delta \varphi_\varepsilon / 2$ and the azimuth is set to either 0 (for odd rows) or $\Delta \varphi_\alpha / 2$ (for even rows). The procedure is completed when $\varepsilon \geq 90^\circ$, i.e. when the poles are reached. $\Delta \varphi_\varepsilon$ is a function of $\varepsilon$. It is minimal at the equator ($\Delta \varphi_\varepsilon = 1.5^\circ$) and increases linearly with $\varepsilon$ (and $-\varepsilon$) to a maximum of $\Delta \varphi_\varepsilon = 3.5^\circ$ at both the dorsal and ventral poles.

Since the bee’s interommatidial angles were measured on great circles, whereas the coordinate system is based on parallels of latitude, the angle by which $\alpha$ has to be increased between each ommatidium is actually

$$\Delta \varphi_\alpha = 2 \sin^{-1} \left( \frac{\sin (\Delta \varphi_\alpha / 2)}{\cos \varepsilon} \right).$$  \hspace{1cm} (2.8)

As a result the rows close to the poles (where $\varepsilon$ approaches $90^\circ$) contain only a few ommatidia, although $\Delta \varphi_\alpha$ is maximally $3.7^\circ$ and $\alpha$ covers a full $90^\circ$.

The output of the procedure described above is an array of 6011 distinct ommatidia.\(^9\) Figure 2.4a, is a view onto the visual field of the model eye (positioned in analogy to Figure 2.3a). Each dot represents the intersection of an ommatidial axis with an

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\(^9\) The algorithm actually creates 6143 ommatidia. Because of the symmetry of the algorithm, ommatidia on the equator and the vertical midline are duplicated, i.e. two ommatidia share the same axis. This has been taken into account in the subsequent processing and is therefore of no relevance here.
Figure 2.4: Ommatidial array of BEOS. a: View of the hemisphere representing the frontal visual field of the bee, oriented as in Figure 2.3a. Each dot represents an ommatidial axis intersecting the hemisphere. b: Ommatidial array in Cartesian coordinates.
(invisible) hemisphere centred around the model bee’s head. Figure 2.4b represents the same ommatidial array, but plotted in Cartesian coordinates. Notice the higher density along the equator ($\varepsilon = 0^\circ$) and at $\alpha = \pm 45^\circ$.

Each model ommatidium has a Gaussian acceptance function with a halfwidth $\Delta \rho = 2.6^\circ$, simulating the visual fields of real ommatidia in the central eye region (Laughlin and Horridge 1971, Eheim 1972). This acceptance function is realised by an array of 441 sampling points, arranged in concentric circles around the axis of the ommatidium (Figure 2.5a). The weight ($g$) with which each sampling point contributes to the intensity the ommatidium perceives is a function of the angular deviation ($\delta$) from the optic axis. $g$ is modelled by a Gaussian with halfwidth $2^\circ$ (Figure 2.5b):

$$
g = 0.0109 \cdot e^{-0.6932 \cdot \delta^2}.
$$

(2.9)

The sampling array is later scaled radially so that this halfwidth fits the set $\Delta \rho$ (default $\Delta \rho = 2.6^\circ$) of the ommatidium. The sum of the weights of all sampling points is 1. That is, if an area of a uniform intensity $I$ is sampled, the output of the sampling array is $I$.

### 2.2.2 The process

BEOS is a DOS program written in C on a PC with 4 Mb RAM (which should explain the relatively low resolution and some other limitations). The program provides a help screen (see facing page) which lists all the options available. Here, the information provided in the help screen might serve as an introduction to the features of the program. (In the usage line, text in brackets denotes optional parameters. Default values are given in the list that follows.)

The input BEOS requires is a greyscale image. There are two reasons not to use colour images: (i) most experiments reported here are based on black and white patterns, and (ii) the colour perception of bees is different from human colour perception, making
BEOS simulates a bee's view of a given pattern (on the level of the retina). Its input can be any image in RAW format, maximally 720x720 pixels. Its output is either a view of the frontal visual field of the bee, projected onto a hemisphere, or the hexagonal array of ommatidia.

Usage: BEOS infile.raw outfile.raw [x000] [y000] [m000] [d000] ...

[x512] [y512] x and y dimensions of infile (max. 720)
[m240.0] x dimension of infile in mm (in reality)
[d100.0] distance bee-pattern in mm
[h0.0] [v0.0] x and y shift of bee's position in mm
[b255] background intensity (max. 255 = white)
[a-30] [e20] viewing angles (azimuth and elevation) for visual field
[r720] resolution in pixels per 180 deg (max. 720)
[t2.6] delta rho of ommatidia (deg)
[t1] text version (no graphics, ergo faster)
[p] geometrical projection of pattern only
[n] no filling up of non-hexagonal output
[w242] hexagonal output, (max. 242 = x dimension, x:y = 1:2)
[f] output hexagonal array as file for other programs (60x160 cells)

The interpretation of a coloured output of BEOS (in terms of what the bee really sees) very difficult. The most important parameters are s, m, d, h and v. The parameter s determines the acceptance angle Δφ of the ommatidia. Unless stated otherwise, s has the value 2.6°. The parameters m (horizontal dimension in space of the input pattern), d (shortest distance between the bee and the pattern plane), h and v (horizontal and vertical position relative to the centre of the pattern) together determine the angular size and position of the pattern as seen from the position of the bee.

The pattern plane around the pattern has a uniform intensity (grey level), which can be set with b. The parameters a and e determine the direction from which the visual field of the bee is viewed on the screen and in the output images. The default output of BEOS is a greyscale image showing the frontal visual field of the bee (as a hemisphere), in which the individual ommatidia are represented by areas of different intensity covering the whole hemisphere. This output demonstrates the "grain" of the retinal image, but not the distortions resulting from the eye's anatomy, i.e. the differences between (and variations of) Δφ_h and Δφ_v. The type of output can be controlled with the parameters p (output: visual field of the bee; no optical processing), n (representation of individual ommatidia by small dots on hemisphere), w (ommatidia arranged in hexagonal array representing the retina) and f (non-pictorial output). The hexagonal output (see below for details) is doubtlessly the type of output best suited to represent the image on the bee's retina.

The parameters x and y define the size of the input file. The parameters t (suppressing graphical display during processing) and r (resolution of the initial projection; see below) are options that save processing time, but are of no relevance here. All of the outputs discussed here were obtained using the maximal resolution.

10 A colour version of BEOS exists, but will not be discussed here.
Figure 2.6: Stages of BEOS. a: Input pattern. The height of the "R" subtends 331 pixels. b: Projection stage. The hemisphere of the bee's frontal visual field is viewed from outside, from the same direction as in Figure 2.3a. The bee is positioned directly in front of the pattern at a distance 1.2 times the height of the "R". The background grey level was set to 225 (white).

The first step of BEOS – after interpreting the command line arguments, reading in the input file and displaying the pattern on the screen – is to project the pattern plane onto a hemisphere representing the bee’s\textsuperscript{11} frontal visual field. Since the bee is always facing the pattern plane the posterior part of the visual field is always empty, i.e. there is no need for BEOS to look backwards. Under the highest resolution the hemisphere is divided into 720 pixels both in the horizontal and the vertical direction.\textsuperscript{12} Each pixel is allocated a rectangle in the pattern plane which corresponds to the exact radial projection of the pixel onto the plane. The grey level of the pixel on the hemisphere is set to the mean grey level within the rectangle on the pattern plane. If that rectangle (or part of it) lies outside the pattern, the background intensity (parameter $b$) is used. Functionally this projection step is not really necessary, but it reduces processing time for the next step dramatically. Figure 2.6b shows the result of this first step. By setting the parameter $p$ BEOS can be terminated at this stage.

The next step – after the ommatidial array and the Gaussian sampling array have been created following the procedure described earlier – is where the actual processing, that is the simulation of the optics, is implemented. From the centre of the hemisphere each ommatidium now "looks" at the projected pattern. This is achieved by centring the appropriately scaled Gaussian sampling array on the ommatidium's axis and multiplying the grey levels at each sampling point by the respective weights. The sum of the weighed grey levels is then taken as the grey level the ommatidium perceives.

If the parameter $n$ was set, BEOS stops here and puts out Figure 2.7a. This is a view of the (transparent) hemisphere, on which the perceived grey level of each ommatidium

\textsuperscript{11} In this section "the bee" always denotes the model bee.
\textsuperscript{12} While all of these pixels are of the same height, the pixels at the equator are much wider than the ones close to the poles.
Figure 2.7: Stages of BEOS. a: Output corresponding to Figure 2.4a. Each dot represents the grey level perceived by an ommatidium. b: Same situation, but the dots in (a) have been expanded to fill the blank spaces. Inset: Detail (framed area in b), enlarged 4 times.

is displayed at the intersection of the ommatidium’s axis with the hemisphere. In the default output (Figure 2.7b) these points are expanded until they touch. The shape of the resulting patches is a consequence of the algorithm and has no biological significance. In fact, the arrangement on the hemisphere is potentially deceiving: on the hemisphere the patches are in close contact with their neighbours in the vertical direction, but are separated from the neighbouring patches in the horizontal plane. However, the ommatidia in the retina of the real bee follow the opposite pattern: they touch their horizontal neighbours, but are separated from the vertical ones. In order to overcome this problem, BEOS can display the ommatidial array as it is in the eye, i.e. as a (pseudo-)hexagonal array\(^\text{13}\) (parameter \(w\); Figure 2.8). Each hexagon in this array represents an ommatidium. The white area in Figure 2.8a corresponds to the frontal hemisphere of the bee’s visual field. This area is oval because, due to the difference between horizontal and vertical interommatidial angles, the number of ommatidia in the row viewing the horizon is smaller than the number of ommatidia viewing the frontal median.

\(^{13}\) In a rectangular array, such as a pixelated computer image, it is impossible to draw perfect hexagons. In the "hexagonal" BEOS output hexagons are approximated by squares of 4 by 4 pixels with the corner pixels missing (see Figure 2.8b for a close-up). This results in a distortion of the hexagonal array. To obtain a proportionally perfect representation of the retina, the hexagonal output of BEOS would have to be scaled in the vertical direction by the factor \(f = 1.156\).
2.3 Discussion

BEOS is a model. As such it has to make several assumptions and approximations:

The model eye consists of a single eye in which all optical axes radiate from the centre. However, the real bee has two eyes, 2–3 mm apart (Seidl 1982). Furthermore, the real bee's eye does not have a single point of convergence. Rather, the intersections of optical axes of neighbouring ommatidia form a funnel-like surface within the compound eye (Baumgärtner 1928; del Portillo 1934). However, because the model ommatidial array does not correspond directly to the bee's ommatidial array anyhow (see below) this approximation of using a single, radial eye does not introduce any additional error. The ommatidial array of BEOS is not an exact representation of the bee's ommatidial array. In other words, we cannot identify individual ommatidia in the model eye and relate them to particular ommatidia in the bee's eye. For our purposes, however, that is not necessary. We want to know what the filter characteristics for different regions of the patterns are, so the only important thing is that the model faithfully reproduces the relevant parameters ($\Delta \varphi$ and $\Delta \rho$) and their variation in different eye regions.

