Sensory Ecology of
Foraging in Bumblebees

Sensory Constraints and the Effect of Scaling

Dissertation

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Sensorische Ökologie bei Sammelnden Hummeln

Sensorische Limitierungen und Körpergrößeneffekte

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Erlärung:

Hiermit erkläre ich ehrenwörtlich, dass die vorliegende Dissertation von mir selbständig und nur unter Verwendung der angegebenen Quellen und Hilfsmittel angefertigt wurde.

Diese Dissertation wurde weder in gleicher noch in ähnlicher Form in einem anderen Prüfungsverfahren vorgelegt.

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Wiser far than human seer,

Yellow-breeched philosopher!

Seeing only what is fair,

Sipping only what is sweet,

Thou dost mock at fate and care,

Leave the chaff, and take the wheat.

Emerson, The Humble-Bee

Contents

Introduction

One of the fundamental insights in animal behavior from the founders of ethology was the idea that every animal has its own *umwelt,* formed by the kinds of information its senses can process (Uexküll, 1934/1957). Consequently, when behavioral scientists attempt to study the behavior of an animal, they have to regard how this animal specifically processes sensory information (Shettleworth, 1998).

Every animal must be able to respond appropriately to its own food, offspring, mates and predators. The cues that it can use to do so are determined by the environment characteristic of its species (Dusenbery, 1992). Sensory capacities of a species are biological traits which reflect some balance between adaptation and constraint. Natural selection may act to optimize sensory capabilities of a species for living and acting in a certain environment, but phylogenetic, developmental and structural limitations may keep the traits far from optimum (Chittka *et al.*, 1999a; Dukas, 1989). Moreover, an animal is not only restricted by the sensory characteristics of its species but possesses also individual-specific modifications of its sensory system due to developmental history, physical stage or experience. However, most of the behavioral and sensory-physiological studies in the past did not attach importance to the existence of individuality (Thomson and Chittka, 2001).

The subject of my thesis is the sensory ecology of foraging in bumblebees, *Bombus terrestris*, an important pollinating insect. For behavioral biologists, bumblebees and honeybees have served as a model system for a long time to test predictions of the optimal foraging theory (Cresswell *et al.*, 2000; Heinrich, 1983; Pyke, 1978), and many studies on foraging behavior and bees' pollinating efficiency were conducted (for review see Alford, 1975; Heinrich, 1979; Plowright and Laverty, 1984). Bumblebees are important pollinator for several agricultural crop plants like apple, almond, tomato, canola, red clover and blueberry (McGregor, 1976; Parker *et al.*, 1987; Thomson, 1993) and much efforts is attempted to augment natural pollination by bringing bumblebees to the crops and to improve the commercial rearing of bumblebee colonies (De Ruijter and Van den Eijnde, 2000; Van den Eijnde, 1990).

In a further field, distinct from behavioral biology, physiologists investigated the sensory capabilities of bumblebees and honeybees, starting at the beginning of the last century, when Karl v. Frisch discovered for the first time that bees can discriminate colors and concluded that they possess a color vision system (Frisch, 1914). Only a few years later, Kühn found that the visual spectrum of bees extend to the UV, a part of the light spectrum which is completely imperceptible to humans (Kühn, 1924). Since this time we clearly know that bees and humans life in a distinct separated sensory *umwelt*. The bee's visual system, for example, possesses in comparison to humans a worse spatial resolution but a higher temporal resolution (Giurfa *et al.*, 1996; Srinivasan and Lehrer, 1988). Bees and humans differ in quantity and quality of odor perception (Frisch, 1919), and a multitude of studies reveal differences in several other senses like thermo-, CO_2-, vibration- or polarized light perception (Dettner and Peters, 1999; Penzlin, 1991).

Despite this long tradition in the bee research and an expended knowledge of the bee's foraging behavior and sensory capabilities, only few biologists have attempted to combine the knowledge of both fields for a more integrative understanding of the bee's behavior. Thus, the aim of my study was to integrate knowledge from both biological fields gaining deeper insights in the interplay of sensory capacities and the foraging behavior of bees.

In the first part of my thesis I focus on the sensory-perceptual processes that may constrain foraging behavior of bumblebees. Bumblebees collect pollen and nectar to satisfy the requirements of their colony. Behavioral ecologists have made intriguing predictions on how pollinators should behave in complex situations where flowers of different species differ in detectability (Dukas and Clark, 1995), but the perceptual dimensions that underlie search behavior, and the floral parameters involved, have been little addressed. Possibly for this reason, predictions of optimal foraging theory are often inconsistent with observations of natural foraging behavior (Heinrich, 1983; Schmid-Hempel, 1993; Varjú and Núñez, 1991; Wells *et al.*, 1986). In my experiments I attempt to identify the neuronal channels used in the natural approach of a bee towards a flower. I also evaluate the bees' flight behavior to see whether the temporal limitations imposed by the underlying neural processes can account for the bees' observed searching strategy.

The next part of this study deals with the inter-individual variation of the sensory system among bumblebees. A bumblebee individual is not only constrained by its phylogenetic based settings of its sensory-perceptual system, but also by its individual-specific modifications due to an individual developmental history. Till this day, the importance of individuality in foraging behavior of bees was mostly ignored. Behavioral or physiological data from different individuals were pooled and intra-specific variation was regarded to be noise and was eliminated (Thomson and Chittka, 2001). In bumblebees, workers of the same colony exhibit a pronounced size polymorphism (Michener, 1974). In my thesis I examined for the first time inter-individual differences in the capabilities of the visual and olfactory system of bumblebees due to scaling and possible constraints on foraging behavior.

Differences in the visual and olfactory system predict different capacities in flower detection between large and small bumblebees, and thus impact on the nectar and pollen foraging performance. In the last part, I survey possible effects of size variation among workers of the same colony on nectar foraging rate in freely foraging bumblebee colonies.

This study hopes to bring two things forward. First, that considering the sensory-perceptual processes underlying flower detection is crucial for understanding foraging behavior of bumblebees. And second, that the visual and olfactory system of a bumblebee are not only determined by its species-specific properties but also strongly modified due to scaling.

Visual constraints in foraging bumblebees: flower size and color affect search time and flight behavior

Flowers visited by bumblebees

Abstract In optimal foraging theory, search time is a key variable defining the value of a prey type. But the sensory-perceptual processes that constrain the search for food have rarely been considered. Here I evaluate the flight behavior of bumblebees (*Bombus terrestris*) searching for artificial flowers of various sizes and colors. When flowers were large, search times correlated well with the color contrast of the targets with their green foliage-type background, as predicted by a model of color opponent coding using inputs from the bee's UV, blue, and green receptors. Targets which made poor color contrast with their backdrop, such as white, UV-reflecting ones, or red flowers, took longest to detect, even though brightness contrast with the background was pronounced. When searching for small targets, bees changed their strategy in several ways. They flew significantly slower and closer to the ground, so increasing the minimum detectable area subtended by an object on the ground. In addition they used a different neuronal channel for flower detection: instead of color contrast, they now employed only the green receptor signal for detection. I relate these findings to temporal and spatial limitations of different neuronal channels involved in stimulus detection and recognition. Thus, foraging speed may not only be limited by factors such as prey density, flight energetics and scramble competition. The results show that understanding the behavioral ecology of foraging can substantially gain from knowledge about mechanisms of visual information processing.

INTRODUCTION

Choosing flower types that involve minimal search times is critical in flower visitors for several reasons. Flight is energetically the most costly activity in insects (Wolf *et al.*, 1999), and even though pollinating insects often operate at the limit of sustaining their flight activity, their fitness depends on the surplus forage brought home to provision their young (Heinrich, 1979; Schaffer *et al.*, 1979). Most flowers offer only small quantities of nectar reward, to keep pollinators moving between plants and so maximize pollen transfer. Activities of many competing flower visitors further reduce those rewards. Bees have been widely used to study foraging decisions, and behavioral ecologists have made intriguing predictions on how pollinators should behave in complex situations where flowers of different species differ in detectability (Dukas and Clark, 1995). But the perceptual dimensions that underlie search times, and the floral parameters involved, have been little addressed. Possibly for this reason, predictions of optimal foraging theory are often inconsistent with observations of natural foraging behavior (Heinrich, 1983; Schmid-Hempel, 1993; Varjú and Núñez, 1991; Wells *et al.*, 1986). In my experiments, I attempt to identify the neuronal channels used in the natural approach of a bee towards a flower. I also evaluate the bees' flight behavior to see whether the temporal limitations imposed by the underlying neural processes can account for the bees' observed searching strategy.

To estimate the color contrast a flower makes with its background, which is critical for its detectability, I need to know the color receptor types of the animal in question, and I need a model to predict how color difference is computed on a neuronal level. Most species of bees have 3 color receptor types most sensitive in the UV, blue, and green part of the spectrum (Chittka, 1996; Menzel and Backhaus, 1991). The responses from these are evaluated by two color opponent processes, and bees appear to ignore brightness cues when identifying flowers (Backhaus, 1991; Vorobyev and Brandt, 1997).

The spatial resolution of bee vision is not only limited by the interommatidial angle (which should allow for a resolution of about 2.8° in the vertical and 5.4° in the horizontal direction in honeybees (Autrum and Wiedemann, 1962; Eheim and Wehner, 1972), and of about 5° in bumblebees (Meyer-Rochow, 1981)), but also by subsequent processing. When a target subtends at least 5° (and no more than 15°), bees employ green contrast, i.e. the difference in signal provided by the green receptor between background and target, for detection. The receptive fields of color coding neurons are comparatively large, so that an area of 15° (equivalent to 59 ommatidia of its compound eye; Giurfa *et al.*, 1996) must be subtended for a honeybee to identify a flower by its color – thus from a distance of 1m, a flower must be 26 cm in diameter so that a bee can recognize its color, or to detect a flower by using color contrast! In this view, flowers would inevitably be first detected by using the green signal as the bee approaches a flower, unless it moves towards very near flowers whose visual angle exceeds 15° at the start of the flight (Giurfa *et al.*, 1996; Lehrer and Bischof, 1995).

These results, however, were obtained with bees making choices at a constrained distance from the target (the fork of a Y-maze), and under the assumption that both the bee and the target are stationary. Times to make a choice, which are crucial in foraging, were not recorded. When the

bee is in motion, as during natural foraging, temporal constraints of the respective neuronal channels might become relevant for the detection process. As a bee moves across a meadow with flowers, the contrast each flower makes with its background is reduced, and spatial resolution also decreases (Srinivasan and Lehrer, 1985). With increasing flight speed, the amount of time a flower passes through the receptive field of a visuo-neuronal channel is reduced. Beyond a critical speed, this time window may be too short for the flower to be resolved by the temporal sensitivity of a receptor or neuronal channel, and the bee may fail to detect the object. In experiments with flickering stimuli, Srinivasan & Lehrer (1985) concluded that a bee needs 10 ms to compute the color of an object. The green receptor channel, which also drives the bees' movement avoidance response, has been reported to have about half that integration time, which appears to be close the photoreceptors' temporal resolution (Srinivasan and Lehrer, 1984). Whether these limitations apply when a single target suddenly appears in the visual field of a bee, and moves across the retina, is unknown.

