BEHAVIOURAL ASSESSMENT OF VISUAL ACUITY IN BUMBLEBEES (*BOMBUS IMPATIENS*)

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Summary

The present study used previously developed techniques to measure resolution acuity in bumblebees (*Bombus impatiens*). Bees were required to discriminate between horizontal and vertical gratings in a Y-maze apparatus. The gratings had a mean luminance of $9\,\mathrm{cd\,m^{-2}}$ and a Michelson contrast of 84 %. For different bees, either the horizontal or vertical grating was rewarded. Rewarded gratings were associated with a sucrose and water solution (30 % sucrose by volume) and unrewarded gratings with plain water. Acuity estimates were established at several different viewing distances over several sessions using a method of constant stimuli. Visual acuity functions were generated from the performance data, and acuity thresholds were interpolated at a performance level of 65 % correct. When corrected for viewing distance, best angular acuity obtained for horizontal and vertical gratings was 0.35 and $0.36\,\mathrm{cycles\,degree^{-1}}$, respectively. These results are compared with those of the honeybee and discussed in the context of the bumblebee's foraging behaviour.

Key words: bumblebee, vision, acuity, resolution, discrimination, *Bombus impatiens*.

Introduction

While all insects have compound eyes, their designs vary substantially in ways that may influence visual acuity. Among other things, the dimensions of the ommatidia, the interommatidial angle and the presence and location of a foveal region all influence visual resolution (Land, 1997). Dafni and Kevan (Dafni and Kevan, 1995; see also Dafni et al., 1997) have suggested that the differential designs of insect eyes might represent adaptations to different kinds of habitat.

The use of an adaptive approach to explain the structure and function of an organism's visual system has been particularly informative for many different insect species (Land, 1997). The evolutionary solution to the relatively poor resolution conferred by the design of the compound eye has been the development of regions of high acuity or 'acute zones', consisting of densely packed ommatidia with larger facets. An analogous structure in vertebrates is the fovea. The location of these foveae or acute zones tends to correlate with the ecological limitations experienced by different insects (Land, 1997; Land and Eckert, 1985). For example, among sexually dimorphic species, the anterodorsal acute zone sometimes observed in males has been implicated in the sexual pursuit of females (Stavenga, 1992; van Praagh et al., 1980; Zeil, 1983). Among non-dimorphic species, such an acute zone serves predation (Sherk, 1978). For fast-moving, foraging insects, such as honeybees and butterflies, the acute zone tends to be located anteriorly. This frontal, equator-centred fovea is probably useful for foraging on different flower types and for reducing the effects of blur when moving through the environment (Land, 1989; Land, 1997).

A bee's natural environment is rich with visual cues that can be exploited for a number of purposes, including the location and identification of food sources, navigation and mating. On the basis of the evidence presented above, it is reasonable to argue that the environmental limitations (e.g. photic conditions, mating and foraging requirements) experienced by honeybees and bumblebees may have influenced the development of their respective visual systems. While many similarities exist between these pollinators, there are also some differences. For instance, bumblebees and honeybees differ in their foraging strategies and preference for certain flower types. Honeybees (*Apis mellifera*) prefer radially symmetrical flowers over bilaterally symmetrical ones (Free, 1970). This preference is reversed for bumblebees (*Bombus* spp.) (Leppik, 1953; Leppik, 1972; Lunau, 1991). Bilaterally symmetrical flowers are more complex, with greater visual detail (e.g. contour, shape, etc.) than radially symmetrical flowers, and a visual system with better resolution may be required to discern this additional detail (Neal et al., 1998). Because these disparate foraging strategies are thought to influence visual abilities, it is possible that bumblebees differ from honeybees in their visual acuity.

Although there is a large literature describing different

aspects of vision in honeybees, few comparative data are available for other hymenopteran insects. With respect to bumblebees, many studies have examined their foraging strategies (see Heinrich, 1979), but there are no data on their visual system and visual capacities. We have taken the first step in rectifying this situation by measuring their resolution acuity.

Estimates of spatial resolution acuity in insects have been made using a variety of methods. These include (i) anatomical, measuring the interommatidial angle and lens diameter to calculate potential acuity (Land, 1997); (ii) neurophysiological, recording the responses of visual neurones in the optic lobe and related visual structures to visual targets (Catton, 1998; Catton, 1999); and (iii) behavioural, using the optomotor reflex or a free-flying discrimination task (Götz, 1965; Srinivasan and Lehrer, 1988). Although behavioural estimates of acuity correlate well with physiological ones, few studies have used such an approach to measure acuity in insects. Further, while anatomical and physiological measurements may provide a theoretical estimate of visual performance, behavioural measurements provide an assessment of the overall capacity of an insect's visual system and the extent to which it may use the information available to it.