The model eye assumes a constant ommatidial acceptance angle of $\Delta \rho = 2.6^\circ$. This is the value measured optically and physiologically in the central region of the bee's eye, i.e. in ommatidia pointing to the side with respect to the head (Laughlin and Horridge 1971, Eheim 1972). We do not know whether, and if so, how $\Delta \rho$ varies across the eye. If discovered, such variations can be incorporated into refined versions of the model.

A model is not of much worth without controls. Ideally the model and the design of the associated simulation should be based on one set of data, while a completely different set of data should be used to test the model. In the case of BEOS the model is based on findings of histological, physiological and optical studies of the bee's eye. To test the model we will be using results of behavioural experiments. But first we will have to test the simulation as such, i.e. the program implementing the model.

2.3.1 Testing the simulation

To demonstrate the functionality of the projection of the pattern plane onto a hemisphere (first step of BEOS) two test images were used as input and BEOS was terminated after the projection stage. In Figure 2.9 the resulting output is shown along with the test images. Test image A consisted of a circular disc filled with a fine checkerboard (unit size one pixel) and a central calibration pattern as shown in Figure 2.9a (representing the central 60×60 pixels of the pattern only). This image, assumed to be 24 cm wide, was viewed by BEOS from a distance of only 2 mm, producing the output depicted in Figure 2.9b and c. This test demonstrates that the projection is accurate down to very small scales. Test image B was a black and white pattern containing radial edges and circular, concentric edges. The radii of the latter were chosen such that, if the 50 cm wide image was viewed from a distance of 67 mm, all the concentric edges would be separated by 15\(^\circ\), the outermost edge being 75\(^\circ\) off axis. In Figure 2.9e the edge positions in BEOS' output for this situation are compared with the expected positions of points on the horizon of the hemisphere, separated by 15\(^\circ\) (grey lines intersecting with the horizon). This comparison shows that the angular projection is correct. Finally, Figure 2.9f
Figure 2.8: Stages of BEOS.  

a: Hexagonal output. See text for explanation.  
b: Central area of hexagonal output, enlarged.

demonstrates the accuracy of the representation of the hemisphere on the screen and in the output file, respectively. Here, the same hemisphere as in Figure 2.9e was rotated to the right by 15° and downwards by 30°. (In this Figure the bee’s visual field is represented as a full sphere with the posterior half appearing in white, the background colour.) The grey lines represent the position of the horizon and the vertical midline
Figure 2.9: Test of the projection stage of BEOS. a: Central 60x60 pixels of test image A (total size of image: 512x512 pixels). b: Frontal view of hemisphere with projected test image A. c: Same hemisphere oriented as in Figure 2.3, i.e. in the default position of a-30 e20. d: Test image B. e: Frontal view of hemisphere with projected test image B (see text for details). f: Same hemisphere oriented with a15 e30 (see text for details).
before the rotation. The fact that these lines form tangents to the first two concentric edges (with radii of 15° and 30°, respectively) demonstrates that the modification of the viewing angle for the visual field (parameters a and e) is implemented correctly. Note that the rough edges in the central region of the projection of test image B (Figure 2.9e and f) are not a deficiency of the projection. They are, rather, the result of BEOS resolving the pixelation of the input image (which was 500x500 pixels in size) in those parts of the image that were close to the viewing position.

The Gaussian sampling array for each ommatidium was tested in two different ways. The first test was employed to ensure that the sampling density of the array was
Figure 2.11: Test of the procedure creating the Gaussian sampling array. 

**a:** Detail of BEOS' output. The intensity perceived by each ommatidium was multiplied by 50. 

**b:** Rationale of the test. The innermost two rings of sampling points (grey dots on circles) are displayed for two different ommatidia (hexagons). The white square represents the light source viewed on a black background. See text for explanation.

sufficient for the tasks BEOS would normally encounter. This was accomplished by rewriting BEOS to double the sampling frequency and running a few selected inputs through both the modified version and the original version. If the latter was undersampling, we would expect to find a difference between the two outputs (the modified version producing a more realistic output). If the original version's sampling density was adequate (or higher), the modified program would simply be oversampling, which should have very little effect on the output. Figure 2.10 presents the comparison of the two versions' outputs for a vertical grating ($\lambda = 4$ cm) viewed from four different distances (20, 30, 40 and 50 cm; See caption for details). With the mean error (defined as the mean absolute difference in grey level as a fraction of 255, the difference between black and white) varying between 0.59% and 0.95% the two versions produce very similar outputs. We therefore conclude that the sampling density of the Gaussian array of the original version (i.e. of BEOS as discussed in this chapter) is at least sufficient.

To test for faults in the procedure creating the Gaussian sampling array we can make use of the array's discrete sampling. If we set the halfwidth of the acceptance function ($\Delta\rho$) to 26°, i.e. ten times the default value, the scaling of the sampling array results in big gaps between the individual sampling points. (Since the halfwidth of the acceptance function is covered by 9 sampling points (see Figure 2.5) the gaps along the radius will be 3.5°.) If we then have BEOS look at a small light source, eg. a square of 2×2°, the
Figure 2.12: BEOS output simulating tests performed by Srinivasan and Lehrer (1988). A 2 cm grating oriented horizontally (top row) and vertically (bottom row) viewed from distances of 10–50 cm. Only the central part of each output is shown. The background around the pattern was set to 127 (50% grey).

A sampling array will hit this square with only one or no sampling point for each ommatidium. The perceived intensity of any ommatidium is then either 0, if the square slipped through the gaps of the sampling array, or equal to the weight of the one sampling point that hit the square. Figure 2.11 shows the result of the described test, along with a graphical visualisation of the rationale of the test. The output of BEOS in this test situation (Figure 2.11a) represents exactly what we would expect for BEOS’ sampling array. (Compare, for example, the two ommatidia represented in Figure 2.11b with their output.) This shows that the Gaussian sampling array has been implemented correctly.

Several more tests were performed on every stage of the program to check for other errors in the code. Special emphasis was placed on the geometry of the ommatidial array and the accuracy of the program’s output.
2.3.2 Testing the model

The study at hand to test the model BEOS is based on the series of discrimination experiments reported by Srinivasan and Lehrer (1988). In these experiments bees were trained, by reward, to discriminate between a periodic grating consisting of black and white stripes of equal width and a uniform grey of the same mean intensity. In other words, the bees, in order to find the reward, had to choose the pattern displaying some modulation in intensity (or the one without modulation, depending on the training). After successful training the patterns were moved farther down two separate tunnels in a Y-maze. This shift increased the angular spatial frequency of the grating as seen from the entrance of the tunnel. It was then recorded at what distance of the patterns the bees entered the two tunnels equally often, i.e. could not tell the two patterns apart anymore. The interpretation is that at that distance (and the corresponding spatial frequency), the intensity modulation in the pattern was no longer transmitted onto the bees' retina, so that both patterns looked the same — uniformly grey — to the bees.

We can now put BEOS through the same test and see how well the intensity modulation in the pattern is transmitted by our model eye. Of course, BEOS simulates only what the bee's retina "sees", and there are several stages of processing between that and the behavioural response of the bee. But this test would at least tell us if the model's resolution was too poor.

Srinivasan and Lehrer (1988) tested the bees on gratings of two different periods (2 and 4 cm), at five different distances (10, 20, 30, 40 and 50 cm) and oriented either vertically or horizontally. A simulation of the bees' view of a 2 cm grating in these situations is shown in Figure 2.12. The bees' choice frequencies for the rewarded pattern in tests on 2 cm gratings versus a uniform grey field are summarised in Figure 2.13a. To compare this to the output of BEOS, Figure 2.13b shows the maximal contrasts.
Figure 2.14: Actual and apparent movement of gratings of different spatial frequencies. The format of this Figure was chosen to match Figures 1–7 in Hertz (1934). Each set of three panels shows a time course of the position of the grating, the theoretical prediction for the processed image (based on non-overlapping, rectangular acceptance functions for the ommatidia), and the output of BEOS in the given situation. See lower right for notation. a: \( \lambda = 4\Delta \varphi \). b: \( \lambda = 3\Delta \varphi \). c: \( \lambda = 2.5\Delta \varphi \). d: \( \lambda = 2\Delta \varphi \). e: \( \lambda = 5\Delta \varphi / 3 \). f: \( \lambda = 4\Delta \varphi / 3 \). g: \( \lambda = 2\Delta \varphi / 3 \).

contained in the individual panels in Figure 2.12, ie. the contrast between the darkest and the brightest ommatidium.

Figure 2.13 demonstrates two points: (i) In both the behavioural experiment and BEOS the horizontal and the vertical gratings yield the same results (see below for discussion), and (ii) the shape and position of all the curves are, with some variability comparable to the variability in Figure 2.13a, very similar.\(^{14}\) On the basis of this

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\(^{14}\) The saturation of the behavioural choice frequency (at ca. 95%) is a phenomenon common to all learning experiments: even with the best training on the easiest task a sustained 100% correct performance cannot be achieved.
similarity we can conclude that the results of the behavioural experiment and the output of BEOS are comparable.\textsuperscript{15} Hence my bee eye model has passed this first test.

There are two points I would like to mention as an aside. Both are nicely demonstrated by the output of BEOS shown in Figure 2.12. The first point is that the bee’s eye undersamples the visual world. In other words, the visual fields of individual ommatidia are narrow enough for them to transmit some contrast even when the spatial frequency of the grating exceeds the sampling limit. That is why the transmitted contrasts are the same for horizontal and vertical gratings, respectively, despite the different interommatidial angles in the vertical and the horizontal plane.

The second point is that the orientation of a grating can theoretically be detected even if the grating’s spatial frequency is above the eye’s sampling frequency. Even in the rightmost panels of Figure 2.12 we can make out what the orientation of the input grating was, although the grating’s period in this situation subtended an angle of only 2.29° which is far beyond the bee eye’s resolution limit. What these panels show, however, are not the gratings as such, but some geometrical interference between the grating and the ommatidial lattice of the eye. In stationary images, such as the ones in Figure 2.12, these interference patterns could be mistaken for a grating of lower spatial frequency. However, if the image is moving – as is usually the case for the real bee – the interference patterns move in the wrong direction and/or at the wrong velocity (Hertz 1934, Kunze 1961, Götz 1964, 1965). This is demonstrated in Figure 2.14 both theoretically (after Hertz 1934, Figures 1–7) and with BEOS.

Figure 2.14 shows that the perceived movement follows the actual movement when \( \lambda > 2 \Delta \varphi \) (a–c). When \( \lambda = 2 \Delta \varphi \), i.e. at the resolution limit, no movement is perceived (d). Reducing \( \lambda \) further results in a reversal of the movement direction (e, f) until, at \( \lambda = 2 \Delta \varphi / 3 \), the grating is perceived as stationary again (g). At this point, BEOS is not able to detect any intensity modulation any more.

\[\text{Table 2.2: Summary of the results of Baumgärtner's acuity experiments. "Distance" is the threshold distance for the detection of the stimuli, i.e. the bees' behavioural response. (Taken from Baumgärtner 1928).} \]

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\textsuperscript{15} This comparison assumes that the relationship between behavioural response and contrast is linear which is not necessarily the case. However, a nonlinear contrast-response function might actually improve the fit between the curves in Figure 2.13b.
Figure 2.15: BEOS’ output simulating tests performed by Baumgärtner (1928). a: Rectangles. b: Squares. The numbers denote width and height of the stimuli and viewing distance (all in mm).