MATERIAL AND METHODS

Flight arena and flowers

All experiments were performed with individually marked bumblebee workers from four different *Bombus terrestris* colonies. The colonies were housed in wooden nest boxes, connected to a flight arena with a plastic tube. The flight arena measured $120 \times 100 \times 35$ cm. It was covered with a UV-transparent Plexiglas cover. The floor consisted of two layers of plastic boards. The upper board was colored green (for spectral reflection see Fig. 1), 1mm thick, and was punctured with 575 holes in 25 rows and 23 columns, 2 mm in diameter and 4 cm apart. The lower board contained an equal number of holes, 4 mm in diameter at the same positions as in the upper board. Into the wells in the lower board, small plastic caps for sugar solution with a maximum volume of 50µl could be placed. Artificial flowers were made of round pieces of Plexiglas 1 mm thick with a central hole ($\varnothing$=1mm), painted with pigment colors. I used seven flower colors (see below) and five flower sizes with diameters of 5, 8, 15, 22 and 28 mm respectively. The two smallest sizes did not have holes, but were placed so that the holes with rewards were placed directly adjacent to the stimuli.

Color analysis

Spectral reflectance functions of the stimuli and the background was measured using a spectrometer (Ocean Optics S2000 with a Deuterium/Halogen light source). The color parameters (relative excitation values in the bees' UV, blue, and green receptors, color contrast, green contrast, and brightness; Table 1) were calculated according to Backhaus (1991) using the

color hexagon (Fig.1; Chittka, 1992); for alternative models, see Vorobyev & Brandt (1997). The relative amount of light absorbed by each photoreceptor color type is:

$$P = R \int_{300}^{700} I_S(\lambda)\, S(\lambda)\, D(\lambda)\, d\lambda \qquad (1)$$

$I_S(\lambda)$ is the spectral reflectance function of the stimulus; $S(\lambda)$ is the spectral sensitivity function of the receptor (I used the functions of Peitsch *et al.* (1992) for the *Bombus terrestris* UV, blue, and green receptors). $D(\lambda)$ is the illuminant (in my case, a standard neon light filtered through the Plexiglas cover combined with natural daylight). The sensitivity factor R in eqtn. 1 is determined by:

$$R = 1 / \int_{300}^{700} I_B(\lambda)\, S(\lambda)\, D(\lambda)\, d\lambda \qquad (2)$$

$I_B(\lambda)$ is the spectral reflection function of the background to which the receptors are adapted (Fig.1). With this model, it is assumed that the photoreceptors display half their maximal response when stimulated by the light reflected from the adaptation background. When the maximum excitation E_{max} of the photoreceptors is normalized to 1, the photoreceptor excitation can be described by

$$E = P / (P + 1) \qquad (3)$$

where P is the stimulus strength (eqtn. 1), in units such that for P = 1, E = 0.5 (i.e. half the maximum potential; for details see Backhaus (1991); Vorobyev and Brandt (1997)). Thus, for the adaptation background, E equals 0.5 in each photoreceptor. Green contrast, then, is the degree to which any given stimulus generates an excitation value different from 0.5 in the green receptor. Because excitation can range from 0 to 1, the maximum green contrast is 0.5. Stimulus brightness is defined as the sum of all three photoreceptor excitations, so it can have any value from 0 to 3. Because the background, by definition, has a brightness of 1.5, brightness contrast can have any value up to 1.5.

Table 1. Color properties of the artificial flowers

| Color	Distance to background (Hexagon units)	Brightness contrast	Green contrast
UV-absorbing white	0.16	0.87	0.33
Blue	0.23	-0.22	-0.16
Yellow	0.35	-0.07	0.21
Lemon	0.31	0.31	0.30
Turquoise	0.10	0.32	0.08
Red	0.06	-0.94	-0.29
UV-reflecting white	0.07	0.77	0.26

Color distance is measured in hexagon units, brightness as the sum of the excitations of all three receptor types after adaptation to background, and green contrast as the specific excitation of the green receptor. In the analysis the absolute values of green contrast and brightness contrast were used.

For calculation of hexagon color loci from receptor excitation values, see Chittka (1992). Color distance in the color hexagon is correlated with the degree to which two stimuli are perceived as differently colored. The background color locus lies in the center of the color hexagon. Distance from the center to any of the hexagon's corners is unity. Therefore, color contrast of a given stimulus with its backdrop can range from 0 to 1.

Experimental procedures

Before the experiments, bees were allowed to familiarize themselves freely with the arena, and to feed from transparent plastic dishes containing 1 molar sucrose solution. Prior to experiments, bees were not exposed to colored targets. During an experiment, only one bumblebee at a time was allowed to enter the arena. During a search bout (a round trip from the nest to the flowers and back), I offered three flowers in the flight arena. The flowers were arranged in an equilateral triangle with a side length of 30 cm. Each flower disk was positioned exactly above a hole in the floor, filled with 30 µl 1.5 molar sucrose solution. In each bout the triangle was randomly arranged on the floor and the flowers were cleaned with 30% alcohol after each visit to eliminate scent marks by bees. The floor was cleaned in the same way after each third bout (Chittka *et al*., 1999b).

An experiment started by training a single bee to search for the flowers and feed on the sugar solution provided by the cap under each flower. Each bee was tested on one color only, but on different flower sizes. During the training phase, I presented the largest size (28 mm in diameter) for 15 bouts. The subsequent test phase for the 28mm flowers comprised five
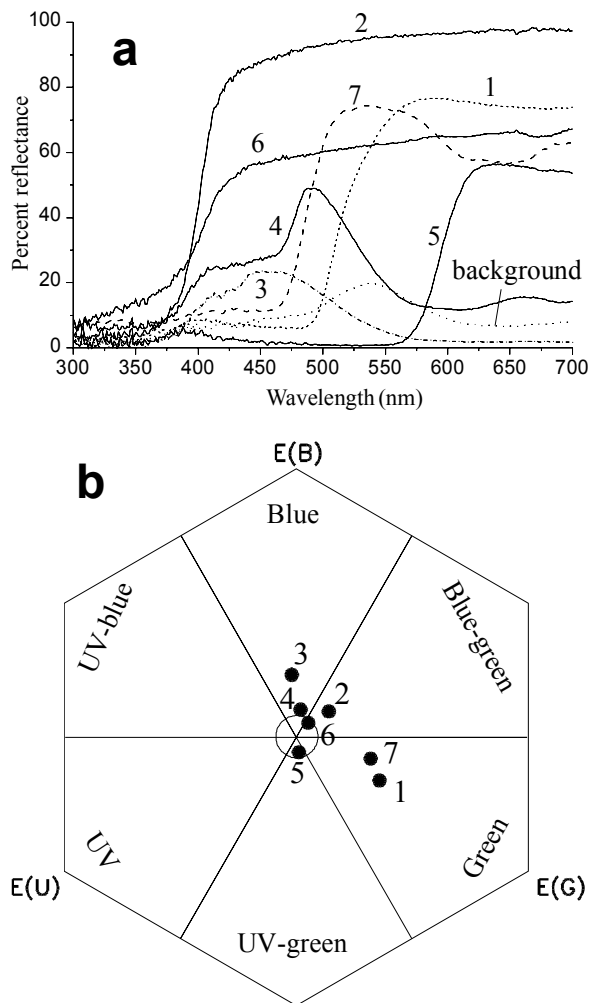
Fig. 1.
Color stimuli employed in the study: a) Spectral reflection curves of the artificial flowers and background. b) Color loci of the stimuli in the color hexagon. The color space inside the central circle (< 0.1 hexagon units) appears achromatic for the bees. 1 yellow, 2 UV-absorbing white, 3 blue, 4 turquoise, 5 red, 6 UV-reflecting white, 7 lemon.

foraging bouts. After that I reduced the size for the next six bouts, and then reduced it further. I did not evaluate the first bout of each new size to exclude phases when bees first familiarized themselves with a new foraging situation. In each bout I measured the search time from entering the flight arena until landing on the third flower excluding the feeding times. In order to reduce high variation in search time due to different distances between the arena entrance and the first flower, I used only search times *between* flowers. I also excluded the time between the second and the third flower, since bees sometimes returned to the first flower. I tracked the bees' behavior using the computer program Observer® which allowed us to record behavioral observation data with defined push-button combinations on a laptop.

In the first experiment, I determined how flower size affected search time. Each of 6 bees was tested on blue flowers of five sizes (28, 22, 15, 8 and 5 mm in diameter) in descending order. For all sizes and for each bee, I calculated the mean search time between the first and the second flower of all five bouts.

In my second experiment, I tested the influences of color properties on search time. I trained bees of seven groups to forage on one color of flower each. The flowers had the colors blue (bee-blue), yellow (bee-green), red (bee-uncolored), turquoise (bee-blue), UV-reflecting white

(bee-uncolored), lemon (bee-green) and UV-absorbing white (bee-blue-green). The bees were tested on three flower sizes (28, 15 and 8 mm respectively) in a descending order. For the UV-absorbing white flowers, I also tested the effect of flower size on flight speed and height for three flower sizes (28, 15, and 8 mm respectively). The final foraging bout on each floral size was video-taped. The flight path was recorded by a digital camera (Sony DCR-VX 1000E, 25 frames / s) from the side of the arena through a transparent Plexiglas sheet. Because one camera was used it was only possible to measure velocity in the vertical x-y plane. Using this method, the recorded velocity and flight height of a bumblebee on a video tape depends on the distance between the bee and the camera lens. Therefore, I evaluated only video tape sequences during which the bee flew in a defined distance to the camera. I mounted a light emitting diode in front of the camera. The experimenter observed the flying bee from above and switched the diode on when the bee was flying above a defined area which had been marked on the arena floor. This area had the shape of a narrow strip of the arena floor (10 cm width, but covering the entire width of the arena). The strip was arranged perpendicular to the direction in which the camera was pointing; its distance to the camera was 60 cm. I excluded all recordings one second before and after landing on a flower to avoid confounding search behavior and landing maneuvers. For each bee and floral size I obtained a mean number of 195 frames (32 to 384) of the flight paths within the marked area. This method enabled me to assess the real mean velocity (assuming that the velocity in the x-y plane is equal to the velocity in the x-z plane) and to determine differences between foraging flights for various flower sizes. For digitizing and analyzing video recordings, I used a computer-based video analysis system (WINanalyze®).

RESULTS

A decrease in flower size prompted a drastic increase in search time, from 10.4 s ± 8.5 s at a size of 28 mm to 124.3 s ± 86.0 s at 5 mm size (Fig. 2). Overall, search time is highly negatively correlated with size (Spearman rank test: $r_s = -1.0$, $p < 0.0001$, N = 5). For the bees, it is substantially harder to detect the smaller flowers. Such flowers involve longer search times and thus lower foraging efficiency.
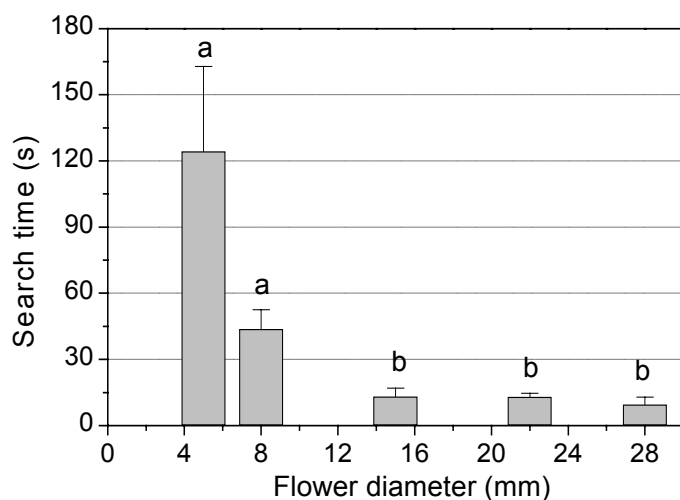
Fig. 2.
Search time for detecting blue flowers of various sizes. Same letters indicate no significant differences (Wilcoxon-matched-pairs test); Mean ± S.E.; N = 7; $p < 0.05$.