Srinivasan and Lehrer (Srinivasan and Lehrer, 1988) obtained behavioural estimates of visual acuity of honeybees by exploiting a natural free-flying response that is of practical significance in foraging. They used a Y-maze in which the bees were required to make a visual discrimination between two patterns, horizontal and vertical square-wave gratings. Using a performance criterion of 65 % correct, they obtained an acuity estimate of 0.26 cycles degree^{-1}. In the present study, we used a similar technique to measure resolution acuity in bumblebees. By adopting the procedure of Srinivasan and Lehrer (Srinivasan and Lehrer, 1988), we hoped to obtain data that could be compared directly with those on the honeybee.

Materials and methods
Subjects

Eight bumblebees (*Bombus impatiens* Cresson) maintained in an indoor hive were trained to collect food from an experimental apparatus. Foraging bees were marked with different colours of the correction fluid Liquid Paper and were tested on an individual basis. For this experiment, bees from five different hives were used (Biobest & Koppert, Canada).

Apparatus and stimuli

The apparatus was constructed from transparent, ultraviolet-transmitting Plexiglas to allow illumination across the full visible spectrum of the bees (Menzel and Backhaus, 1989). It consisted of a Y-maze with two tunnels branching from a decision chamber (see Fig. 1). Bees entered the decision chamber through a small aperture 2.5 cm in diameter connected to the hive by a short length of wire mesh tubing. A gating system within this tubing allowed us to test bees individually. The decision chamber was a box 41.5 cm wide, 40.5 cm high
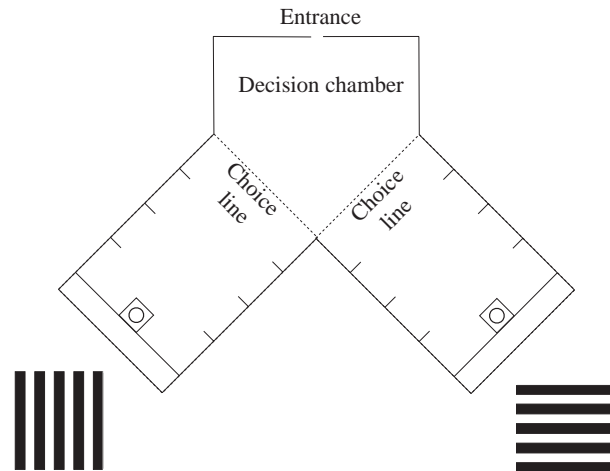

Fig. 1. Schematic view of the apparatus shown from above.

and 20.5 cm long. The tunnel arms were 31.5 cm wide and 40.5 cm high, and their length was determined by the conditions of the experiment. The stimuli were a series of black-and-white square-wave gratings with linear spatial frequencies ranging from 0.286 to 1.42 cycles cm^{-1}. The angular spatial frequency was determined by the distance from the entrance to the tunnel arms to the stimuli. In the main part of the experiment, the bees were tested at three distances: 20, 30 and 40 cm. The mean luminance of the gratings was 9 cd m^{-2}, with a Michelson contrast of 84 %. They could be oriented either horizontally or vertically and were mounted in a black Plexiglas frame that filled the tunnel. A central 19 cm square aperture directly behind a sheet of transparent ultraviolet-transmitting Plexiglas allowed a view of the gratings. In the centre of each display window there was a 5 cm square ledge. These allowed for the placement of small Petri dishes that contained either sucrose-water (30 % sucrose by volume) for the positive stimulus or plain water for the negative stimulus.

Procedure

During preliminary training, bees entered the decision chamber through the wire mesh tubing and simultaneously viewed horizontally and vertically oriented gratings placed at the entrance to each tunnel. Gratings with the lowest spatial frequency, 0.286 cycles cm^{-1}, were used. Initially, bees were trained to enter the decision chamber by placing a Petri dish containing sugar-water at the end of the wire mesh tubing. As training progressed, bees were transferred to a spatial two-alternative forced-choice discrimination task. In the horizontal condition, four naive bees were rewarded for visiting the horizontally oriented gratings, and in the vertical condition four naive bees were rewarded for visiting the vertically oriented gratings. The positions of the positive and negative stimuli were randomly changed from trial to trial to prevent the stimuli from becoming associated with the left or right maze tunnel. In addition to reducing the usefulness of olfactory cues by presenting the positive stimulus an equal number of times

in each tunnel, the entire apparatus was thoroughly cleaned between blocks of trials and between sessions.