What other studies can we draw upon to test our model? Hertz’ optomotor experiments are hard to simulate with BEOS, since they involved panoramic patterns. Furthermore, as mentioned earlier, the acuity of the movement detection pathway as a whole might be poorer than the maximal (optical) acuity that BEOS characterises.

One possible test for BEOS are Baumgärtner's experiments (training on colours and testing with rectangles of varying dimensions and at different distances; see Section 2.1.2). Table 2.2 summarises the results of these experiments, stating the maximal distance at which each stimulus could be detected. These distances, together with the corresponding stimulus dimensions, were used to produce BEOS' view of the stimuli presented in Figure 2.15.

It is immediately apparent from Table 2.2 that the detectability of the rectangles does not vary linearly with either the width, height or area of the rectangles. Increasing the width of a 10 mm high rectangle from 4 to 5 mm or from 30 to 35 mm increases its threshold distance by a factor of at least 2, while rectangles with a height of 10 mm and a width of 5, 10 (see squares), 20 and 30 cm all elicit similar visibility. Baumgärtner's interpretation was that from a distance of 11 cm the 5×10 mm rectangle stimulated the
"smallest receptor unit" just above its threshold, while a rectangle wider than 30 mm stimulated additional units (Baumgärtner 1928).

The results for the squares, on the other hand, show a strong, direct correlation of the threshold distance with the area of the square. This is incompatible with the above notion of a "receptor unit", since here the squares, as seen from the threshold distance, vary considerably in size (Figure 2.15b).

Since Baumgärtner's results are so inconsistent, they do not seem to be well suited as test for BEOS. Fortunately, Lehrer and Bischof (1995) recently performed similar experiments, but under more controlled conditions. In this study bees were trained to detect a disc placed at the end of one of two parallel channels. The unrewarded stimulus (in the other channel) was a disc of the same colour as the background. The distance at which the bees had to make their decision as to which channel to enter was kept constant, but the size of the discs was varied. Several different luminance and colour

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\[\text{Figure 2.16: Simulation of tests performed by Lehrer and Bischof (1995). a: Comparison of behavioural data (curve e, left ordinate), maximal contrast in BEOS' output (curve k, right ordinate), and pooled contrast (curve p, right ordinate; see text for explanation). b, c: Effect of varying the vertical (b) and horizontal (c) halfwidth of the pooling function. See text for details.} \]

\[\text{Lower part of Figure: BEOS' view of black discs of different angular sizes.} \]
contrasts were tested, but here we shall consider only the situation of a black disc on a white background.

Lehrer and Bischof recorded the variations in the bees' choice frequency for the rewarded channel as the disc's size (i.e. visual angle, since the viewing distance was always the same) was varied. The results are shown in Figure 2.16a (curve e). The lower part of Figure 2.16 depicts the imaging of these stimuli on the eye, as simulated with BEOS.

One possible parameter to describe the bees performance in this task is the perceived contrast between the disc and the background. Since the bees have to distinguish the channel containing the disc (i.e. with some contrast present) from the channel without the disc (i.e. with no contrast present), the perceived contrast must have some influence on the behaviour. Curve k in Figure 2.16a represents the contrast between the intensity perceived by the central ommatidium and the intensity of the background for the seven situations simulated with BEOS (the six angular sizes shown in the lower part of Figure 2.16, plus 35°). The comparison of the contrasts with the behavioural response of the bees shows that the detectability of the discs increases more slowly with disc size than does the contrast transmission. We therefore cannot explain the bees' behaviour by the perceived contrast alone.

In order to correlate the contrast with the behaviour we have to go one step beyond the output of BEOS (which represents the view of a pattern on the level of the retina). Let us assume that, at higher levels of the bee's visual system, the optical image is processed by neurons with a visual field extending beyond one ommatidium. By further assuming a Gaussian pooling function for this visual field and adjusting the horizontal and vertical halfwidths of this function we might be able to fit the perceived contrast to the behaviour. Curve p in Figure 2.16a shows the result of such an attempt. The halfwidth of the resulting visual field is 3.0° in the horizontal direction and 0.8 in the vertical direction. The difference between the horizontal and vertical halfwidths of the field, however, is not imperative. With a fixed horizontal halfwidth of 3.0, varying the vertical halfwidth between 0.1 and 5.0 has very little effect on the shape of the resulting contrast curve, as shown in Figure 2.16b. The horizontal halfwidth of 3.0, on the other hand, is crucial, as can be derived from Figure 2.16c where the halfwidth was kept constant at 0.1 in the vertical direction, but was varied between 0.1 and 5.0 in the horizontal direction. This asymmetric behaviour can be explained by the difference between $\Delta \phi_h$ and $\Delta \phi_v$ and the resulting distortion of the retinal image of the discs (see lower part of Figure 2.16). Due to this distortion, the only conclusion we can safely draw from the present simulation is that the neurons involved in object detection – under the given experimental conditions – have a visual field with a horizontal halfwidth of 3.0. In real space this halfwidth covers a visual angle of ca. 11.1° (for frontal ommatidia).

To summarise the last few paragraphs we can say that behavioural experiments involving object detection are not suitable for testing our model. The two measures simulated by BEOS – anatomical resolution and contrast transfer – are not sufficient to

---

17 The horizontal and vertical halfwidths of the Gaussian pooling function were varied independently between 0.1 and 5.0 (for unit definition see next footnote). For each combination a contrast curve was computed. A mean square error minimising procedure was then used to determine the best fit to the behavioural data.

18 Unit: center to center distance between neighbouring hexagons.
describe the bees performance in this task. However, by adding another processing stage
the output of BEOS can be brought to agreement with the behavioural data. Neverthe-
less, the study by Srinivasan and Lehrer provides the only direct test of our model.

2.3.3 Comparison between BEOS and the Wiitanen-Varela model

As mentioned earlier, Wiitanen and Varela (1971) attempted to simulate the bee's optics
based on matrix representations of the input image, the acceptance function, and the
retina's output, respectively. A direct comparison between the outputs of their model and
those of BEOS would require an identical input to both simulations. Unfortunately,
Wiitanen and Varela do not specify any dimensions, nor angular sizes, of the patterns
they used. Furthermore, these parameters seem to vary throughout the article, and a
close inspection of their figures reveals several inconsistencies with the authors' descrip-
tion of the input patterns.\(^{19}\) However, to point out the main differences between the
Wiitanen-Varela model and BEOS it is sufficient to consider only one or two situations.
Figure 2.17 compares two patterns presented in Figure 6 of Wiitanen and Varela (1971)
with the output of BEOS for the (possibly) equivalent situations.\(^{20}\)

The effects of the two major shortcomings of the Wiitanen-Varela model are
immediately apparent from Figure 2.17. These are (i) the undistorted retinal image
resulting from the assumption of equal horizontal and vertical interommatidial angles,
and (ii) the excessive blurring of the image arising from the overestimated acceptance
angle of each ommatidium. Note that some (but not all) of the asymmetries in Wiitanen
and Varela's patterns could be explained by the fact that their patterns were centred
between two neighbouring ommatidia.

2.3.4 Limitations of BEOS

BEOS was designed with a very specific application in mind. Its purpose is to simulate
the view of a flat, vertical pattern through the eyes of an approaching honeybee. As a
result, BEOS has several restrictions:

While the position of the model bee can be chosen arbitrarily anywhere in front of
the pattern plane, its viewing direction is always the same, namely perpendicular to the
pattern plane. In other words, the bee is always facing the pattern plane. It cannot turn
about its vertical axis and view the pattern with the lateral part of its eye. Nor can we
define the pattern to be in the horizontal plane, eg. lying on the floor with the bee
hovering above it. This deficiency can be overcome to some extent by distorting the
input image and positioning the bee appropriately. However, this procedure can be quite
cumbersome and, in any case, the pattern has to lie within the frontal visual field.

The second restriction is that the bee's surroundings can be presented in two
dimensions only. The model bee's visual world consists of only one plane, containing
the pattern to be viewed. If we wanted the bee to view a three-dimensional object, we

\(^{19}\) In their Figure 8, for example, several of their output patterns do not show 60° rotational symmetry
which would have to be expected with equal horizontal and vertical interommatidial angles, respectively.
Also, the constraint of equal surface area for the patterns in the Schnetter series is not satisfied.

\(^{20}\) The halfwidth of the intensity distribution of Figure 2.17a spans 10 ommatidia, ie. 15°. This was
taken as the angular size of the disc.
Figure 2.17: Comparison between outputs of the Wiitanen-Varela model and BEOS, respectively. a, c: Outputs of the Wiitanen-Varela model, modified from Figure 6 of Wiitanen and Varela (1971). The non-white background in these panels is due to the output being represented with 10 intensity classes only. b: Output of BEOS for a disc with angular size of 15°. d: Output of BEOS for a star with same area as the disc and angular size of 28.14° (max. diameter). Insets: Input images used for BEOS.
would have to present it with a two-dimensional projection of that object, which could be viewed from one position only, the centre of projection.

The third point I would like to mention here is not really a restriction, but something we have to keep in mind when interpreting the output of the simulation: BEOS' output is a static image, the view of a pattern from one point in space. By contrast, a real bee's visual surroundings are always moving, since bees hardly ever hover stationary in mid air. As we have seen above (movement of geometric interference patterns, see Figure 2.14), a temporal sequence of images can contain more information about a pattern than a single, stationary image. Of course, using BEOS such a sequence of images can easily be obtained by viewing the same pattern from a number of different positions in space.

In principle, it would be possible to implement the full visual field of the real bee, model the environment in three dimensions, and automatically produce sequences of images (as a movie or even in real time) and thereby create something like a bee flight simulator. However, the amount of programming and subsequent processing involved in such a project would far exceed the scope of this thesis. After all, BEOS was created only as a tool for the design of behavioural experiments.

2.3.5 Concluding remarks

BEOS is a simulation of the optics of the bee's eye. The output of this simulation is an image of the outside world as it is seen by the retina. Any processing that is been done on this image in later stages, i.e. in the optic lobes and the brain, is not included in BEOS. Therefore, it cannot tell us what the bee really sees of our patterns. What it does tell us is the anatomical limit to resolution. If the retina cannot resolve a pattern, there is nothing neural circuitry can do to restore resolution. Information lost during the transfer through the optics is irretrievable. The principal utility of BEOS is that if we run a pattern through the simulation and the output is a uniform grey, we know that the real bees will not resolve the pattern either.

Furthermore, the output of BEOS can be used as input to higher processing stages, as exemplified at the end of Section 2.3.2. The knowledge of what precisely the eye's optics transmit to the retina allows us to infer properties (such as the pooling size, i.e. the visual field) of neurons at higher levels of processing in the bee's visual system.

The main aim of BEOS, however, is to assist in the design of experimental stimuli. In addition, it will be helpful in interpreting the experimental results presented in some of the following chapters of this thesis.
Chapter 3

General methodology

3.1 Setup, stimuli and environment

All the experiments reported in this thesis were performed indoors, in a laboratory illuminated by natural (stray) light through large windows. The bees entered and left the setup via an aperture in a window, which could be closed by means of a hinged flap. Although the setup was bee-tight, bees did occasionally escape the setup and enter the laboratory proper while the patterns were changed or the setup was opened for some other reason. Such escaping bees were captured and released to the outdoors via a second aperture in the window, also fitted with a flap.