For the tested flower sizes of 28, 15 and 8 mm diameter, mean search time differs significantly among flower colors (Kruskal-Wallis H-test: 28 mm: $H = 16.3$, $p < 0.01$; 15 mm: $H = 23.6$, $p < 0.001$; 8 mm: $H = 12.8$, $p < 0.05$). For large flowers, search time ranges from 2.0 seconds (lemon flowers) to 14.2 sec (red flowers) or even 21.7 seconds (UV-reflecting white flowers; Table 2). For small flowers search times are more than doubled, ranging from 15.4 seconds (lemon flowers) to 46.1 seconds (turquoise flowers). The mean search times for each color and size are plotted in Fig. 3a-b as a function of color contrast and green contrast provided by the flowers. For large flowers, Spearman's rank test reveals a significant negative correlation between search time and color contrast ($r_s = -0.93$, $p < 0.01$, $N = 7$). No correlation with the achromatic properties brightness ($r_s = -0.71$, $p = 0.08$) or green contrast ($r_s = -0.11$, $p = 0.82$) was found. The same picture is obtained for the medium flower size (Color contrast: $r_s = -0.86$, $p < 0.05$; brightness contrast: $r_s = 0.64$, $p = 0.12$; green contrast: $r_s = -0.32$, $p = 0.48$). This means that with an increase in color contrast, search time decreases.

Table 2. Mean search times for flowers of different colors and sizes

Color	N of bees	28 mm diameter		15 mm diameter		8 mm diameter	
		Search time (s)	S.E.	Search time (s)	S.E.	Search time (s)	S.E.
UV-absorbing white	9	11.8	1.4	17.4	2.5	26.2	4.9
Blue	6	10.4	3.9	15.1	3.5	44.4	9.3
Yellow	5	9.7	3.3	9.5	2.0	45.0	11.9
Lemon	5	2.0	0.9	6.3	1.5	15.4	5.0
Turquoise	7	13.3	3.7	31.1	5.5	46.1	11.4
Red	9	14.2	2.3	29.2	4.4	42.3	8.1
UV-reflecting white	5	21.7	4.3	28.7	9.3	41.8	7.0

Mean search times for flowers of different colors and sizes. Time was measured from leaving the first flower to landing on the second flower.

An entirely different picture is obtained for small flowers (8 mm). Here, a significantly negative correlation is found between mean search time and green contrast ($r_s = -0.89$, $p < 0.01$), but no correlation with color contrast ($r_s = 0.00$, $p = 1.0$) or brightness contrast ($r_s = -0.36$, $p = 0.43$). For this size, a larger green contrast leads to a shorter search time.

The switch from one neuronal channel to the other is particularly striking when comparing search times in the yellow and UV-absorbing white flowers: yellow flowers exhibit a higher color contrast to the background, but only approximately two thirds of the amount of green
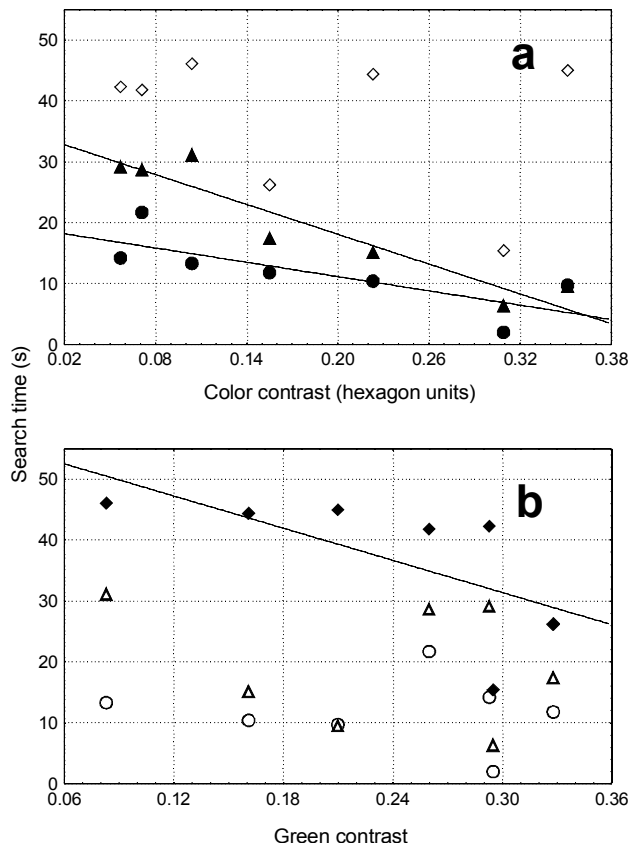
Fig. 3.
Relation between search time and (a) color distance and (b) green contrast respectively for the three different flower sizes. Circle indicates 28 mm, triangle 15mm, and rhombus 8 mm flower diameter. Filled symbols indicate significant correlation (for details see text).

contrast. Bees take longer to search for the large UV-absorbing white flowers (22% longer compared to yellow flowers), suggesting that color contrast is the relevant parameter. But search times are reversed in the small flowers: here they are longer for the yellow flowers (72% longer compared to the white flowers), presumably because bees now use green contrast instead of color contrast.

I find a significant decrease in flight height and velocity with decreasing flower size (Fig. 4).
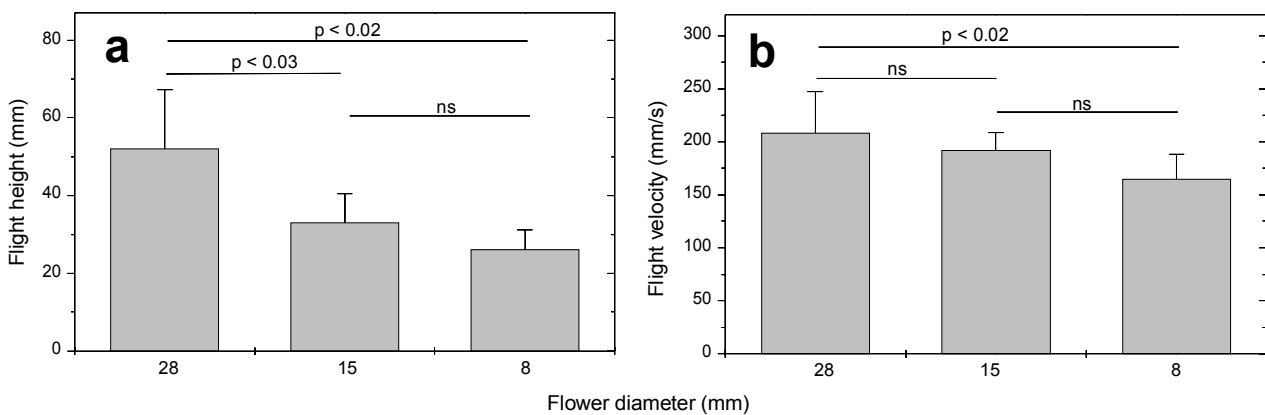
Fig. 4.
Flight height (a) and flight velocity in the vertical x-y plane (b) while searching for flowers of different flower sizes (Wilcoxon-matched-pairs test; numbers indicate p-level). Mean ± S.D.; N = 7. ns, not significant.

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The mean flight height drops from 52.0 mm (± 15.2) for 28 mm flowers to 26.1 mm (± 5.2) for 8 mm flowers. The velocity declines from 208.6 mm / s (± 38.8) to 165.1 mm / s (± 23.2).

DISCUSSION

I tested whether the optical properties of a flower, measured as color contrast, green contrast and size, affect search time and flight behavior of foraging bumblebees. The results reveal a strong influence of these properties on search time and thus foraging costs. I discuss these findings in the light of optimal foraging behavior.

Floral color properties and detectability

Both chromatic and achromatic color properties of a flower affect search time, depending on flower size (Fig. 3 and Table 2). In large flowers search time is a function of color contrast, whereas in small flowers it is correlated with green contrast. Consequently, the bees seem to be limited alternatively by chromatic or achromatic features, depending on the visual angle subtended by the flower.

At first glance, these findings are nicely consistent with the results obtained in dual choice experiments for honeybees (Giurfa *et al*., 1997; Giurfa *et al*., 1996). In these experiments, bees were trained to discriminate between two objects, providing various visual angles and either chromatic or achromatic contrast or both. When provided with an angle between 5° and 15°, the bees' choice behavior was governed by green contrast. When the angle was >15°, the bees used solely chromatic cues. At close inspection, however, my results are not so easily explained by these earlier findings. As a bee approaches a flower, that flower will inevitably exceed the 5° threshold before the 15° threshold. Therefore, detectability should always be correlated with green contrast, unless such contrast is not available, or unless flowers are extremely close to one another. Identification by color would always happen subsequent to detection by green contrast. The results indicate that bumblebees use color contrast to detect large flowers. This result is not explicable by the possibility that each flower already subtends more than 15° when seen from the other flowers: from 30 cm away, the largest flower type covers only 5°, even if presented vertically. I suggest that bees may be selectively using color contrast when they expect large flowers, and ignore the signal from the green receptor channel. This may enable bees to identify flowers with more certainty, simply because color contrast uses three input variables, whereas green contrast is only defined by one. Thus, bees face a tradeoff between reliable identification and rapid detection, and the relative benefits of both might change depending on floral size. To estimate the theoretical increase in search costs if bees would use the color channel for small flowers, it is useful to consider the size of the area inside which the bee is able to detect a flower from a given flight height.
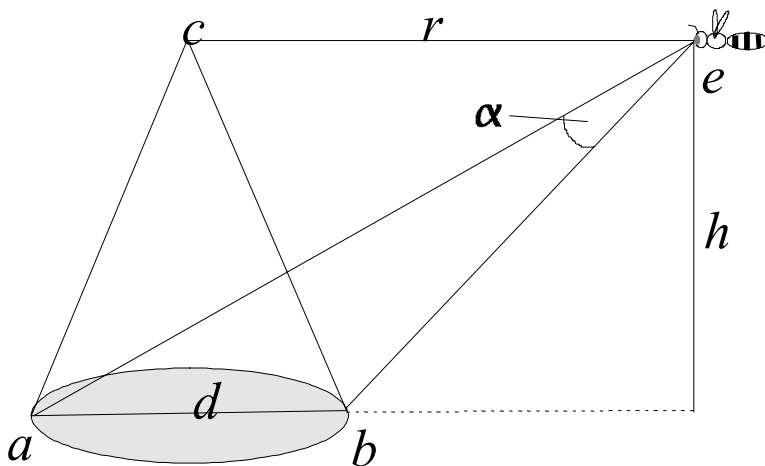
Detection area and color recognition area

As a bee searches the arena for flowers, the probability of its success does not depend directly on stimulus size. Rather, at any given flight altitude, this probability is dependent on the likelihood that she enters the area inside which the flower subtends either a visual angle of ≥ 15° for using the color channel or ≥ 5° for using the green contrast channel. I will henceforth designate the circular area (with radius = r, see Fig. 5) directly above the flower, inside which detection is possible, as *detection area*, and that inside which an assessment of color contrast is feasible, *color recognition area* (Fig. 5). Considering the mean flight height (h), the corresponding flower diameter (d), and the minimum visual angle (α) of 5° or 15° respectively, I calculated these areas as follows:

$$A = r^2 * \pi \tag{4}$$

$$\text{where} \quad r = \sqrt{\left| -\frac{2h^2 - \dfrac{d^2}{2}}{2} \pm \sqrt{\frac{\left(2h^2 - \dfrac{d^2}{2}\right)^2}{4} - \left(h^4 + h^2\frac{d^2}{2} + \frac{d^4}{16} - \left(\frac{dh}{\sin\alpha}\right)^2\right)} \right|} \tag{5}.$$

(For explanation see appendix).