Once the bees were visiting the appropriate stimuli at the tunnel entrances consistently, the stimuli were moved back to a distance of 15 cm from their respective tunnel entrances. The 0.286 cycles cm^{-1} gratings were used first, providing an angular spatial frequency of 0.075 cycles degree^{-1}. Bees were tested individually by using the gating system described above. A trial began once a bee entered the decision chamber. Bees flew through the maze when making their choices. We used several behavioural criteria to record the bees' choice behaviour. The initial choice of the right or left tunnel was defined as the point at which the bee crossed the demarcation line between the decision chamber and the tunnel. The trial was terminated once the bee landed on one of the Petri dishes, and this was termed the final stimulus choice. On some trials, bees failed to land on the feeder on their initial choice and made several passes through the maze. Such choice behavior was recorded as intermediate tunnel choices. Response biases were dealt with by using a correction procedure in which positive stimuli were placed on the non-preferred side until the bee responded appropriately, typically in 6–10 trials. Correction trials were excluded from the data analysis.

The bees were trained at the initial viewing distance for 20–25 trials per session and were required to reach a performance level of 80 % or higher over three consecutive sessions before they advanced to the testing phase. After each bee had achieved criterion on the training pattern, formal threshold measurements were begun. The bees were tested at three different viewing distances, 20, 30 and 40 cm. The order in which they were tested at these viewing distances was selected randomly.

A method of constant stimuli was used to obtain threshold estimates. For each of the viewing distances, five spatial frequencies were chosen that spanned the range 0.1–0.5 cycles degree^{-1}. It was expected that this would include the threshold value. The order of presentation of these gratings was randomized, and the bees were given four trials at each frequency until all five gratings had been presented. Within each session, bees completed 8–12 trials at each of the five spatial frequencies. Testing at each viewing distance continued over several sessions until the bee had accumulated a total of at least 32 trials for each spatial frequency.

Results
Preliminary training

All the bees learned the initial discrimination training task with the 0.286 cycles cm^{-1} grating efficiently. Fig. 2 shows the percentage of correct visits plotted as a function of the number of training sessions. The results of a two-way analysis of variance (ANOVA) revealed a significant main effect for sessions ($F_{8,54}$=33.26, $P<0.001$). However, the main effect for orientation of training pattern ($F_{1,54}$=2.63, not significant) and the interaction term ($F_{8,54}$=0.43, not significant) were not significant. Because the performance of individual bees was
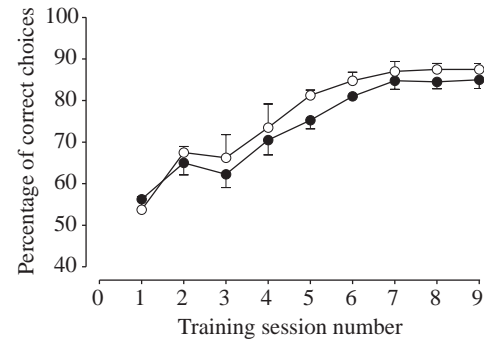

Fig. 2. Preliminary training: discrimination performance as a function of the number of training sessions for horizontal (open symbols) and vertical (filled symbols) positive conditions. Values are means ± 1 S.E.M. (*N*=8).

similar to that of the group, and because there were no differences between the horizontal and vertical conditions, the data from all the bees in both the horizontal and vertical conditions were averaged. Most of the bees required approximately seven sessions to reach criterion.

Acuity estimates

As described in the Materials and methods section, three separate choice measures were recorded: initial tunnel choice, intermediate tunnel entries and final stimulus choice. These data were analysed separately. The final stimulus choice showed uniformly high performance levels regardless of viewing distance or spatial frequency. This suggests that the bees were obtaining information about the orientation of the gratings during their initial tunnel choices and using that information to guide their final decision to approach the rewarded stimulus. This relationship was so strong that, as testing progressed, bees made very few intermediate tunnel choices. For this reason, all subsequent analyses were carried out using only the initial tunnel choice data.

For each bee, we plotted the total percentage of correct choices as a function of the grating spatial frequency. Fig. 3 shows these functions, averaged across bees, for the linear and angular spatial frequencies and for the horizontal and vertical conditions, plotted separately. Each of the panels shows that performance declined as the spatial frequency increased, leveling off at the chance level of 50 %. When the data are plotted as a function of linear spatial frequency (Fig. 3A,B), performance on each grating was systematically poorer as the viewing distance was increased. However, once the correction had been made for viewing distance, by plotting as a function of angular spatial frequency (Fig. 2C,D), the curves overlapped, indicating that the bees' behaviour was under stimulus control and that their responses were determined by the visibility of the gratings.