All the different experimental setups used for the studies presented here were modifications of the same basic setup depicted in Figure 3.1. The walls and ceiling of this Y-shaped box were of transparent perspex, while its floor was made of white, laminated wood. This "Y-maze" could be divided by two imaginary "decision lines" into a "vestibule" or "decision-chamber" (the trunk of the Y) and two "tunnels" (the branches of the Y) bearing the training and test stimuli at their back walls. These tunnels were perpendicular to each other and were each 28 cm wide, 26 cm high and 27 cm long. Their back walls consisted of removable perspex panels covered with white or grey paper. The length of the tunnels could be reduced by shifting these panels into the setup and closer to the tunnels' entrances (ie. the decision lines). A perspex tube (outer diameter 1.9 cm) protruding from the centre of each panel led from the inside of the setup through the panel and was either blocked (at a depth of 3 cm) or opened into the reward box. This reward box was made from opaque, black perspex and contained a feeder offering a sucrose solution ad libidum. The inside of both the reward box and the blocked tube appeared completely dark from inside the setup. Thus, the tubes in the two tunnels were visually indistinguishable.

In most experiments the training and test stimuli were presented at the end-walls of the two tunnels. They usually consisted of circular paper patterns fixed to cardboard discs with a diameter of 24 cm and a round hole in their centre. These discs were held in place by the protruding tubes in the back walls of the tunnels, which fitted tightly into the holes in the patterns.

Two types of paper were used to prepare the patterns: white photocopying paper and coloured paper. The white paper was either used as such or printed with a range of grey levels (including black) on a high quality laser printer. In one study (Chapter 4) photocopies of 'Letratone' grey papers (LT 10–70%) were used. For the coloured paper 'Spektrum' blue 57, green 63 and yellow 8 were used. Figure 3.2a shows the spectral composition of the light reflected by the different papers (for details of the meas-
urements of these spectra see Chapter 4). This is a function of both the spectral reflectance of the papers and the spectral composition of the ambient light. By combining the curves of Figure 3.2a with the sensitivity curves of the bee's three receptor types (Figure 3.2b) the colour locus of each paper in the bee's colour triangle can be calculated (Figure 3.2c).

3.2 Pre-training, training and test procedures

All behavioural experiments presented in this thesis consisted of a training on two stimuli, of which one (termed "positive") was rewarded and the other one (termed "negative") was not rewarded, and subsequent dual-choice tests. Each experiment could be roughly divided into three phases, namely the pre-training, training and test phase.

During the pre-training phase a group of 10–20 honeybees (Apis mellifera) from a nearby hive was attracted to and familiarised with the experimental setup. To that end a feeder offering sucrose solution was moved, in small steps, from outside through the opening in the laboratory window into the decision chamber of the setup. Those bees that had followed the feeder on this relocation were then individually marked with coloured dots of shellac paint to allow them to be identified and distinguished from untrained bees recruited later on in the experiment. The reward was then moved further, again in small steps, through the tube and into the reward box behind the back wall of one of the tunnels. During and after this relocation, the reward and the pattern associated with it were moved from one tunnel to the other at regular intervals (usually ten minutes) to make the bees associate the reward with a particular pattern, rather than with a particular tunnel. At the end of the pre-training phase (which typically took about 2–3 hours) the bees had learnt to enter the setup, fly into one of the tunnels and crawl through the tube at its end to collect the reward.

The training phase usually overlapped with the pre-training phase. Its beginning was marked with the introduction of the training patterns as soon as the feeder reached the back walls of the tunnels. During the training phase the bees were trained to discriminate between the two training stimuli and to associate the reward with the positive training stimulus. While the interchanging of the rewarded and the unrewarded tunnel was maintained, the patterns were interchanged as well, such that the reward was always offered behind the positive training pattern. This was easily achieved by swapping the whole back-wall panels of the two tunnels. The bees' discrimination performance was monitored continuously (or, in the case of the "close-up" experiments, with training tests at regular intervals; see below). The training phase was completed when the bees' learning curve reached a plateau (i.e. when their choice frequency for the positive pattern

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1 While the overall intensity of the ambient light varied through the day and with the weather conditions (e.g. cloudy versus clear sky) the spectral composition of the ambient light and, thus, the light reflected from the papers was found to change very little.

2 The appearance of this colour triangle (i.e. the position of the monochromatic colour loci and the colour loci of the papers) depends on the data used for the spectral sensitivity of the bee's three receptor types. The sensitivity curves used here (Figure 3.2b) were obtained from a combination of published and unpublished data (see Srinivasan and Lehrer 1984) and differ slightly from curves used by other authors.

3 To move the reward through the narrow tube, the feeder was temporarily replaced with a rolled up tissue paper soaked in sucrose solution.
had stopped increasing), or when it was clear that the bees were not able to learn the required discrimination.

Unless the training was unsuccessful, the learning phase was followed by the test phase. During this phase the continuing training was interspersed with tests at intervals of 50–60 minutes. In these tests the bees were presented either with the same stimuli as in the training ("learning tests"), with one of the training stimuli and a novel stimulus, or with two novel stimuli.

The test procedures varied for the different experiments reported here. In the case of "close-up" experiments (in which the patterns were presented at the entrance to the tunnels of the Y-maze) the tests were unrewarded, i.e. neither of the test patterns gave access to the feeder. Before each test the reward box and both tubes were removed and replaced with two clean tubes identical to the blocked tube used in the training. The patterns were also replaced with fresh, unscented patterns. This procedure prevented the bees from using possible olfactory cues. Then, during a period of 4 minutes, the bees'}
Figure 3.2: Spectral properties of the papers used for the construction of training and test patterns, in the context of the bee's trichromatic vision. a: Spectral composition of the light reflected by the papers (in 10 nm steps). b: blue. g: green. l: photocopy of 'Letratone' LT 30%. p: laser printer grey (40% black). s: laser printer black. w: white. y: yellow. b: Spectral sensitivity of the honeybee's UV, blue (B) and green (G) receptors (from Srinivasan and Lehrer 1984). c: Colour triangle of the honeybee. Open circles: loci of monochromatic colours in 10 nm steps. Solid circles: colour loci of the papers used for constructing the visual patterns in this thesis.
performance was measured by counting the number of times they touched or landed on either of the tubes, and quantifying these by giving landings a weight of 2 and touches a weight of 1. The effects of any side preferences were ruled out by interchanging the patterns' positions halfway through the test period. Each test was repeated several times until the total number of choices was sufficient for statistical analysis.

In the case of "distant" experiments (in which the patterns were presented further back in the tunnels, so that the bees had to discriminate between them at a certain distance) the reward was retained during the testing period. These tests differed from the training only in the patterns with which the bees were confronted. Because of the distance between the decision line and the patterns, the scent of the tube leading to the feeder – which was a major consideration in the close-up experiments – could be excluded as a discrimination cue (for control experiments see 3.4). Furthermore, due to the short duration of the tests (usually about ten minutes) relative to the duration of the training periods between them, the bees were unlikely to learn the test patterns (ie. associate the rewarded test pattern with the reward). The bees' performance in these tests was determined by recording which pattern they approached first (ie. which decision line they crossed first) after entering the setup. The effect of any side preference was eliminated by swapping the patterns (with the reward) halfway through the test.

In some distant experiments (see Chapters 4 and 5) testing was continuous. In these cases the relevant cue (ie. the cue the bees had been trained on) was present – to variable extents – in all the test patterns. More specifically, in any particular test the two test patterns (chromatic gratings in Chapter 4 and moving gratings in Chapter 5) differed only in their orientation. Thus, they acted both as training and test stimuli, so that the tests could be conducted without dedicated training periods in between. However, since these experiments all lasted for several days, the training was refreshed with the training patterns each morning before testing was resumed.

The bees' behaviour, both in close-up and distant experiments, was recorded and analysed by means of a computer program. This facilitated the acquisition and storage of the data and offered instant access to basic statistical analysis. The program also allowed the easy examination of the time course of the bees' response (eg. learning curves), because it recorded not only the choices of each bee individually, but also the exact time for each record.

### 3.3 Data analysis

The bees' choices recorded during the tests (ie. landings×2 and touches in the close-up experiments and decision line crossings in the distant experiments) were used to calculate the bees' choice frequency $\alpha$ in favour of the positive pattern. $\alpha$ is defined as the ratio of the number of positive choices (ie. choices in favour of the rewarded stimulus)

---

4 Landing on the tube (defined as touching the tube with all six legs) is presumably a stronger response – indicating a more powerful attraction by the test pattern – than just touching it (usually with the antennae or the first pair of legs only).

5 The duration of the tests was defined either by time (eg. 10 minutes) or by number of choices (eg. until 20 bees were counted).
to the total number of choices. In the result sections of this thesis, \( \alpha \) is usually stated as 
\( \alpha \pm \sigma_{\alpha} \), where \( \sigma_{\alpha} \) is the standard deviation of the mean, estimated as (Scheffler 1979, van Hateren et al. 1990)

\[
\sigma_{\alpha} = \sqrt{\frac{\alpha (1-\alpha)}{n}}. \tag{3.1}
\]

Statistical evaluation of the test results was usually based on the assumption that the binary choice behaviour of the bees follows a binomial distribution. If this is the case, then the probability that of a total of \( n \) choices exactly \( x \) choices are in favour of the positive test pattern is

\[
P_{n,0.5}(x) = \binom{n}{x} p^x q^{n-x} = \frac{n!}{x!(n-x)!} \ p^x q^{n-x}, \tag{3.2}
\]

where \( p \) is the probability for each choice to be in favour of the positive pattern, and \( q = 1 - p \) (Sachs 1982). If \( p = q = 0.5 \), this formula can be written as

\[
P_{n,0.5}(x) = \binom{n}{x} 0.5^n = \frac{n!}{x!(n-x)!} \ 0.5^n. \tag{3.3}
\]

This binomial distribution specifies the probabilities of obtaining \( x = 1, 2, 3, \ldots \) choices in favour of the positive stimulus, in \( n \) trials, when the bees are choosing randomly between the two stimuli (ie. \( p = 0.5 \)). To determine whether a measured \( \alpha \) is significantly different from 0.5, we need to compute the probability that \( x = (n \alpha) \) will fall within the two (symmetrical) tail regions of this distribution, defined by \([x > n(1-\alpha)]\) and \([x > n(1-\alpha)]\), when the bees are in fact choosing purely randomly. This probability is given by

\[
P_{\text{bin}}(x) = 2 \sum_{k=x}^{n} \binom{n}{k} 0.5^n = 2 \sum_{k=x}^{n} \frac{n!}{k!(n-k)!} \ 0.5^n, \quad \text{with} \quad x \geq \frac{n}{2}. \tag{3.4}
\]

The \( p \) values (ie. significance levels) given in the result sections of this thesis refer to \( P_{\text{bin}}(x) \) as calculated according to equation 3.4.