It follows that the *color recognition area* (visual angle ≥ 15° at the bee's eyes) is 91.9 cm² for 28 mm flowers and 3.6 cm² for 8 mm flowers. The *detection area* (a minimum visual angle of 5° is subtended by the flowers) is 352.2 and 50.5 cm² for 28 mm and 8 mm flowers respectively. This means that, using color contrast, bees would have to face a roughly 25 fold increase in search times (because the probability of a searching bee to enter the *detection area*

Fig. 5.
This sketch illustrates the geometry necessary to calculate the radius of the *detection area* r, within which a bee flying at a given height h will be able to detect a flower with diameter d, given a resolution of α ≥ 5° (α ≥ 15° for the *color recognition area*; r = radius of the circular detection area, α = visual angle subtended by the flower when the bee is situated at point e).

is proportional to its surface) when searching for 8 mm instead of 28 mm flowers. If bees switch from color contrast to green contrast (and thus from a 15° to a 5° receptive field) when searching for smaller flowers, I would expect an increase in search time by only a factor of 1.8, which is almost within the range of factors (1.9 to 4.6) which I empirically determined (Table 2). Note that, while the *detection area* is always larger than the *color recognition area*, its relative advantage decreases with floral size. In 28 mm flowers, the *color recognition area* measures 26% of the *detection area*, whereas in 8 mm flowers, color contrast can only be used in 7% of the total *detection area*. I conclude that, with increasing floral size, the increase in search time by using color contrast is compensated by acquiring higher precision of floral recognition.

Flower size and flight behavior

I showed that the size of an artificial flower strongly affects the time a bee needs to detect this flower (Fig. 2). Two scenarios might explain this increase in search time: (1) the bee's flight height and velocity stay constant while searching for flowers of different sizes. This leads to a reduced *detection area*. In this case, the probability of a randomly searching bee to enter this area decreases, and thus the time until she detects the object increases. (2) The bee adapts her flight height to the sought objects in such a way that *detection area* is maintained constant. Here the *detection area* becomes larger compared to the first scenario, but the total area scanned by the bee per time decreases. The results indicate that bees use a strategy which is closer to second possibility: the bees sacrifice total area scanned per unit time to increase *detection areas*. When the bees search for small flowers they decrease their flight height from 52 to 26 mm, close to the theoretical height of 15 mm when they would keep the *detection area* exactly constant.

With a decrease in flower size, flight velocity also decreases. This means that bees forgo even more of area scanned per time, suggesting that temporal constraints play a role in floral detection as well. The results of Srinivasan & Lehrer (1985) suggest that bees take about 10 ms to compute the color of an object seen in flight. They concluded that bees flying at a speed of 7 m/s at a height of 2 m can still resolve two objects spaced 12.5 cm apart on the ground.

Can the 10 ms limit explain the strategy used by bees searching for small flowers? Yes. Consider a bee flying at a speed of 30 cm/s at a height of 5.2 cm – these are the values obtained for bees searching for flowers with $\varnothing$ = 28mm. For a neuron with a receptive field of 5°, this flower would "appear" for 48 ms (and 78 ms for a receptive field of 15°) – a value large enough for processing color information. But bees even further reduce their speed when flowers are smaller (23 cm/s for flowers of $\varnothing$ = 8 mm). In this case, a flower would appear for 24 ms for a receptive field of 15°, but only for 5ms for a receptive field of 5° - too short for processing color information!

Hence the bee can only rely on the green contrast for detecting small flowers at a speed of 23 cm/s, or they would have to fly even slower and thus further increase search times. Note that energetic constraints appear to play a marginal role in adjusting foraging velocity: Ellington et

al. (1990) found that, in bumblebees, energetic expenses are constant over a very large range of flight speeds. Together with my findings, these results suggest that temporal limitations of visual processing are critical to determine the optimal flight speed.

Implications for studying foraging behavior

Studies of foraging behavior in bees have treated visual cues, such as color and shape, as stimuli with which the bees can associate and discriminate between different rewarding units (Heinrich *et al*., 1977; Hill *et al*., 1997; Real *et al*., 1982; Smithson and Macnair, 1996; Wells *et al*., 1986; Wells and Wells, 1983). Most of these authors paid attention to effects of the amount of nectar provided and the density of nectar dispensers, but not the possible effects caused by the cues themselves. I emphasize that, for assessments of the energetic value of a prey type, it is essential to include the costs of detecting this type. In the optimal foraging literature, there is a wide range of papers that deal with optimal foraging speed. As possible factors contributing to adjustments of speed, these papers discuss energetic considerations (Hedenström and Alerstam, 1995; Kunze and Chittka, 1996), motivation (Roces, 1993), and scramble competition (Shaw *et al*., 1995). My study shows that understanding the mechanisms of visual information processing is also crucial to interpret optimal search behavior. For example, optimal foraging theory predicts that two flower types with equal nectar rewards, and with equal density, should be chosen equally frequently. The results do not only show that the net caloric value of a flower type will be fundamentally determined by its color, which has strong effects on search time. More intriguingly, my findings lead to the prediction that the bees' relative preference for flowers of two colors may switch depending on floral size; one floral color may be easier to detect at large size, but the same color may be harder to detect than the other color when flowers are small. This is because different neuronal channels with different spatio-temporal properties are used for detection of large and small flowers. Floral advertising strategies may respond to these perceptual constraints: a strong color contrast with the background (independently of direction) may be favored in large flowers, whereas small flowers should strive to optimize green contrast only.

Optical Scaling and Spatial Resolution in Bumblebees
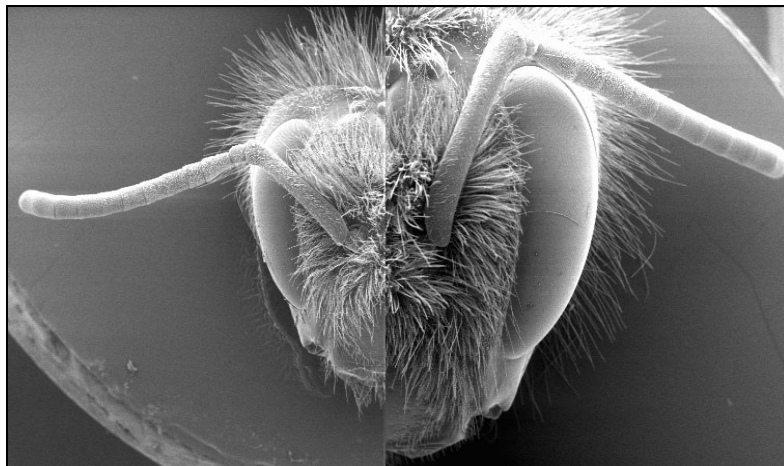
Head of small and large bumblebee worker from the same colony

Abstract   Foraging efficiency in bees is strongly affected by proficiency of detecting flowers. Both floral display size and bee spatial vision limit flower detection. In chapter one I have shown that search times for flowers strongly increases with decreasing floral display size. The second factor, bee spatial vision, is mainly limited by two properties of compound eyes: (a) the interommatidial angle $\cap\Sigma$ and (b) the ommatidial acceptance angle $\cap\langle$. When a pollinator strives to increase the resolving power of its eyes, it is forced to increase both features simultaneously.

This chapter examines the effect of body size variation in bumblebees on the optical properties of the compound eyes by means of morphological and behavioral investigations. Bumblebees show a large variation in body size. I found that larger workers with larger eyes possess more ommatidia and larger facet diameters. Large workers with twice the size of small workers (thorax width) have about 50 % more ommatidia, and a 1.5 fold enlarged facet diameter.

In a behavioral test, large and small workers were trained to detect the presence of a colored stimulus in a Y-maze apparatus. The stimulus was associated with a sucrose reward and was presented in one arm, the other arm contained neither stimulus nor reward. The minimum visual angle a bee is able to detect was estimated by testing the bee at different stimuli sizes subtending angles between 30° and 3° on the bee's eye. Minimum visual detection angles ranged from 3.4° to 7.0° among tested workers. Larger bumblebees were able to detect objects subtending smaller visual angles, i.e. larger bees were able to detect smaller objects than their small conspecifics. Thus morphological and behavioral findings indicate an improved visual system in larger bees. I suggest that larger workers can decrease their search times for flowers and increase their foraging rates due to their superior visual system.

INTRODUCTION

The ability to detect flowers and discriminate between flowers of different species strongly influences foraging efficiency in pollinating insects. Flowers serve as carbohydrate and protein sources, and optimal foraging theory predicts that pollinators strive to exploit these resources efficiently. As flowers differ in the quantities of their nectar and pollen rewards and in the frequency of their occurrence, pollinating insects are expected to restrict their visits to a subset of profitable flower species (Chittka *et al.*, 1999a). Flowers display several optical features like color, size and shape which pollinators can use to detect and identify a certain flower type. Thus, the optical system of pollinators is an important sensory modality which may limit foraging efficiency (see also chapter 1).

All insect pollinators possess the same optical system, namely compound eyes, but the design, and thereby spatial resolution, varies substantially among different species. Compound eyes comprise a varying number of ommatidia, the receptor units of the eye, and must be capable of two basic things. First, the eye must be able to collect sufficient light, and second it must be able to reliably determine the direction of light sources and objects, respectively. Therefore, the array of the receptor units and the dimension of the ommatida are the two most important factors which affect both cases of spatial resolution, single object and grating resolution (Land, 1997b; Warrant and McIntyre, 1993). The angle between two ommatidia (= interommatidial angle $\cap\Sigma$) in a compound eye is one of the fundamental determinants of its spatial resolution. When a compound eye, for example, faces a grating of black and white strips the eye can resolve the two black (or white) strips as two distinct objects only when the visual angle between them subtends a minimum of $2\cap\Sigma$. In that case each black and white strip projects onto at least one ommatidium. However, this minimum resolution of $2\cap\Sigma$ can only be realized when the intensity contrast perceived by two neighboring ommatidia is large enough to be detectable. If the two alternate strips present only a weak contrast (e.g. light and dark gray strips), resolution is additionally limited by ommatidial diameter (and thus by ommatidial acceptance angle $\cap\langle$; Land, 1997a). The larger the ommatidial diameter, the smaller the contrast differences which can be detected. Beside the amount of absorbed light quanta, ommatidial diameter mainly limits the visual field of an individual photoreceptor by the phenomenon of diffraction (Land, 1997b). This phenomenon is caused by an important physical characteristic of light itself (for explanation see Warrant and McIntyre, 1993). As a consequence, light which passes a convex lens and focuses in the combustion point always induces a complex diffraction pattern (Airy disc), a central intensity maximum and a series of rings of minima and maxima of sharply decreasing intensity, rather than a single point. The wider the lens aperture (= lens diameter) the narrower the Airy disc and hence the finer the image resolution (Land, 1997a).

For these reasons, increasing the resolution of a compound eye of a particular size results in a dilemma. The interommatidial angle $\cap\Sigma$ can be increased by increasing the number of ommatidia. Unfortunately, in this case ommatidial diameter drops too, and the contrast detection of the eye is reduced and diffraction is increased. On the other hand, increasing ommatidial diameter and thereby increasing contrast detection entails an increase of $\cap\Sigma$. The

only way to improve resolution without the cost of decreased contrast detection or increased $\cap\Sigma$ is, therefore, enlarging eye size (Land, 1985).

Additionally, when identifying the color of a flower, pollinators are not only limited by the optical properties of their eyes as mentioned above, but additionally by subsequent neuronal processing. In honeybees, for example, the receptive field of color coding neurons is comparatively large, and a colored target must subtend at least 15° (equivalent to a minimum of 59 ommatidia of bee's compound eye; see chapter 1 and Giurfa *et al*., 1996) so that the bee can identify its color. In the case of a target subtending less than 15° but at least 5° (equivalent to a minimum of 7 ommatidia) bees can only use the achromatic green contrast channel for detection, i.e. the difference in signal provided by the green receptor between background and target (Giurfa *et al*., 1997).