To obtain a threshold estimate, best-fitting sigmoidal functions were applied to the data, and thresholds were interpolated as the spatial frequency corresponding to the 65 % correct level. This value was chosen to provide a direct comparison with the data of Srinivasan and Lehrer (Srinivasan
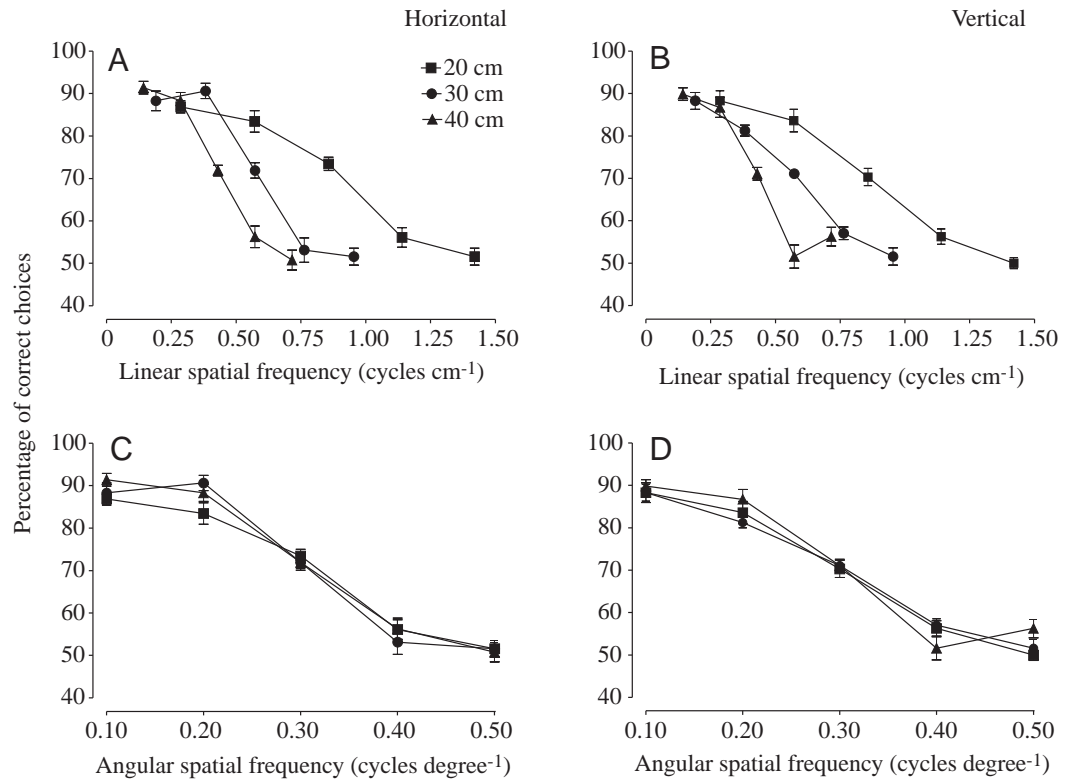
Fig. 3. Visual acuity functions showing the percentage of correct choices as a function of spatial frequency for the horizontal (A,C) and vertical (B,D) positive conditions. (A,B) The data plotted as a function of the linear spatial frequency of the gratings. (C,D) The same data plotted as a function of angular spatial frequency, now corrected for viewing distance. Values are means ± 1 S.E.M. (N=8).

and Lehrer, 1988). The pattern of results was similar for both the horizontal and the vertical conditions. For each of the viewing distances for both the horizontal and vertical conditions, the interpolated thresholds fell between 0.35 and $0.36 \, \text{cycles degree}^{-1}$. The thresholds calculated for individual bees were all very close to the group average, as indicated by the small standard errors in Fig. 3.

Discussion

The data suggest that bumblebee resolution is approximately 25 % better than that of honeybees (Srinivasan and Lehrer, 1988). This estimate may be a conservative one, however. Although the general procedures between these two studies were similar, there were two differences that may have influenced the final acuity estimates in each study. First, while the luminance contrasts of the stimuli of Srinivasan and Lehrer and our stimuli were almost identical (82.7 % *versus* 84 %), the mean luminances were different (e.g. $46.4 \, \text{cd m}^{-2}$ *versus* $9 \, \text{cd m}^{-2}$). The effect of decreasing luminance levels is to produce a similar decrease in acuity estimates (Pick and Buckner, 1979). Thus, the luminance difference between the two studies would favour higher acuity estimates in the honeybee. As mentioned above, it was clear that the bumblebees were using information from their initial choice to make subsequent tunnel choices, so our choice criterion was the initial tunnel visit. Srinivasan and Lehrer (Srinivasan and Lehrer, 1988), however, measured the probability that a bee would enter the tunnel containing the correct stimulus. Although there is no indication that the honeybees were relying

on memory to determine their choice following their initial tunnel choice, any effect of memory would tend to enhance the acuity estimate. Thus, it is possible that the difference between honeybee and bumblebee acuity might be greater than suggested here if they were tested under identical conditions.