The binomial distribution is based on the assumption that the individual choices are independent of each other. To fulfil this requirement, none of the recorded choices should be influenced in any way by the preceding choices of the same bee or the choices of other bees. In the distant experiments this assumption was almost certainly valid, provided only the first choice after entering the setup was recorded. In the close-up experiments, on the other hand, it is conceivable that the individual choices were not independent. Because not only the first choices but all consecutive choices within a certain time period were recorded, the decisions of one bee could have been influenced by its own decisions immediately beforehand. Furthermore, because in these tests both tubes in the centres of the patterns were blocked, the setup was not continuously emptied of bees. As a result, it often happened that several bees were being tested simultaneously. Thus, it is possible that the decisions of one bee were influenced by the presence of other bees in front of one of the patterns. In an attempt to overcome this potential flaw, the test procedure in some close-up experiments (Chapter 7) was modified slightly, in that the number of sub-tests of each test was fixed to nine, and each sub-test was terminated as soon as nine choices had been recorded (9x9 testing regime). The outcome of each sub-test was regarded as in favour of the pattern which was chosen.
more often (after weighting landings with 2 and touches with 1).\textsuperscript{6} If each sub-test was treated as a single, truly independent event, the assumption of a binomial distribution was legitimate.

### 3.4 Controls

While performing trainings and tests as described above, a number of potential artefacts have to be kept in mind. The effects of most environmental variables (such as illumination or landmarks inside or outside the setup) are either cancelled out because they apply to both patterns equally, or can be eliminated by a careful design of the experiments. In particular, different tests that are to be compared directly should be performed as simultaneously as possible (to ensure comparable conditions), and the sequence of tests should be randomised.

Bees learn the location of a food source very easily. In my experiments this cue was eliminated, as mentioned above, by the continuous swapping of the reward from one tunnel to the other. The other very important potential source of artefacts when working with bees is olfactory cues, in particular pheromones with which the bees mark a lucrative food source to attract other bees. The effect of this signalling behaviour in the setup can be directed or undirected. In the former case, one pattern (or tunnel) is scented more strongly than the other, influencing the bees' choices. In the latter case, the whole setup accumulates the bees' scents. This seems to impair the bees' ability to "concentrate" on the task at hand, resulting in a poor training.

Undirected effects of scent could easily be reduced by assuring a good ventilation of the setup. In some experiments (eg. aperture experiments in Chapter 7) ventilation could also prevent directed effects, while other setups required more extensive measures (particularly in the close-up experiments; see above). In any case, to ensure that the bees were not using olfactory cues to discriminate between the patterns control experiments were run frequently during the course of all experiments. These controls were usually done by testing the bees with two identical patterns. If the bees chose randomly between the two patterns, that indicated that non-visual cues were not important.

As already mentioned in section 3.3, the bees' choices can be influenced by interactions with other bees. When looking for a food source, bees tend to be attracted by the sight of other bees, especially if these have alighted somewhere on the floor (or a pattern).\textsuperscript{7} The only way to completely prevent the simultaneous presence of more than one bee in the setup would be to train one bee at a time. However, such a procedure would be too time consuming, considering the large volume of data that has to be collected for studies like those presented here. Therefore, groups of individually marked bees were trained instead. The size of these groups varied between the different experiments, depending of the bees' activity level, the degree of difficulty of the tasks at hand,

\textsuperscript{6} If, as a result of the unequal weighing of landings and touches, the first nine choices of a sub-test resulted in a draw, one more choice was recorded.

\textsuperscript{7} It should be noted that such interactions between bees tend to worsen the bees' discrimination performance, rather than enhance it. Bees entering the rewarded tunnel soon crawl into the reward box and out of sight, while bees entering the unrewarded tunnel often spend some time searching for the reward. Therefore, newly arriving bees are more likely to see (and be attracted to) other bees in the unrewarded tunnel.
and the peculiarities of the setup. The effects of these parameters had to be balanced such that the number of bees inside the setup at any time was kept to a minimum, while the data that could be collected within a few days\(^8\) was still sufficient for statistical analysis.

During the course of an experiment the trained bees often recruited new bees to the setup, which – being untrained – tended to interfere with the experiment in several ways. The number of new recruits could be minimised by (i) keeping the concentration of the sucrose solution in the feeder to a minimum, (ii) preventing the bees' pheromones from accumulating inside the setup (and subsequently leaking to the outside, attracting other bees) and (iii) placing a second feeder offering a slightly weaker sucrose solution outside the laboratory window, which bees arriving at the site for the first time would accept as the food source to which they were recruited. Untrained bees that found their way into the setup despite all of these measures could easily be recognised (as unmarked bees) and eliminated.

Although the bees in Canberra are moderately active throughout the year, experiments were performed during the warmer months (October–April) only. This enabled experiments to be carried out relatively quickly, and also minimised differences in temperature, illumination and experiment duration (as a function of the bees' activity level) as well as possible seasonal changes in the bees' physiology that might have had an effect on the bees' behaviour.

\(^{8}\) Due to the continuous loss of trained bees, the duration of an experiment was usually limited to ca. 5 days.
Chapter 4

Chromatic properties of orientation analysis

4.1 Introduction

The honeybee's retina contains three types of photoreceptor, each being maximally sensitive to a different wavelength of light (Menzel and Blakers 1976). These three receptor types (see Figure 3.2b) are labelled "UV receptor" (with its spectral sensitivity function peaking at ca. 330 nm), "blue receptor" (peaking at 430 nm), and "green receptor" (peaking at 550 nm). The appropriate combination of outputs of these receptors provides the honeybee with excellent trichromatic colour vision (rev. Menzel and Backhaus 1989).

However, not every subsystem of the bee's visual system receives input from all three receptor types. It is now well established that the detection of motion (Kaiser 1975), as well as other visual tasks that rely on motion information such as range estimation (Srinivasan et al. 1989) and figure-ground segregation (Zhang et al. 1995) are "colour blind", using information only from the green receptor channel. Srinivasan and Lehrer (1988) found that the discrimination of fine spatial detail also relies exclusively on input from the green receptor type. While their experiments involved the discrimination of horizontal and vertical gratings, the chromatic properties of pattern orientation analysis in isolation were still unknown. The present study was designed to investigate this topic. In particular, I wanted to find out how many receptor types are involved in the analysis of orientation, and which receptor types these are.

A grating can offer a number of different types of contrast to the bee's visual system. Some of these are the "blue contrast" which is the contrast seen by the blue receptors, the "green contrast" seen by the green receptors, the "luminance contrast" which is the contrast as seen by the summed signals from all three types of receptor, and the "chromatic contrast" which quantifies the colour difference between two areas of the visual field by comparing the signals of – in our case – the blue and green receptors, respectively.¹ By analysing how well bees can discriminate the orientation of gratings offering different magnitudes of the different types of contrast, we can determine which contrasts, and therefore which classes of receptor, are relevant to the bee's orientation analysis.

¹ The UV channel is excluded from these considerations as there was virtually no UV light in the laboratory environment.
4.2 Methods

All experiments of this chapter were performed in the Y-maze described in Chapter 3. The training and test patterns were presented on the white back walls of either tunnel, 27 cm from the entrance to the tunnel. Thus, the bees had to make their decision as to which tunnel to enter whilst viewing the patterns from a distance.

The training patterns were ten random black and white gratings, each consisting of twelve 2 cm wide bars, presented on cardboard disks 24 cm in diameter (Figure 1.1d). Each bar had an equal probability of being black or white. The phase shifts of the gratings in the ten training patterns were also randomised. The test patterns were periodic square-wave gratings with a period of 8 cm, again presented on cardboard disks 24 cm in diameter. They consisted of 4 cm wide stripes of coloured or grey paper (representing one half-phase of the grating) pasted onto a background of grey paper (representing the other half-phase) (Figure 4.1a). For the background either photocopies of 'Letratone' paper (LT, 10–70% black) or high quality laser printer printouts (60–100% black) were used. Blank photocopying paper was used for white backgrounds. The coloured papers were 'Spektrum' blue 57, green 63 and yellow 8. These papers were chosen such that gratings constructed from specific pairs of colours (Figure 4.1b, see Section 4.3.2) appear uniform (ie. do not provide any contrast) to specific spectral classes of photoreceptors.

The bees were trained to discriminate horizontally oriented patterns from vertically oriented patterns. During training, one of the ten training patterns was chosen as the positive pattern (with the bars oriented horizontally) and another one as the negative pattern (with the bars oriented vertically). Every twenty minutes the patterns were replaced by another pair of patterns. This procedure prevented the bees from using cues based on eidetic imagery or information on position of edges to distinguish between the positive and negative stimuli, and ensured that the only parameter relevant to the discrimination task was the orientation of the patterns. As mentioned in Chapter 1, van Hateren et al. (1990) showed that bees trained in this way can abstract the orientation of the positive pattern and use this as a parameter to discriminate between patterns they have never encountered before. That study also demonstrated that rewarding the vertical orientation, instead of the horizontal one, leads to similar performance in discriminating orientation.

Once the training was complete, the bees were tested on the coloured gratings. One set of test patterns consisted of either blue, green, yellow or grey (Letratone, 40% black) stripes on one of eight grey Letratone backgrounds ranging from 0% to 70% black. Another set consisted of blue or green stripes on one of five different laser printer created backgrounds ranging from 60% to 100% black. A third set consisted of blue/green, blue/yellow and green/yellow gratings. Tests were performed continuously on pairs of identical patterns differing only in their orientation (horizontal or vertical). In the tests, the first choice made by each arriving bee was recorded by noting which tunnel it entered first (positive or negative). Each pair of patterns was tested until 25 choices had been recorded. Then, the patterns were replaced by the next pair in a (pseudo-) random sequence, and testing continued. Since the tests were rewarded, and the bees were able to discriminate the orientation of most of the test patterns, these patterns simultaneously acted as training patterns, ie. the training was constantly reinforced during testing. Nevertheless, the training was refreshed at regular intervals throughout
testing by using the random black/white training gratings. Each pair of test patterns was tested three times (four times in the case of the dual-colour gratings). The bees' preference for the positive orientation was measured in terms of the average choice frequency in its favour (α; see Chapter 3).

All in all three different groups of bees were used, each group being trained and tested the same way for 4–6 days. To ensure that light conditions were as nearly uniform as possible, tests were only performed with direct sunlight on the entrance to the apparatus. Care was taken, however, to ensure that the patterns were illuminated diffusely and were not intercepted by shadows.

Because of the large scale of this study, the behavioural data was collected in three separate experiments with either Letratone paper (0–70% black) or laser printer printouts (60–100% black) used for the grating backgrounds. The choice frequencies obtained in the second and third experiment were then scaled to match the choice frequencies in the first experiment, using the maximal responses (tested with black/white gratings, data not shown) as the reference.

In order to calculate the excitation produced by each colour or grey paper in each of the three receptor types, it was necessary to measure the spectrum of the light reflected by each paper onto the eye (Figure 3.2a). This spectrum, S(λ), was measured in photometric (quantal) units using a PC1000 Fiber Optic Spectrometer. The measurements were made with the papers in the Y-maze exactly as in the experimental situation and under normal ambient illumination. The sensor was positioned at the entrance to a tunnel of the Y-maze, i.e. at the bees' decision point. The measurements covered a range of 290–830 nm, spanning 1035 data points with a resolution of 0.52 nm on average. Each paper was measured twice, shortly before and after noon. The two resulting curves were smoothed (Gaussian filter with a half width of 6 data points) and averaged.

\[ \text{(2)} \] Measurements of the ambient light at different times of the day revealed variation in overall intensity, but very little change in the spectral composition of the light. The spectra measured around noon can therefore be assumed to be applicable to the whole period of experimentation each day.