In this chapter I examine possible effects of size variation on flower detection in pollinators. I use bumblebees as ideal study objects, since workers exhibit a pronounced size variation (Garófalo, 1978; Inouye and Kato, 1992; Knee and Medler, 1965). Large workers can reach up to 7 times the body weight of small workers in a colony (Cumber, 1949). Increasing body size results in an increase in eye size and thus the question arises if large workers possess a higher spatial resolution compared to their smaller nestmates. I assess scaling effects on (1) anatomical features of workers' compound eyes and (2) behavioral performances in single object detection. The first aspect provides a theoretical estimation of the impact on visual resolution, the second facilitates an assessment to which extent the visual information perceived by the compound eye can be used by the bumblebees in detection tasks.

MATERIAL AND METHODS

Animals and body measures

I used bumblebee workers from three *Bombus terrestris* colonies reared in the lab at the University of Würzburg. For morphometrical measurements bumblebee workers were selected according to their size and killed by cooling them in a freezer at -20°C. Head and thorax of each bee were mounted on a table with a micrometer screw. Size measurements were carried out with a stereomicroscope (Wild TM M3Z, Switzerland) at twenty fold magnification. I determined head width (from eye to eye), thorax width (intertegula span) and length of left eye from each worker (for anatomical terminology see Michener, 2000).

*Scanning electron microscope (SEM)*

For estimating ommatidia number and diameter I removed the left eyes of freshly killed bees with a razor blade and glued them with their inner side on a SEM table. Afterwards they were air-dried, gold-palladium coated (Balzers sputter coater SCD 005, Liechtenstein) and viewed with a scanning electron microscope (Zeiss DSM 962, Germany). On the SEM photos I marked

a 1 mm^2 area in the center of each eye and counted all ommatidia inside this area. Of 15 randomly selected ommatidia I measured facet diameter (tip to tip distance of the hexagonal lens). I scanned the photos of each eye into a computer and measured eye surface using a imaging program (Scion image, Scion corporation, USA). Number of ommatidia per eye was calculated by counting ommatidia per 1 mm^2 multiplied with eye surface (in mm^2).

Experimental setup and stimuli

All colonies were housed in small wooden boxes (15 * 28 * 11 cm) inside the lab. Each colony was connected to a flight cage (0.45* 0.45 m, 0.3 m height; Fig. 2) via a Plexiglas tube (2 cm diameter, 0.3 m length). Shutters between nest and arena allowed to control access of selected workers. The flight cage was directly connected to the experimental apparatus, a Y-maze with two tunnels (0.3 m width, 0.2 m length and 0.3 m height) branching from a trilateral decision chamber (0.3 m * 0.3 m * 0.42 m, 0.3 m height), similar to that used by Srinivasan & Lehrer (Srinivasan and Lehrer, 1988 and Fig. 2). The two back walls of the tunnels consisted of white plastic boards (0.3 m * 0.3 m) with a central hole (1 cm diameter). On the back side of each board a small plastic tube (1 cm diameter, 6 cm length) with a feeder (20 ml) was mounted at its end connected to the central hole (Fig. 2). Both, back walls and feeders, could be interchanged independently between the two arms. The arena was covered by a UV-transmitting Plexiglas top.

As flower stimulus I used yellow paper disks (HKS 3N; K +E Stuttgart, Stuttgart-Feuerbach, Germany) 15.9, 7.9, 5.5, 3.9, 3.1, 2.4, and 1.6 cm in diameter, respectively. Spectral reflectance functions of the stimulus and the background was measured as described in chapter one (Fig. 1). I measured color contrast, green contrast and brightness contrast between stimulus and background (see chapter one and Chittka, 1992). The color disc was glued with plasticine onto the back wall of one arm of the Y-maze (termed positive arm). Large discs (up to 5.5 cm diameter) were perforated at their center with a hole (1 cm diameter) that fitted to the hole in
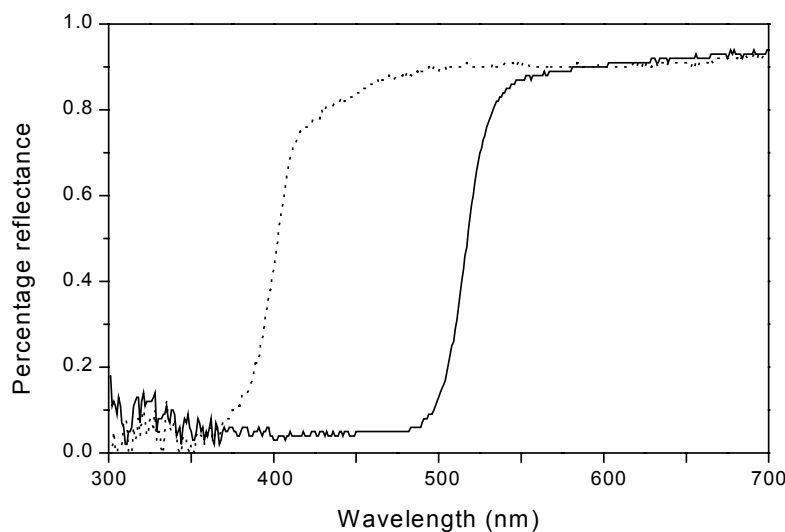
Fig. 1.
Spectral reflectance curves of the yellow stimulus and back wall. Dotted line, back wall; solid line, stimulus.

the back wall (Fig. 2). Smaller color discs without perforation were mounted directly above the hole. During all training and test phases color stimulus was always associated with a reward of sucrose solution in the feeder behind the back wall. The back wall of the second arm (termed negative arm) contained no stimulus and always an empty feeder (except during pre-training phase; see below).

Flight cage

Decision point

Entrance

Decision chamber

Choice line

D

Sucrose reservoir

+

-
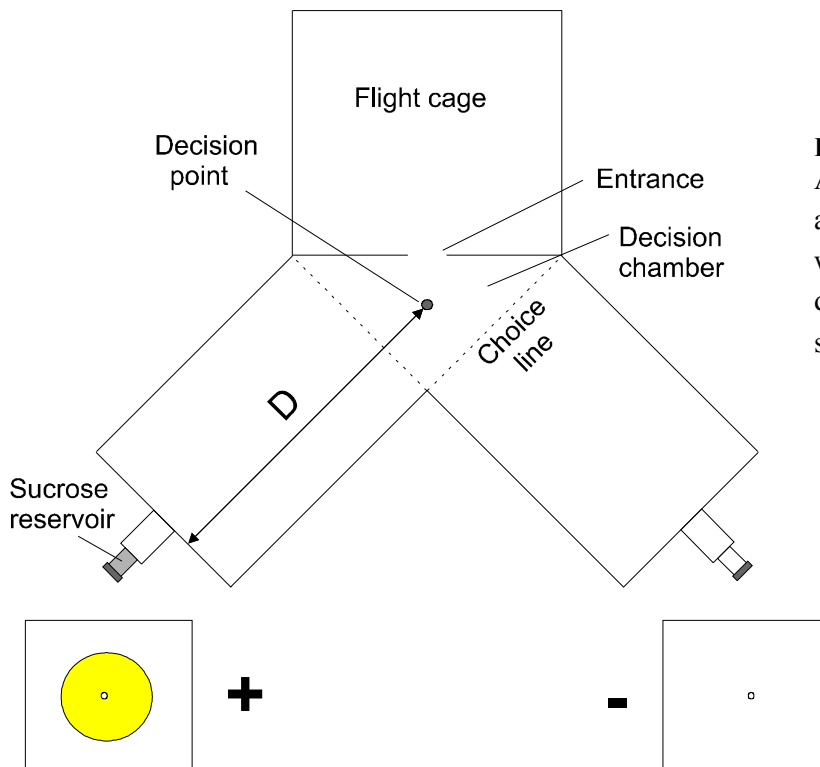
Fig. 2.
Assembly drawing of the Y-maze apparatus. Plus indicates back wall with stimulus and filled feeder. D, distance between decision point and stimulus (= 30cm).

Procedures

During the pre-training phase, both arms presented a color disc (15.9 cm diameter) associated with a reward. Bees had free access to the Y-maze for several days. Bees discovered the entrances to the feeders in both arms within one day and started collecting sucrose solution for their colony. I marked foragers with numbered 'Ophalitplättchen' on their thorax. When marked bees continuously visited the feeders in both arms I started training and testing foragers individually.

During training and test phases only one arm presented the stimulus associated with the reward. Back wall, stimulus and feeder were interchanged randomly and independently between both arms after each trial. After each fifth trial the disc was replaced by a new one. In order to exclude that bees used possible olfactory cues I cleaned the back wall and the entrance tunnel to the feeder after each third trial with alcohol. Each training and test trial started when the bee entered the decision chamber. I defined the initial choice of the bee as the point when she crossed the choice line of the positive or negative arm for the first time (see Fig. 2). After each

visit at the feeder the bee returned to the colony and the next trial started when she initialized a new foraging trip.

Table 1. Visual angle of stimuli calculated with $D = 30$ cm.

Disc diameter (cm)	15.9	7.9	5.5	3.9	3.1	2.4	1.6
Visual angle (°)	30	15	10	7.5	6	4.5	3

During training phase only the largest stimulus (15.9 cm diameter) was presented. Each bee was trained until she reached a performance level of 80% or higher for the largest stimulus (at least for a minimum of 20 trials) before test phase started. During the test phase, I presented the stimuli in a descending order, starting with a color disc of 15.9 cm in diameter. The bee was tested for ten trials at each stimulus size when she made no mistake (wrong choice), twenty trials when she made one or two, and a maximum of thirty trials when she made more than two mistakes. To obtain a threshold estimation for the minimum visual angle of a stimulus the bee was able to detect I first calculated the visual angle subtended by each tested stimulus at distance $D = 30$cm, the distance between stimulus and the 'decision point' (defined as the mean distance between the point where the bee entered the Y-maze and where she crossed crossed the decision line; see Table 1 and Fig. 2). Second, for each bee I plotted the percentage of correct choices as a function of the visual angle of the stimulus. I employed the learning criterion established by Giurfa et al. (1996): a bee is able to detect a given stimulus when she chooses the positive arm of the Y-maze with a probability of correct choices greater than 60%. Therefore, detection threshold was interpolated as the visual angle corresponding to the 60% level.

After testing, the bee was killed and size was measured as described above. Thus, for each bee I obtained body size and minimum visual angle at which the bee was able to detect the stimulus with a probability of 60%.

Data analysis

I tested possible correlations between measured parameters with a nonparametric test for association (Spearman's rank correlation) except for body size versus eye length, where I used a least square regression (model I; Sokal and Rolf, 1981). All p-values above 0.05 were considered as not statistically significant.

RESULTS

Scaling of optical properties

In order to test whether eye size scales with body size, I measured eye size of 34 bumblebee workers whose thorax widths varied between 2.6 and 4.0 mm. Larger workers possess larger eyes (Fig. 3). Data reveal a linear correlation of eye size with body size (N = 34; r = 0.91; p < 0.0001).