If the differences in acuity between the bumblebee and honeybee are valid, then it is appropriate to consider what factors might account for them. Unfortunately, there are no available data on the structure of the bumblebee eye, so the following account must be considered to be speculative. One possibility is that the acuity differences are a simple consequence of size differences between the two species. Recently, Zollikofer et al. (Zollikofer et al., 1995) examined the influence of body size variation on the optical properties of conspecific *Cataglyphis* ants. They found that, with up to a twofold variation in head size, the extent of the visual fields remained constant. This was accomplished by ommatidial scaling changes. While the larger ants had more ommatidia, the interommatidial angles were reduced proportionately, giving a constant field of view. A direct consequence of this scaling is that the smaller ants have a lower visual acuity than the larger ones. Although honeybees and bumblebees are not conspecific, their similar ecological limitations suggest broadly similar optical requirements, although the details might differ. If their visual fields are similar, then the higher acuity of the bumblebee would follow directly.

Our data are neutral with respect to the presence and location of an acute zone. However, given their performance, it seems reasonable to assume that bumblebees do have a foveal region comparable with that of honeybees. As described above, the

resolution of a compound eye is increased by establishing an acute zone of densely packed ommatidia (e.g. smaller interommatidial angles) with larger facets and high acuity (Land, 1989; Land, 1997). In the honeybee, the interommatidial angle in this region is 2.1° in the horizontal plane and 1.05° in the vertical plane (Seidl and Kaiser, 1981; Srinivasan and Lehrer, 1988). Bumblebees are larger than honeybees and have larger eyes. Even if both species had the same optical organisation, a larger eye is likely to have larger facets and confer superior optical resolving power (Zollikofer et al., 1995). The present data are consistent with this idea, although they do not address the question of interommatidial angle; anatomical studies are necessary to determine the optical organization of the bumblebee eye.

A meridional anisotropy between the interommatidial angles in the horizontal or vertical plane leads to a prediction of a similar anisotropy in acuity for vertical and horizontal grating targets. This was not investigated in the present study. For practical reasons, to minimize potential brightness artefacts and to provide a direct comparison with Srinivasan and Lehrer (Srinivasan and Lehrer, 1988), we used a horizontal/vertical discrimination. This means that, while the threshold measured represents the maximum acuity level achievable by the insect, it does not provide information about the orientation of maximal acuity. Additional studies are needed to establish the possible existence of such an anisotropy and its relationship to interommatidial angle.

A grating acuity of 0.36 cycles degree^{-1} suggests a minimum resolvable visual angle of 1.39°. This level of resolution is consistent with that of most insects (Land, 1989; Land, 1997). Given that the level of acuity determines an organism's ability to recognize fine detail in an object, how well does the resolution capacity observed in bumblebees serve them in a real-world task such as foraging? Nectar guides serve as a marker for successful retrieval of nectar, allowing the bee quickly to locate the nectar source. If we consider that nectar guides in flowers are approximately 1–11 mm apart, the maximum viewing distance required to resolve such structures based on our grating acuity would be 4–45 cm (Lunau, 1991). However, bumblebees are probably attracted to flowers by global visual cues such as size, colour and symmetry rather than the nectar guides themselves (Anderson, 1977; Gould, 1993; Menzel and Backhaus, 1989). Their level of acuity easily affords them the ability to deal with these larger global cues. For instance, a flower 2 cm in diameter would be resolvable at a distance of 82 cm. By using these global cues, bees should be able to orient themselves at a working distance consistent with our calculated viewing distance (Wehner, 1981). It should also be noted that the nectar guides themselves are single lines rather than gratings. It is well-established in humans that line or point acuity is superior to grating acuity. This is because the point-spread function of a target imaged on the retina reduces the task to one of contrast detection rather than resolution (Rubin, 1972). Even if bees were located at a distance further than that required to resolve detail on the basis of their grating acuity, they should still be able to make effective use of nectar guides. Therefore, in the natural setting, an acuity level of 0.35–0.36 cycles degree^{-1} will serve bumblebees well when foraging.

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