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**Figure 4.1:** Examples of test patterns used in this study. **a:** Four examples of colour/grey and grey/grey gratings with different background grey levels. **b:** Dual-coloured gratings.
**Figure 4.2:** The bees' orientation discrimination performance in the experiments with colour/grey and grey/grey gratings. Abscissa: relative background grey level in % black. (This Figure and Figure 4.3 are plotted using the effective background grey levels calculated from sums of the excitations of all three receptor types. These values differ slightly from the nominal grey values of the papers.) Ordinate: choice frequency for the positive pattern in % (n = 75 for each data point). Data points that are not significantly different from random choice ($\alpha = 0.5$) are depicted by unfilled circles, and points that are significantly different from random choice ($p < 0.01$) are depicted by filled circles. **a:** Results of control experiments with grey stripes (43% black) on grey backgrounds of varying intensity. **b:** Results of experiments with colour/grey gratings. **b:** blue/grey gratings. **g:** green/grey gratings. **y:** yellow/grey gratings.

The excitation of each of the bee's three receptor types (UV, blue and green receptors) for a given paper was calculated by multiplying the normalised spectral sensitivity of the receptor (Figure 3.2b) by $S(\lambda)$ for the paper (Figure 3.2a) at intervals of 10 nm over the range of 380–620 nm and summing the products. The receptor spectral sensitivities were obtained from a combination of published and unpublished data (see Srinivasan and Lehrer 1984). These values were then used to calculate specific channel contrasts, luminance contrasts and chromatic contrasts (blue-green) of the gratings according to the formulas given in Srinivasan and Lehrer (1988). The specific channel contrast is the contrast perceived by a particular receptor channel alone. The luminance contrast is the contrast perceived by all three receptor channels together (their individual excitations are summed before the contrast is computed). The chromatic contrast is a measure of colour difference independent of intensity. The chromatic contrast for a given pair of receptor types is 0 if the two papers are of the same colour (even if they differ in intensity) and 1 if the two papers stimulate the two receptor types in a mutually exclusive way.
4.3 Results

4.3.1 Colour/grey gratings

Bees trained on horizontal pattern orientation versus vertical pattern orientation as described in the previous section were tested on gratings consisting of stripes of coloured paper pasted onto backgrounds of different grey levels (Figure 4.1a). Figure 4.2a shows the result of a control experiment performed with grey stripes. In both the positive (horizontal) and negative (vertical) gratings these stripes with a constant grey level of 43% black were presented on a background with a grey level varying between 0% and 72% black. (The grey levels specified in the figures are not the nominal values, but the excitations of the bees’ luminance channel relative to black (100%) and white (0%), calculated using the S(λ) measured for the grey papers used in the experiment.) As expected, the dip in the curve occurs at a background grey level of 43% black when the contrast in the gratings is zero.

The results of tests with colour/grey gratings are depicted in Figure 4.2b. Here, coloured stripes of constant luminance were presented on grey backgrounds varying in grey level between either 0% and 72% black, or 62% and 100% black. The discrimination curve obtained with yellow/grey gratings (y) exhibits a minimum at a background grey level of 10% black, while the curves for the blue/grey gratings (b) as well as the green/grey gratings (g) reach a minimum at a background grey level of circa 80% black. The finding that the two latter curves are very similar in shape is a first indication that, as far as the analysis of orientation by the bee’s visual system is concerned, there is no perceptual difference between the blue and the green coloured papers.

A comparison of the blue, green, luminance and chromatic contrasts produced by all of the gratings used in this experiment is presented in Figure 4.3. The figure shows the variation in blue contrast (panel a), green contrast (panel b) luminance contrast (panel c) and chromatic contrast (panel d) produced by each of the grating types (g: green/grey, b: blue/grey, y: yellow/grey, n: grey/grey) as the intensity of the grey background in each of these gratings is varied (abscissa). The following observations can be made by comparing the behavioural data shown in Figure 4.2 with the four panels of Figure 4.3.

First of all, it is evident from Figure 4.3d that there is no significant variation in chromatic contrast across the range of background grey levels, for any of the gratings. Therefore, chromatic contrast cannot be the crucial parameter in determining orientation discrimination performance. Dips occur, however, in the curves for green, blue and luminance contrasts (Figure 4.3a–c), and I shall consider each of these contrasts in turn below. For each of these three contrasts the curves for the grey/grey gratings always show a minimum at a background grey level of 43% black, which is the intensity of the constant grey stripes. This is to be expected and is, of course, borne out by the behavioural data (Figure 4.2a). A comparison of the curves for the grey/grey gratings in Figure 4.2a and Figure 4.3a–c, respectively, reveals a relationship between the calculated contrast and the behavioural choice frequency that is monotonically increasing, but not linear (see last paragraph of this section). Therefore, if we wish to compare Figure 4.2 and Figure 4.3, as I shall do below, we should concentrate on the position of the curves’ minima rather than their overall shape.

With luminance contrast (Figure 4.3c) the curves for the blue/grey and the green/grey gratings show minimum contrast at ca. 74% black (the actual minimum lies somewhere between the sampling points at 66% and 78% black) and 83% black,
Figure 4.3: Contrasts calculated for the gratings used in the experiments of Figure 4.2. In each panel the curves are labelled as follows: b: blue/grey gratings. g: green/grey gratings. y: yellow/grey gratings. n: grey/grey gratings. The abscissa denotes relative background grey level in % black. Data points from 0–60% black represent experiments using Letraset papers, data points from 70–100% black experiments using laser printer printouts. a: Contrasts experienced by the blue receptor channel. b: Contrasts experienced by the green receptor channel. c: Luminance contrasts. d: Chromatic contrasts (blue-green).

respectively. The curve for the yellow/grey gratings shows a minimum at a grey level of ca. 40% black and is virtually indistinguishable from the curve for the grey/grey gratings. The location of the minimum for the yellow/grey gratings is not consistent with the behavioural data in Figure 4.2b. Thus, we can rule out luminance contrast as a parameter governing orientation discrimination.

In the case of blue contrast (Figure 4.3a) the curves for the yellow/grey and the green/grey gratings are similar and show a prominent minimum at 86% black. The blue/grey gratings exhibit minimum contrast at ca. 60% black. The blue contrast curves for the blue/grey and the yellow/grey gratings are very different from the bee’s choice frequencies in Figure 4.2b. Therefore, blue contrast is not a likely candidate.

However, a good match is obtained when we consider the variation of green contrast for all of the gratings (Figure 4.3b). Here, the curve for the yellow/grey gratings shows a
Figure 4.4: Contrast-response function, calculated for blue contrast from data obtained using grey/grey gratings (details in text). Abscissa: blue contrast (see Figure 4.3a; n). Ordinate: normalised behavioural response (see Figure 4.2a). The scale, ranging from 0 to 1, is equivalent to a choice frequency range of 0.50–1.0. Responses above 0.32 (dashed line) are significantly different from 0 (p < 0.01, n = 75). The response for contrasts greater than 0.4 was assumed to be saturated and equal to 0.92, the experimentally measured value for a contrast of 0.38. The response for contrast 0.01 was set to 0 (choice frequency in behavioural test: 0.43, not significantly different from 0.5). The slope of the fitted curve was determined by calculating a linear regression through all points with contrasts < 0.3.

minimum at ca. 20% black, while the curves for the blue/grey and the green/grey gratings are very similar and show minima at background grey levels of 78% and ca. 80% black, respectively. It is clear that the green contrast curves provide by far the best fit to the behavioural data of Figure 4.2b. This suggests that orientation discrimination performance is dominated by the green receptor channel.

As mentioned above, the relationship between the contrast of the gratings and the bees' choice frequency for the correct orientation is not linear. In Figure 4.4 this relationship is illustrated by plotting the choice frequencies for the grey/grey gratings (Figure 4.2a) against the blue contrasts$^3$ of the same gratings (Figure 4.3a; n). The resulting contrast-response function is approximated by a steep linear function saturating at a level of 0.92. This function can be used to predict the bees' choice behaviour on the basis of the calculated contrasts (Figure 4.3) as shown in Figure 4.5 for the blue, green and luminance contrast channels. (Chromatic contrast is not considered again here, as it has been shown to be not relevant). The panels in Figure 4.5 can now be directly compared with the bees' choice behaviour (Figure 4.2b). In doing so we notice, again, that the green contrast (Figure 4.5b) provides the best match. With the blue contrast (Figure 4.5a) the curves predicted for the blue/grey and yellow/grey gratings do not match the behavioural data; and with the luminance contrast (Figure 4.5c) the curve predicted for the yellow/grey gratings does not match the behavioural data.

$^3$ The choice of blue contrast for this calculation was arbitrary: I could have equally chosen green contrast or luminance contrast, since the blue, green and luminance contrasts for the grey/grey gratings are all virtually identical, as illustrated by the curves labelled n in Figure 4.3a-c.
Figure 4.5: Predictions of behaviour based on calculated contrasts (Figure 4.3a–c) and the contrast-response function (Figure 4.4). a: blue/grey gratings. b: green/grey gratings. c: yellow/grey gratings. a: Prediction by the blue receptor channel. b: Green receptor channel. c: Luminance channel.

4.3.2 Dual-coloured gratings

Alternating with the tests mentioned above, the same bees were also tested on three different dual-coloured gratings (Figure 4.1b). Table 4.1 shows the choice frequencies for the correct orientation for each of these gratings, along with the contrasts calculated for each grating. Orientation was discriminated well with the green/yellow grating as well as the blue/yellow grating. Each yielded a choice frequency of at least 0.8 for the correct orientation. In each case the contrast provided to the green channel was greater than 0.5, but the blue channel contrast, luminance contrast and chromatic contrast were different. With the blue/green grating, however, orientation discrimination broke down completely. In this case the green channel contrast was very low, whilst the blue channel contrast and chromatic contrast were significant. This finding suggests, in agreement with the results of Figure 4.2 and Figure 4.3, that orientation discrimination in the honeybee is mediated by the green receptor channel.

One could argue, in principle, that the luminance contrast might play a role in the bees' choice behaviour, since its value is somewhat lower in the blue/green grating than
in the other two gratings. However, according to the contrast-response function in Figure 4.4 the minimal contrast to elicit a significant response is 0.1. Yet, the orientation of the blue/green grating could not be discriminated, although it offered a luminance contrast of 0.15. The green contrast in the blue/green grating, on the other hand, is only 0.06. Thus, the most reasonable interpretation of these results, as well as those of Figure 4.2 and Figure 4.3, is that orientation discrimination in the honeybee is mediated by the green receptor channel.

Table 4.1: Orientation discrimination performance of bees with respect to three different dual-colour gratings. Each measurement of choice frequency ($\alpha$) is based on 100 choices.