As a next step I tested what factors underlie the increase in eye size. I estimated ommatidia number and mean ommatidial diameter of ten workers. Both, ommatidia number (Fig. 4A) and size (Fig. 4B) correlated with eye surface. Ommatidia number ranged from 2963 to 4132 per eye, ommatidial diameter from 19.3 µm to 29.4 µm. These data show that eye size of large workers increased due to more and larger ommatidia. However, total ommatidia number per eye might be underestimated because facet diameter in the dorsal and ventral area of the eye might be smaller than in the central area (Meyer-Rochow, 1981).
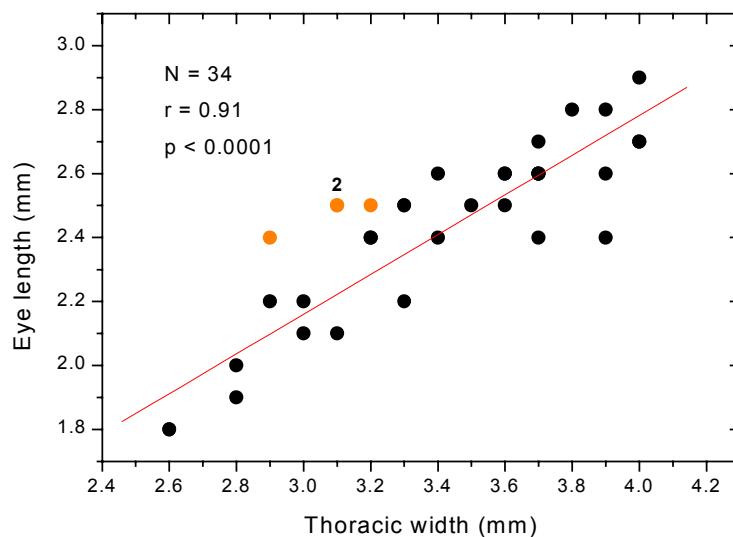
Fig. 3.

Thorax width of 34 bumblebees plotted against eye length of left eye (r = 0.91, p < 0.0001). Red dots show data from four honeybee workers for comparison.

Minimum visual angle

Color contrast, green contrast and brightness contrast between stimulus and back wall was measured as described in chapter one. The tested stimuli provided substantial contrast of all measured parameters (color contrast: 0.301; green contrast: -0.105; brightness contrast: -0.912). In the first test phase, during which the largest stimulus size (subtending 30°) was presented, bees reached performance levels between 80% and 100%. Up to a visual angle of 10° performance level stays constant. Performance level decreased for stimuli smaller than 10° visual angle. I plotted minimum visual angle of all tested bees as a function of thorax width.

Minimum visual angle ranged from 3.4° to 7.0°. I found a significant negative correlation between minimum visual angle and body size (Fig. 5). The data clearly show that larger bees possess a higher single object resolution and are therefore able to detect objects of a definite size from a larger distance than their smaller nestmates.
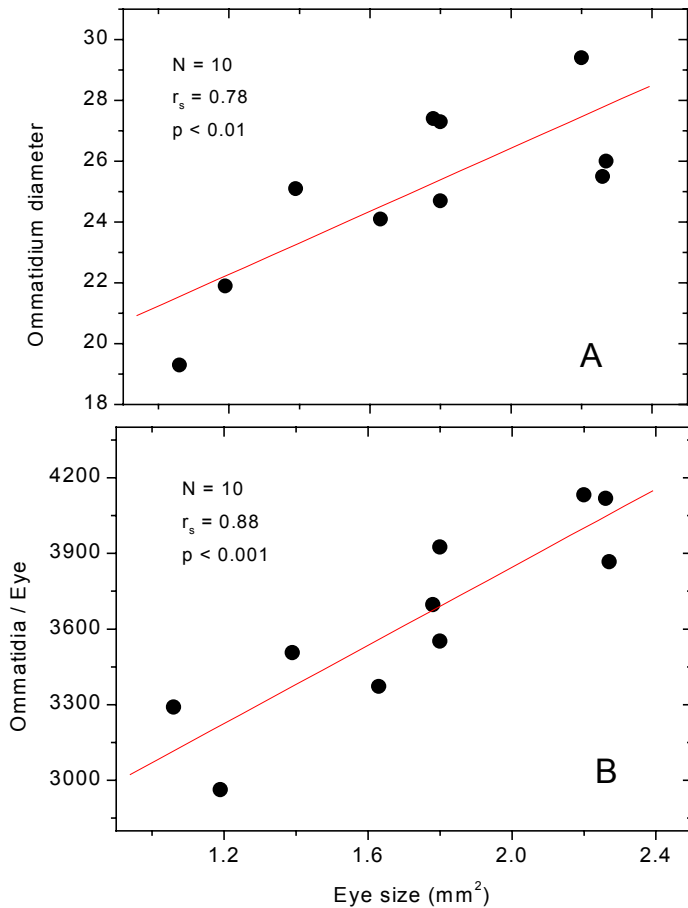
Fig. 4.

Eye size (measured as eye surface; see material and methods) plotted against **A** ommatidia diameter and **B** ommatidia number per eye.
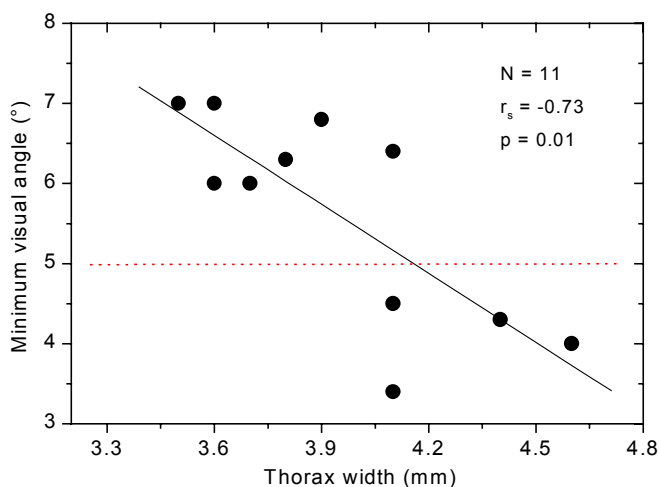
Fig. 5.

Data reveal a significant negative correlation between thorax width and minimum visual angle of bumblebee workers (N = 11, r_s = -0.73, p = 0.01). Dotted line corresponds to the minimum visual angle found in honeybees.

DISCUSSION

The data suggest that the size of a bumblebee affects both investigated properties of its visual system, anatomical eye design and behavioral performance. Larger bumblebee workers seem to possess a superior visual system for object detection compared to their smaller conspecifics.

Optical features

As mentioned above, an increase in eye size can be due to an increase in ommatidia number or ommatidia size. In large bumblebees both ways are realized. Larger workers therefore possess the anatomical condition which is required for an improved eye resolution (Warrant and McIntyre, 1993).

In the case of bumblebees only few data on eye anatomy are available. Meyer-Rochow (1981) investigated ommatidia number and diameter of an "average bumblebee worker" (p. 125) of *B. hortorum*. He estimated that one eye contains at least 6000 ommatidia and that ommatidial diameters range from 26 – 28 μm. Because he gave no data on worker size it is not clear if the higher ommatidia number and diameter he found is due to species-specific differences or to a scaling effect. However, although he did not investigate size effects, he expected that "... dimensions of the eye are adjusted proportionately, correlated with body size" (Meyer-Rochow, 1981; p. 125).

The larger ommatidia number in large individuals indicates smaller interommatidial angles which contribute to a higher spatial resolution. However, the causality between interommatidial angle and ommatidia number is only valid when the shape of the eye stays constant with scaling. In *Cataglyphis* ants, for example, larger individuals possess more ommatidia with smaller interommatidial angles (Zollikofer *et al.*, 1995). This finding is due to an unchanged surface curvature of the compound eye among different sized ants, i.e. the shape of the eye does not change with size. I took cross sections through the lateral meridian of eyes from small and large bumblebee workers and found no noticeable changes in the curvature of the surface (data not shown). However, a direct measurement of the angular spacing of ommatidia by means of the pseudopupil technique would be recommendable (Stavenga, 1979).

Behavioral performances

To estimate how similar or different two colors are perceived by a bumblebee I applied a bee color model described in chapter one. By means of this model I found that the stimulus (yellow disc) provides sufficient color and green contrast to the background. Bumblebees are therefore able to discriminate between stimulus and back wall on the basis of input from both visual channels, the achromatic and chromatic channel. Under these conditions honeybees can detect objects with a minimum visual angle of 5° (Giurfa *et al.*, 1996; Lehrer and Bischof, 1995). The minimum visual angle of tested bumblebee workers ranged from 3.6° to 7.0°, i.e. small bumblebee workers have a weaker object resolution, whereas large workers possess a better object resolution than honeybees. A medium sized bumblebee worker with a thorax width of

approximately 4.2 mm possesses the same object resolution as found in honeybee workers (Fig. 5). In a recent study Macuda et al. (Macuda *et al.*, 2001) tested the visual acuity (= grating resolution) of *Bombus impatiens* workers and found that bumblebees possess a 25% better resolution compared to honey bees. Unfortunately, the authors give no data on worker size, so it is hard to estimate the effect of scaling. My results demonstrate that large *Bombus terrestris* workers can reach a roughly 40% higher object resolution compared to honeybees. Taking into account that in some bumblebee species workers can reach a much larger body size (for example large *B. griseocollis* workers can reach the double weight of large *B. terrestis* workers; Fisher, 1987), visual resolution of these species is expected to be still higher.


Implication on foraging behavior

Spatial resolution of insect pollinators varies among sexes of the same species and among different species. In honeybee drones, for example, the dorso-frontal part of the compound eye comprises a zone of very large ommatidia with small divergence angles (Menzel, 1991), which is assumed to be an adaptation to the mating behavior in honeybees. Drones can detect objects in their dorso-frontal visual field subtending only 0.5° (e.g. a flying queen in a distance of two meter against the sky; Vallet, 1993). Compared to honeybee workers, the density of ommatidial axes per square degree (which is a good measure for spatial resolution) in the dorso-frontal visual field of drones is 7 times higher (Stavenga and Wunderer, 1999).

What are the consequences of differences in the spatial resolution among bumblebee workers for the bearer of a small or a large eye? Large individuals are supposed to be better in all behavioral contexts where vision plays a limiting role. For example, larger bumblebees may fly earlier in dawn and later in dusk when light intensity is still low due to their expected higher light sensitivity. During flight they are able to detect important landmarks in the landscape for orientation at a larger distance than their smaller conspecifics. Spatial resolution also limits search time during foraging on flowers (see chapter one). Bumblebees visit hundred to thousands of flowers in order to fill their crop, because most flowers provide only small quantities of nectar reward. Thus, when search time per flower increases only slightly due to a worse spatial resolution, foraging rates of small workers would drop substantially and their impact on nectar or pollen influx into the colony decreases too. To summarize, all findings indicate that the visual system of large bumblebees is superior in flower detection and orientation during flight than that of their smaller conspecifics.

A bumblebee colony exhibits a size-related division of labor among its members. Larger workers tend to forage outside the nest, smaller ones perform nest tasks (Cumber, 1949; Free, 1955b; Garófalo, 1978). It is assumed that by allocating its largest foragers to foraging a colony will maximize its nectar influx because of the higher foraging rates of larger workers (see chapter four). My data support the idea that not only physical factors may responsible for higher foraging rates in large bumblebees, but also neglected sensory limitations due to different sensory capabilities.

Size Polymorphism in the Olfactory System of Bumblebees
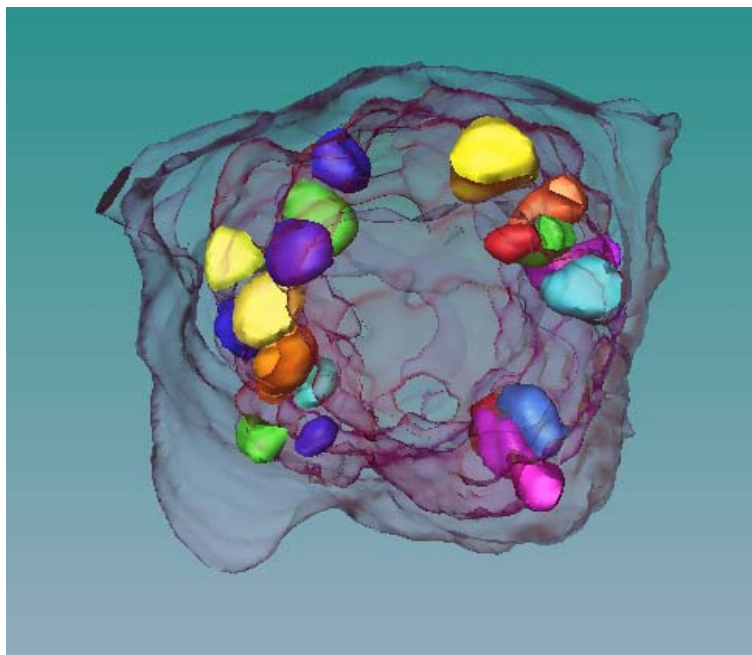
3-D reconstruction of the antennal lobe of a *B. terrestris* worker

Abstract   Olfaction is a very important sensory modality in an insect's life. It is essential in most behavioral tasks. Pollinating insects, for example, utilize species-specific odors for detecting and identifying nectar and pollen rich flowers. I investigated the olfactory system of an important insect pollinator, *Bombus terrestris*, and the effect of scaling on its antennal olfactory sensilla and the first olfactory neuropil, the antennal lobes. Bumblebees exhibit a pronounced size polymorphism among workers of the same colony. I found that worker size (2.7 to 4.3 mm head width) correlates significantly with sensilla number (sensilla placodea; 708 to 2594 per antenna), sensilla density (2377 to 3168 mm^{-2}), volume of antennal lobe neuropil (5.7 to 19.0 * 10^6 μm^3) and volume of single identified glomeruli. The enlarged volume of the first olfactory neuropil in large individuals is caused by an increase in glomeruli volume and coarse neuropil volume. Additionally, beside an overall increase of brain volume with scaling I found that the olfactory neuropil increases disproportionately compared to a higher order neuropil, the central body.

The data predict a higher odor sensitivity in larger bumblebee workers. Thus I propose *B. terrestris* as an excellent model system for testing the implication of sensilla number on odor perception in a behavioral context.

INTRODUCTION

Olfaction is a very important sensory modality in an insect's life. It is essential in most behavioral tasks: orientation (Cardé and Minsk, 1997; Chittka *et al.*, 1999b), feeding (Bernays and Chapman, 1994), mating (Cardé and Minsk, 1997) and communication (Free, 1987; Hölldobler, 1995; Vander Meer *et al.*, 1998). The majority of insect olfactory sense organs (sensilla) are located on the antennae, which are specific modified head appendages (Keil, 1999). The number of olfactory sensilla on the antennae among different species ranges from a few tens up to 100.000 with more than 300.000 sensory neurons (Chapman, 1982). The highest sensilla numbers are found in male insects which need to find females over long distances. Intra-specific differences in the number of sensilla are related to size, sex, different feeding habits or behavioral specializations (Bernays *et al.*, 2000; Chapman, 1982).

Besides differences in sensilla number, insect species also differ in the organization of their first olfactory brain neuropil, the antennal lobes (Rospars, 1988). The antennal lobes are composed of small spherical subunits, called glomeruli, in which the synaptic connections between the invading antennal sensory neurons and the antennal lobe interneurons occur (Boeckh *et al.*, 1990; Boeckh and Tolbert, 1993). It is assumed that the individual glomerulus is a functional unit and the spatial array of the glomeruli in the antennal lobe represents a functional separation of different input channels, i.e. similarly tuned sensory neurons project into the same glomerulus or group of glomeruli (Hansson and Christensen, 1999). Evidence for this hypothesis comes from selective staining of identified sensory neurons (Hansson, 1997; Hansson *et al.*, 1992) and from functional Ca^{++} imaging techniques (Galizia *et al.*, 1999b). The number and spatial arrangement of glomeruli is mostly invariant within species. However, intraspecific differences in the number of glomeruli may occur between sexuals. In many moth species, for example, males possess enlarged glomerular complexes (so-called macroglomerular complexes) which are lacking in females, specialized for the perception of the female's sex-pheromones (Hildebrand, 1996). Additional to variation in glomeruli number, the volume of glomeruli can vary among individuals of the same species. In the hemimetabolous insect *Periplaneta americana*, for example, glomeruli volume increases up to the adult stage due to an addition of sensilla with each larval instar (Prillinger, 1981). Sensilla number as well as glomeruli number and glomerular volume, are thought to be correlated with qualitative and quantitative differences in odor perception (Hansson and Christensen, 1999).

In the present study I examine size-related effects on the number of olfactory sensilla and on the volume of antennal lobe neuropil in adult workers of *Bombus terrestris*. The olfactory system of bumblebees resembles that of the closely related honeybees. The antennae bear the same main olfactory sensilla type, the sensilla placodea (Agren and Hallberg, 1996; Esslen and Kaissling, 1976). In bumblebees, these sensilla are innervated by 13-20 sensory neurons, most commonly 14-15 (Agren and Hallberg, 1996). Electrophysiological recordings in honeybees demonstrated that the poreplate sensory neurons are sensitive to a variety of plant and flower odors as well as pheromones (Vareschi, 1971). All poreplate sensory neurons project into the glomeruli of the antennal lobe. The sensory neurons of a single poreplate project to different

glomeruli, whereas each sensory neuron terminates in only one glomerulus (Brockmann and Brückner, 1995). The glomerular organization of the antennal lobe of bumblebees seems to be very similar to that of honeybees, exhibiting the same four antennal lobe tracts (T1-4) (Fonta and Masson, 1985).

In contrast to honey bees, bumblebees show a pronounced size polymorphism among workers of the same colony (Plowright, 1968; Garófalo, 1978; and Fig. 1). Large workers can reach up to 7 times the body weight of small workers (Cumber, 1949), the largest intra-colonial size-variation in social bees (corbiculate *Apinae*). Bumblebee workers exhibit a size-related division of labor; larger workers are mostly engaged in foraging and smaller workers in performing nest duties like larvae provisioning or cell cleaning (Michener, 1974). Most recently it has been shown, that larger bumblebee workers are more successful foragers than smaller ones, i.e. they have higher foraging rates and thereby contribute disproportionately more to the colony's nectar influx than their smaller nestmates (Spaethe and Weidenmüller, submitted). Besides other physical factors, larger olfactory and visual sensory epithelia may enable larger individuals to detect flowers more rapidly and thus enable them to forage more efficiently.

Here I address the question how scaling within a polymorphic social bee species effects the sensory periphery and the first olfactory neuropil. I measured volume of glomerular neuropil, coarse neuropil and twelve identified glomeruli from different antennal lobe regions. For comparison I measured the volume of a central brain region, the upper and lower part of the central body. The data reveal that in larger bumblebee workers, brains do not simply increase isometrically, but sensory neuropils increase disproportionately. I propose to use bumblebees as a model system for investigating the functional significance of scaling on odor perception and odor elicited search behavior.

MATERIAL AND METHODS

Animals and body measures

I obtained bumblebee workers from two *B. terrestris* colonies bought from a commercial breeder (Koppert, Netherland). Workers from both colonies were selected according to their size and were killed by cooling them in a freezer at − 20 °C. Afterwards bees were decapitated and heads were mounted on a table with a micrometer screw. All size measurements were carried out with a stereomicroscope (WildTM M3Z, Switzerland) at twenty-fold magnification. I determined head width (from eye to eye), flagellum length and width of the right antenna of each worker. I used head width to estimate body size because it correlates very well with body weight and other morphometric measures in bumblebees (Spaethe, unpublished data; Bullock, 1999). For evaluating sensilla density, I estimated flagellum surface by means of the equation for a cylinder (h * 2 * r * ◊, with h = flagellum length, and r = half of flagellum width).

Scanning electron microscope (SEM)

After head measurements, the right antenna was cut, fixed in a wax-colophonium mixture and split in two halves using a razor blade. The wax was removed from the antenna halves with Rotihistol (Roth, Germany). The antennae halves were air dried and mounted with their inner side on an SEM table. Antenna halves were gold-palladium coated (Balzers sputter coater SCD 005, Liechtenstein) and viewed with a scanning electron microscope (Zeiss DSM 962, Germany). I counted total number of pore plates on both flagellum halves and all segments.
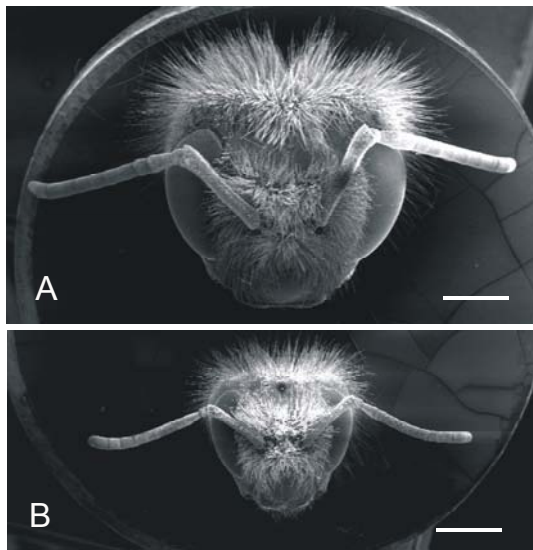
Fig. 1.
Scanning electron micrograph of heads from two *B. terrestris* workers from the same colony. **A:** large and **B:** small worker. Scale bars = 200μm.

Histology and volumetric analysis

Workers were collected randomly from the two colonies and narcotized with CO_2. Heads were cut, fixed with needles on a wax-colophonium plate and head widths were measured (see above). Immediately afterwards the heads were mounted in wax-collophonium and the head capsules were opened to fixate the brain tissue in alcoholic Bouin. After prefixation the brains were detached and completely removed from the head capsules. Fixation lasted overnight in the refrigerator at 4 °C. Afterwards, brains were dehydrated and embedded in paraffin wax. The embedded brains were serial sectioned in 8 μm slices on a rotation microtome (Leitz 1516, Germany) and stained with azocarmin-anilinblue. Drawings of each slice were made using a camera lucida attachment to a microscope (Axiophot, Zeiss, Germany). Line drawings were transferred on tracing paper, aligned to each other (best fit) and digitized. For reconstruction and volumetric measurements I used a 3-d-reconstruction software (Amira, TGS, France).

I obtained volumetric data for total antennal lobe, glomerular neuropil (sum of all glomeruli in the antennal lobe neuropil), coarse neuropil (inner fibrous core), several glomeruli from different tracts of the antennal nerve, and the central body (upper and lower part). For identification of measured glomeruli see results.

Data analysis

Correlations between one of the measured parameters (antennal size, pore plate number, pore plate density and volume of different parts of the brain) and worker size were tested by a nonparametric test for association (Spearman's rank correlation). To test if antennal lobe neuropil and the central part of the brain (upper and lower part of the central body) increase isometrically with body size, I tested for possible correlation between ratio of total antennal lobe neuropil volume divided by central body volume and worker size, applying the same statistical test. All p-values above 0.05 were considered as not statistically significant. Where I performed multiple significance tests, I adjusted p-values applying the sequential Bonferroni correction to control for Type I error (Rice, 1989).

RESULTS

Peripheral olfactory system

Head widths of workers used for morphometric measurements ranged from 2.7 to 4.3 mm. Poreplate number ranged from 708 to 2594, density from 2377 to 3168 mm^{-2}. I found a significant correlation between head width and flagellum length (N = 10, r_s = 0.93, p < 0.001), poreplate number (N = 11, r_s = 0.88, p < 0.001) and poreplate density (N = 9, r_s = 0.86, p < 0.01; all correlations significant after sequential Bonferroni correction), respectively (Fig. 2A-C). Thus, larger workers exhibited larger antennae with about 3.5 times more poreplates compared to their smaller nestmates.