<table>
<thead>
<tr>
<th>Grating</th>
<th>$\alpha$</th>
<th>Specific channel contrast</th>
<th>Luminance contrast</th>
<th>Chromatic contrast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blue</td>
<td>Green</td>
<td></td>
</tr>
<tr>
<td>green/yellow</td>
<td>0.81 (p &lt; 0.001)</td>
<td>0.095</td>
<td>0.536</td>
<td>0.493</td>
</tr>
<tr>
<td>blue/yellow</td>
<td>0.80 (p &lt; 0.001)</td>
<td>0.493</td>
<td>0.579</td>
<td>0.372</td>
</tr>
<tr>
<td>blue/green</td>
<td>0.49 (p &gt; 0.9)</td>
<td>0.561</td>
<td>0.062</td>
<td>0.148</td>
</tr>
</tbody>
</table>

4.4 Discussion

In this chapter I have investigated the chromatic properties of orientation discrimination by the honeybee. Two different kinds of experiments were conducted. In one series, I examined the variation of orientation discrimination as the contrast of gratings composed of alternating grey and coloured stripes was varied by changing the intensity of the grey stripes. The results of these experiments (Figure 4.2, Figure 4.3 and Figure 4.5) suggest that orientation discrimination is governed primarily by the magnitude of the green contrast in the gratings. In another set of experiments, I compared orientation discrimination performance with gratings that (a) carried a significant blue contrast, but negligible green contrast, (b) carried a significant green contrast, but negligible blue contrast and (c) carried significant amounts of blue, green and luminance contrast. The results of these experiments (Table 4.1) are consistent with the above notion: orientation discrimination is good when green contrast is present, and very poor when green contrast is absent. The presence or absence of other kinds of contrast (such as blue contrast, luminance contrast or chromatic contrast) has no effect on orientation discrimination. These findings demonstrate quite clearly, therefore, that the discrimination of orientation by the visual system of the honeybee relies solely on signals from the green receptors. This performance is therefore "colour blind", although the bee's visual system as a whole possesses excellent trichromatic colour vision.

Plotting the bees' orientation discrimination performance with grey/grey gratings against the contrast offered by these gratings it was found that, with increasing contrast,
the bees' response increases linearly up to a contrast of ca. 0.3. Above that contrast the response is saturated.

As mentioned in the introduction to this chapter, Srinivasan and Lehrer (1988) found that visual acuity, measured by investigating the ability of bees to discriminate between regularly spaced horizontal and vertical stripes of various spatial frequencies in a Y-maze, is also dominated by signals from the green receptors. Thus, as the limits of spatial resolution are approached, the bee's visual system behaves as if it is "colour-blind". At that time, it was not clear whether this was due to (a) simply a better visual acuity of the green receptor channel as compared to the other channels, (b) the use of directional movement signals, which are known to be green-receptor driven (rev. Kaiser 1975), to distinguish between horizontal and vertical stripes, or (c) the presence of an orientation-discriminating subsystem that uses only signals from the green receptors. Other studies (Srinivasan et al. 1993b, 1994) have since ruled out possibility (b) and produced evidence for the existence of an orientation-discriminating subsystem. In this study I examined the chromatic properties of that subsystem in isolation, excluding the participation of eidetic imagery or other mechanisms involved in the perception of shape. My results indicate that orientation discrimination is mediated by a colour-blind, green-sensitive subsystem even when the bee's visual system is not operating anywhere near its limits of spatial acuity. Thus, possibility (a) can now be ruled out as well.

Some visual subsystems in the bee seem to be able to use either colour information or luminance information, whichever is available. Object detection, for example, has been shown to be functional with black objects on white backgrounds (providing luminance contrast only) as well as isoluminant object/background combinations containing only chromatic contrast (Lehrer and Bischof 1995, but see Giurfa et al. 1996). On the other hand, orientation discrimination of patterns such as flowers, that do not carry strong orientation signals, appears to be compromised when the colour contrast between the object and the background is increased (Giurfa et al. 1995). In this case, the bees seem to discriminate between the bilateral flower shapes using memorised images (ie. templates; see Chapter 1.1.2) rather than the (weak) overall orientation of the patterns (Giurfa et al. 1995).

Bees have been reported to discriminate between horizontal and vertical rectangles in the absence of green contrast (Zhang et al. 1995). At first sight this result may seem contradictory to my findings. However, in discriminating the rectangles the bees could have been using cues based on the shape of the stimuli, rather than their orientation. If orientation is the only cue available to the bees, pattern discrimination relies on the presence of green contrast.

Colour-blindness is not uncommon among subsystems of visual systems that possess good colour vision in toto. Well known examples are the optomotor response (wide field movement detection) in bees, bumblebees and butterflies (Schlieper 1927, Möller-Racke 1952, Menzel 1973, Kaiser 1975, Kaiser and Lieske 1974), target detection in butterflies (Ilse 1932), the phototactic response in bees (Menzel and Greggers 1985), visual scanning behaviour in bees (Lehrer et al. 1985), evaluation of depth from motion in bees (Srinivasan et al. 1989) and humans (Livingstone and Hubel 1987) and extraction of motion parallax (Zhang et al. 1995). In insects, all of these tasks are

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5 This isolation of orientation analysis was achieved by training the bees on random gratings, as set out in the previous section. For references see van Hateren et al. (1990), Zhang et al. (1995) and Chapter 6 of this thesis.
mediated by the green receptor channel. The orientation-analysing subsystem of the bee must now be added to this list.

The reason for the existence of colour-blind mechanisms in insects that possess colour vision is not entirely clear. It is possible that colour information is unimportant for many routine tasks, if these tasks are performed in a predominantly green environment such as grass or foliage. In this context, it is noteworthy that 50–75% of all receptors in the bee's eye are green receptors (Gribakin 1972). In the ventral region of the eye six of the nine receptors in each ommatidium are green receptors. Therefore, using just the green receptor subsystem would not lead to a dramatic loss in sensitivity.

4.5 Conclusions

In this study, both the experiments with colour/grey gratings and the experiments with dual-coloured gratings indicate that the honeybee's analysis of pattern orientation relies solely on signals from the green receptors and is therefore "colour-blind".

The contrast-response function for the bee's orientation discrimination behaviour – derived from experiments with grey/grey gratings – shows that the bees' response is saturated at contrasts above ca 0.3, and that pattern orientation is perceived at contrasts as low as 0.1.
Chapter 5

Temporal properties of orientation analysis

5.1 Introduction

Image motion plays an important role for a flying insect such as the honeybee. Apart from more specialised applications (e.g. landmark learning; see Zeil et al. 1996) it is used for such crucial navigational tasks as course-stabilisation (optomotor response; see Kunze 1961, rev. Wehner 1981), range perception (Lehrer et al. 1988, Srinivasan et al. 1989, Srinivasan et al. 1991) and figure-ground discrimination (Srinivasan et al. 1990). Much of the image motion a flying bee perceives is self-induced, i.e. the stationary surrounding — including any patterns the bee is being trained on — appears to move as an effect of the bee’s own movement. Although it has been shown (Srinivasan et al. 1993b) that image motion is not vital for the bee to discriminate orientation, it is of interest to find out more about the temporal properties of the bee’s orientation analysis and to compare it to that of other visual behaviours of the bee, such as, for example, the optomotor response. Knowing some of the properties of the orientation discrimination in behavioural experiments should also help constrain the search for neural mechanisms subserving orientation analysis.

This chapter will examine the temporal characteristics of the bee’s behavioural orientation discrimination. The temporal properties of potential neural substrates of this behaviour will be discussed later on, in Chapter 9.

5.2 Methods

Bees were trained and tested on moving gratings generated by two computer-driven CRTs. While the experimental paradigm of the Y-maze was retained, the use of CRTs instead of paper stimuli required a number of modifications to be made to the basic setup described in Chapter 3. The modified setup is depicted in Figure 5.1.

First of all, the removable back wall panels of each tunnel were replaced with white sheets of cardboard taped to the edges of the tunnels. Both of these featured a circular aperture 20 cm in diameter and 2.5 cm from the ceiling of the tunnel. The CRTs were placed behind each tunnel such that their screens were flush against the cardboard apertures. Thus, these apertures served to keep the bees inside the setup, as well as to ensure a consistent presentation of the stimuli.

The use of CRTs for pattern presentation meant that the reward could no longer be offered in the centre of the patterns. Instead, the bees accessed the feeder through a circular hole (1 cm diameter) in the ceiling, positioned above the centre of the pattern.
Figure 5.1: Setup for experiments on temporal properties of orientation analysis. **Top:** View from above of the setup with the left tunnel rewarded. **Lower left:** View into the left tunnel (with the rest of the setup omitted). **Lower right:** Side view of right tunnel. A: fan. B: blocked hole. C: cardboard structure to hide feeder box (identical on both tunnels). D: decision line. E: entrance to setup. G: grating displayed on CRT. H: hole leading to reward. J: CRTs. K: cardboard aperture. R: reward box.
and 2 cm from the back wall. In the rewarded tunnel this hole led to a dark reward box placed on top of the tunnel and hidden from the bees by a cardboard structure (C in Figure 5.1). The unrewarded tunnel also carried an identical cardboard structure and hole, but this hole was blocked by a piece of opaque black perspex. With this arrangement both tunnels appeared identical apart from the training or test patterns.  

Finally, two 12V fans were inserted into the side walls of the tunnels to ensure proper ventilation of the setup. In experiments with paper patterns the air inside the Y-maze was stirred each time the back wall panels were removed to swap or change the patterns. In the present experiment with the fixed back walls, on the other hand, the air inside the setup was stationary and, thus, tended to become saturated with the bees' odours. This, in turn, seemed to impair the bees' learning ability. However, this problem could be prevented with the newly installed fans sucking the contaminated air out of the setup and replacing it with fresh air from outside. An additional benefit of this arrangement is that, because of the inward flow of air at the entrance to the setup, fewer untrained bees were attracted to the experiment by the trained bees' pheromones marking the food source.

The stimuli consisted of moving black and white gratings displayed on two CRTs (Joyce; Cambridge Electronics, UK) at a frame rate of ca. 400 Hz. The CRTs were driven by a PC equipped with an AT-Vista Videographics Adaptor (Truevision, USA). Both CRTs displayed the same stimulus, while the raster orientation (and thereby the orientation and direction of movement of the gratings) could be adjusted manually. Care was taken to reduce reflections on the screens to a minimum.

The spatial and temporal properties of the moving gratings could be adjusted with three parameters: spatial period (in raster lines), jump size (number of raster lines the grating was shifted between successive positions) and delay (number of frames showing the grating in the same position; usually 1). The jump size never exceeded 1/4 of the spatial period, preventing the introduction of lower frequency artefacts. Thus, the maximal contrast frequency that could be created with any spatial frequency was 108.44 Hz (1/4 of the CRTs' frame rate).

All the data on moving stimuli was collected during a single experiment lasting for nine days. A group of initially 17 bees was trained to enter the setup and select the rewarded tunnel on the basis of the patterns presented at the end of each tunnel. The training patterns were horizontal (rewarded) and vertical (unrewarded) gratings with an angular frequency (viewed from the tunnel's entrance) of 0.038 c/deg and moving at a contrast frequency of 4.54 Hz. Every ten minutes the reward was moved to the other tunnel (by swapping the feeder box with the black piece of perspex marked B in Figure

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1 A preliminary experiment with black and white paper gratings was conducted to test whether this new arrangement had any adverse effect on the bees' learning of the patterns which were now physically removed from the reward. I found, surprisingly, that the bees performed even better than with the reward in the centre of the patterns (α = 1, n = 40 with the horizontal grating rewarded; α = 0.97, n = 68 with the vertical grating rewarded). For a discussion of this positive effect see Chapter 7.

2 The term "contrast frequency" is equivalent to the terms "temporal frequency" and "drift frequency" used in the vertebrate vision literature.

3 During the first half of the training phase the patterns were actually kept stationary. This allowed the bees to get used to the setup and to acquire a firm knowledge of the position of the feeder entrance within the tunnels before the apparently disturbing movement of the patterns was introduced.
Figure 5.2: Results of behavioural experiment on temporal properties of orientation analysis. Contrast frequency-response curves plotting the bees’ choice frequency (α) as a function of the contrast frequency (abscissa, in Hz) and spatial frequency (panels a–d) of the gratings. Error bars represent ±1 standard deviation. The sample size for each data point is 31 ≤ n ≤ 38.

5.1) and the gratings (ie. the rasters of the two CRTs) were rotated accordingly. The drift direction of the gratings varied randomly.4

Testing began after the bees' performance in training had reached a plateau, at 0.8, for the choice frequency with respect to the rewarded tunnel (α). A total of 23 different combinations of four spatial frequencies and eight contrast frequencies (including 0 Hz) were tested. Each stimulus was tested for 10–20 minutes (depending on the bees' activity level) before the setting was changed for the next test stimulus in a (pseudo-) randomised sequence. This procedure was continued until the sample size for each stimulus was at least 30. Testing was continuous, since the majority of test stimuli were discriminated by the bees and were thus – with the reward continuing to be present during tests – acting as training stimuli as well. The bees' performance was measured by recording which tunnel they entered first on each visit.

4 The direction of movement of the gratings (eg. up or down for the horizontal grating) in any ten minute period was determined using a random sequence of binary values.
Figure 5.3: Summary of results presented in Figure 5.2. The four spatial frequencies tested are combined in one plot. The two sigmoid curves were fitted to the data for the 0.147 c/deg gratings (lower curve) and the pooled data for the other three spatial frequencies (upper curve). See text for details.

To display the gratings on the CRTs at a sufficient contrast, the ambient light level had to be kept relatively low. However, if the inside of the setup was kept too dark, the bees no longer distinguished between the gratings and tended to show phototactic behaviour towards the bright screens, irrespective of the patterns displayed. Furthermore, it was difficult to convince the bees to come to the setup and forage in such a dark environment. Consequently, the training time was very long, and many bees were lost during the experiment.

5.3 Results

The results for the four different spatial frequencies tested are depicted separately in Figure 5.2 and summarised in Figure 5.3. With all four spatial frequencies the contrast frequency-response curves have a similar shape, differing only in amplitude. Generally, up to a contrast frequency of 36 Hz the bees' performance with moving patterns is the same as with stationary patterns (0 Hz, leftmost data point of each curve). With the contrast frequency increasing beyond 40 Hz, the bees' choice frequencies rapidly drop to random choice level, reaching 0.5 at 60–70 Hz.

While the curves for the three lower spatial frequencies show a remarkable overlap, the bees' response to the finest gratings (0.147 c/deg) was lower at all contrast frequencies. However, the general shape of the curve, characterised by a significant response at
up to 36 Hz and no response at higher frequencies, is the same as for the coarser gratings.

The two sigmoid curves in Figure 5.3 were fitted to the data using a mean square error minimising algorithm with some manual control.\(^5\) The upper curve fits the data points for the three lower spatial frequencies. (Curves fitted individually to the three plots in Figure 5.2a–c are virtually indistinguishable.) The lower curve represents the best fit to the 0.147 c/deg data points. These two curves cross their respective halfheights at 44.3 Hz (\(\theta\)) and 51.6 Hz, respectively.

Several control experiments were performed to ensure that the stimulus on which the bees were basing their decisions was indeed the orientation of the moving gratings. These test and their results are summarised in Table 5.1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sp F (c/deg)</th>
<th>Cont F (Hz)</th>
<th>(\alpha)</th>
<th>n</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Both gratings horizontal</td>
<td>0.074</td>
<td>36.15</td>
<td>0.51</td>
<td>37</td>
<td>p &gt; 0.9</td>
</tr>
<tr>
<td>Horizontal vs. vertical ganzfeld</td>
<td>—</td>
<td>—</td>
<td>0.48</td>
<td>40</td>
<td>p &gt; 0.8</td>
</tr>
<tr>
<td>Moving checkerboards</td>
<td>0.056</td>
<td>13.59</td>
<td>0.53</td>
<td>40</td>
<td>p &gt; 0.8</td>
</tr>
<tr>
<td>Jumping gratings</td>
<td>0.074</td>
<td>9.04</td>
<td>0.87</td>
<td>31</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0.038</td>
<td>9.04</td>
<td>0.84</td>
<td>32</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

In the first of these tests ("both gratings horizontal") both CRTs showed the same grating at the same contrast frequency and the same orientation (horizontal), but moving in opposite directions (up and down, respectively). In this situation the bees entered the two tunnels at random. (In the corresponding test with gratings of different orientations, the choice frequency for the rewarded orientation was 0.88; see Figure 5.2c) This demonstrates that grating orientation was indeed the parameter the bees were using, and that other cues (eg. odour, interactions with other bees etc.) were not important.

However, it could be argued that – at least in a subset of contrast frequencies – the bees could have used the orientation of the stationary raster lines of the CRTs (which were always parallel to the gratings) rather than the orientation of the moving gratings. To control for this possibility the bees were tested on two white screens with their raster lines at perpendicular orientations ("horizontal vs. vertical ganzfeld"). Knowing that the spacing of the CRT raster is far finer than the finest grating the bee’s eye can resolve

\(^5\) To obtain a biologically feasible curve, the slope of the upper curve had to be controlled manually to prevent it from becoming too steep. However, the fitting of the lower curve, as well as the fine adjustment of the upper curve, were done by minimising the mean square error. These curves are of the general form

\[
y = 50 + a \left(1 - \frac{e^d}{1 + e^d}\right), \text{ where } d = b \left(\frac{x}{c} - 0.5\right)
\]

(5.1, 5.2)

and where the fitted parameters are \(a, b\) and \(c\).
(see Chapter 2), we are not surprised that the bees did not discriminate between the two stimuli.

The conclusions of the first two control experiments are supported further by the test labelled "moving checkerboards". Here, both stimuli offered the same orientational information (ie. no overall orientation), but one was moving horizontally and the other vertically. In each case, the CRTs' raster was oriented perpendicular to the direction of movement. Thus, the rasters in the two stimuli were oriented orthogonally. The bees' behaviour in this situation – random choice – demonstrates again that grating orientation is crucial for the task, and that the orientation of the CRTs' raster lines is not important. In addition, it serves as an indication that pattern orientation cannot be simulated by pattern movement (for a more thorough demonstration see Srinivasan et al. 1993b).

All the above tests controlled for the influence of spurious cues that might have improved the bees' performance. The final control experiment ("jumping gratings") was performed to test for the possibility of a different kind of effect, one that could have mitigated against performance. To create the highest contrast frequency tested (108.44 Hz), the gratings had to be shifted by a quarter of their spatial period between each frame. These large jumps, as opposed to a smooth movement of the gratings, might have had an adverse effect on the bees' ability to perceive the gratings as such. To control for such an effect, the contrast frequency of those gratings was reduced to 9.04 Hz, not by reducing the jump size, but by increasing the delay (see Methods). This test was performed with gratings of two different spatial frequencies. In both cases the bees discriminated orientation just as well with the jumping gratings as they did with the gratings that moved with the same contrast frequency, but more smoothly (Figure 5.2a and c). Therefore, the large jumps of the gratings could not have been responsible for the bees' poor orientation discrimination at high contrast frequencies.

5.4 Discussion

This study demonstrates that the neural mechanisms subserving pattern orientation analysis in the bee are operative at contrast frequencies of up to ca. 50 Hz, irrespective of velocity or spatial frequency. Of the four spatial frequencies tested, the lower three produced virtually indistinguishable results, while the highest spatial frequency (0.147 c/deg) elicited a somewhat poorer response across all contrast frequencies.

The most likely reason for this difference is that the finest grating used in this experiment is approaching the limits of the bees' spatial acuity. Since the study of spatial acuity by Srinivasan and Lehrer (1988) we know that bees can resolve black and white gratings with spatial frequencies of up to 0.25 c/deg. Accordingly, the bees should have had no difficulty in resolving any of the gratings used in this study. As an illustration, Figure 5.4 shows the output of BEOS (see Chapter 2) simulating the experimental situation with 0.147 c/deg stimuli. The two images of Figure 5.4a represent the bees' views of maximal contrast gratings and are clearly distinguishable. However, Srinivasan and Lehrer (1988) also showed that the bees' visual acuity deteriorates with decreasing contrast of the gratings. Since the contrast of a grating on a CRT screen is quite low when displayed under fairly high ambient illumination, the 0.147 c/deg grating might

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6 See last paragraph of Section 5.2.
Figure 5.4: BEOS output simulating the bees' view of the (stationary) stimulus with a spatial frequency of 0.147 c/deg. Each stimulus was viewed individually from a distance of 27 cm. Only the central portion of the model bee's frontal visual field is reproduced. The maximal output contrast is given for each view. a: Views of horizontal and vertical gratings with a contrast of 1. b: Views of horizontal (left) and vertical (right) gratings with a contrast of 0.2.

well have come close to the resolution limit of the bee's orientation analysis. Figure 5.4b simulates the bees' view of this grating at two different orientations with a contrast of 0.2. The contrast of the actual stimuli in the experiment is unknown, but is likely to be closer to 0.2 than to 1.

The results of the behavioural experiment described in this chapter were intended to serve as a means for the comparison of the orientation analysis in the freely flying bee with the characteristics of orientation-sensitive cells found in electrophysiological studies. This will be undertaken in Chapter 9. Here, I would like to make another interesting comparison, namely that between the data presented above and the temporal properties of two other visual behaviours of the bee: the optomotor and movement-avoidance responses, respectively.

The best studied visual behaviour of the bee involving pattern movement is the optomotor response. A quantitative investigation of the temporal properties of this response can be found in Kunze (1961). He measured the yaw torque of tethered flying bees positioned in the centre of a rotating cylinder lined with vertical gratings. His results show that the optomotor response depends primarily on the contrast frequency induced by a moving grating, and not on its angular speed. The frequency-response curve for one of the gratings he used (spatial frequency 0.05 c/deg) is reproduced in Figure 5.5 (curve a). This curve has a maximum at 10 Hz and decreases exponentially with both increasing and decreasing contrast frequencies. No significant optomotor response is elicited at contrast frequencies higher than 40 Hz. (For reviews of the optomotor response of insects in general see Reichhardt (1987), Borst and Egelhaaf (1989)).

7 The contrast of the CRT display is dependant on the (variable) ambient illumination and is, furthermore, technically difficult to measure.

8 Qualitative observations, namely that gratings of different spatial frequencies elicit maximal optomotor response at different optimal speeds, had been made earlier by Hertz (1934).