Fig. 2.
A: Flagellum length, **B:** poreplate number and **C:** poreplate density plotted against head width. All measured parameters correlate significantly with head width of workers. N, number of tested workers; R_s, Spearman's rank correlation coefficient; p, p-value.
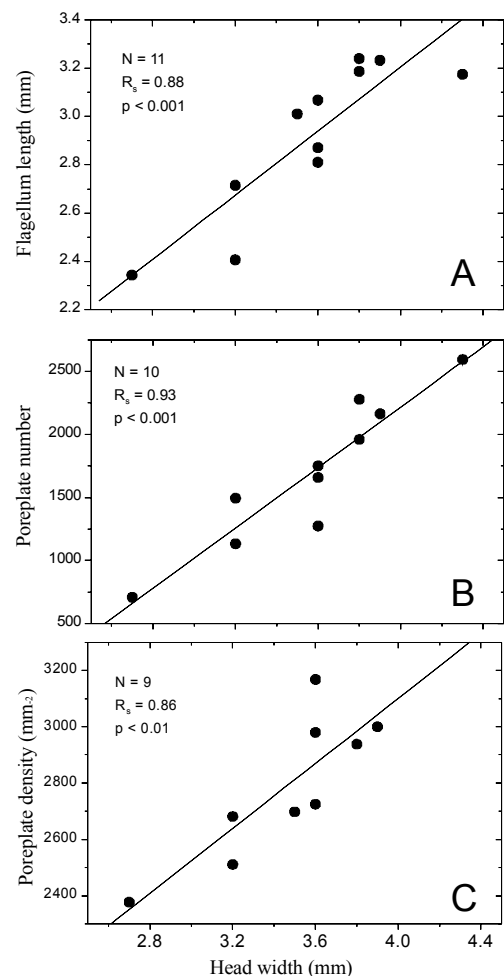
Glomerular organization of the antennal lobe

I found that the glomerular organization of the antennal lobe of bumblebees strongly resembles that of honey bees, as has been mentioned by Fonta & Masson (Fonta and Masson, 1985). All four main antennal lobe tracts show similar routes (paths) as their counterparts (T1-T4) in honey bees and thus can be recognized to be homologous to those.

In the bumblebee worker antennal lobe three prominent glomeruli (T3-a, T2-a, T4-a) were readily identifiable in all investigated individuals due to their characteristic shape and position. The T3-a glomerulus lies medial in the ventral group of glomeruli close to the coarse neuropil and directly posterior to the T1-tract entering the coarse neuropil (Fig. 3A). The T2-a is a

Fig. 3.
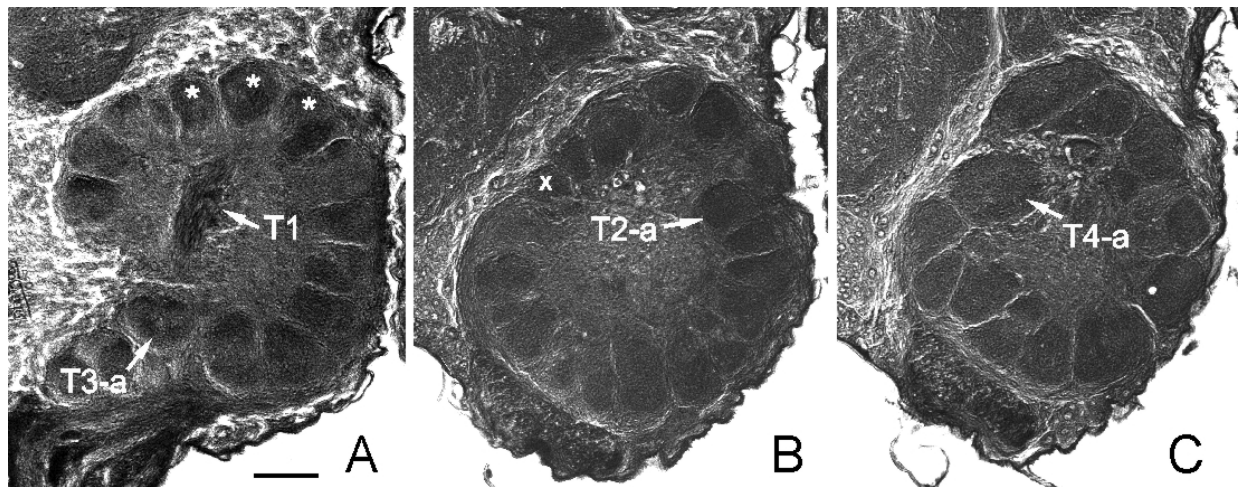Three characteristic frontal sections (from anterior to posterior) of the right antennal lobe of different bumblebee workers showing the position of the three individually identified glomeruli (T3-a, T2-a and T4-a) and the two glomeruli groups (T1-(b-g) and T1-(h-j)). **A**: The T3-a glomerulus is located at a depth of 100-150 μm (depending on body size) from the anterior surface of the brain directly posterior to the T1-tract (T1) entering the coarse neuropil. The glomeruli group T1-(b-g) (*) is lying directly dorsal to the T1-tract. **B**: At a depth of 220-290 μm the T2-a glomerulus appears, which is lying slightly inwards from the ring of glomeruli. Opposite to the T2-a the most posterior glomerulus of the dorso-lateral glomeruli group (Δ) is visible. **C**: The T4-a glomerulus lies at a depth of 250-320 μm just behind the lateral passage; scale bar = 50 μm.

voluminous, roughly spherical glomerulus, which is displaced inwards of the ring of glomeruli (Fig. 3B). The T4-a is a large glomerulus lying posterior to the lateral passage (Fig. 3C). A comparison with the published antennal lobe atlases of honey bees (Flanagan and Mercer, 1989; Galizia *et al.*, 1999a) shows that in the antennal lobe of honey bee workers glomeruli with a similar position and shape have been identified. The bumblebee glomeruli T3-a, T1/2-a and T4-a are anatomically homologous to the T3-23, T2-1(2) and the T4-2(1) in honey bees (Flanagan and Mercer, 1989; labeled C23, B01(2) and D02 in Galizia *et al.*, 1999a). In addition to the individually identified glomeruli I determined two groups of closely neighboring glomeruli within the dorsal T1-population. The glomeruli group T1-(b-g) (Fig. 3A) consists of dorsally

located glomeruli, and the glomeruli group T1-(h-j) consists of three glomeruli dorsally lining the lateral passage (Fig. 3B).

Computerized 3D-reconstruction allows a comparison of the position of all determined glomeruli and visualizes the size variation of the glomeruli between small and large bumblebee workers (Fig. 4).
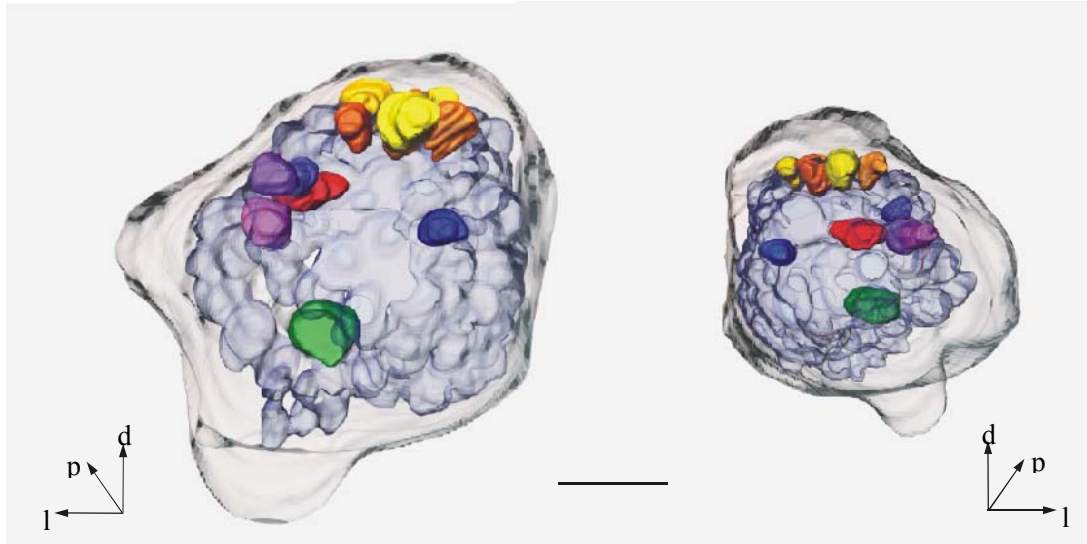
Fig. 4.
Reconstruction of left antennal lobe from a large and a small worker. Left antennal lobe was mirrored for better comparison. Same color represents homologous glomeruli in both antennal lobes. Green, T3-a; single blue, T2-a; red, T4-a; yellow and orange, T1-(b-g) group; violet and blue, T1-(h-j) group. Scale bar = 100μm.

Scaling of the antennal lobe neuropil

Considering the first olfactory neuropil, the antennal lobes, I likewise found a significant correlation between worker head width and volume of total antennal neuropil ($N = 8$, $r_s = 0.86$, $p < 0.01$), glomerular neuropil ($N = 8$, $rs = 0.86$, $p < 0.01$), and coarse neuropil ($N = 8$, $r_s = 0.86$, $p < 0.01$), respectively (Fig. 5A and table 1). I found that the volume of total antennal lobe neuropil of the largest worker ($19.0 * 10^6$ μm^3) was more than three times larger than that of the smallest one ($5.7 * 10^6$ μm^3). Ratio of glomerular and coarse neuropil volume does not change with scaling. Comparison of identified glomeruli of different populations indicates that glomeruli volumes increase proportionally (data not shown).

Both, the volume of the specific glomeruli T2-a and T4-a, and the mean volume of the glomeruli group T1-(b-d) and T1-(e-j) increased significantly with head width (Fig. 6A, B and table 1). Correlation between volume of glomerulus T3-a and head width was not significant (Table 1). All investigated neuropil volumes differed by factor three to four between largest and smallest bumblebee worker.
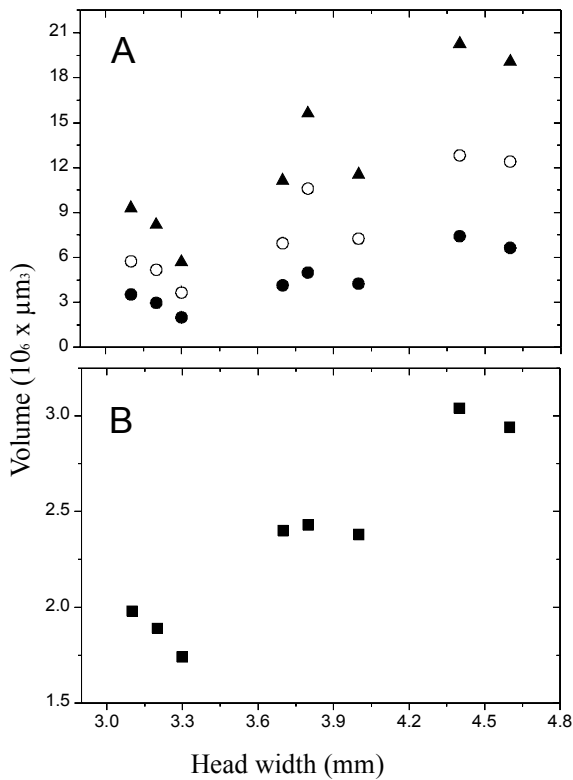
Fig. 5.
Volume of the **A:** antennal lobe neuropils and the **B:** central body neuropil (upper and lower part) plotted against head width. All measured parameters correlate significantly with head width (see Table 1). Triangle, total neuropil volume; open circle, glomerular neuropil; filled circle, coarse neuropil; filled square, central body.
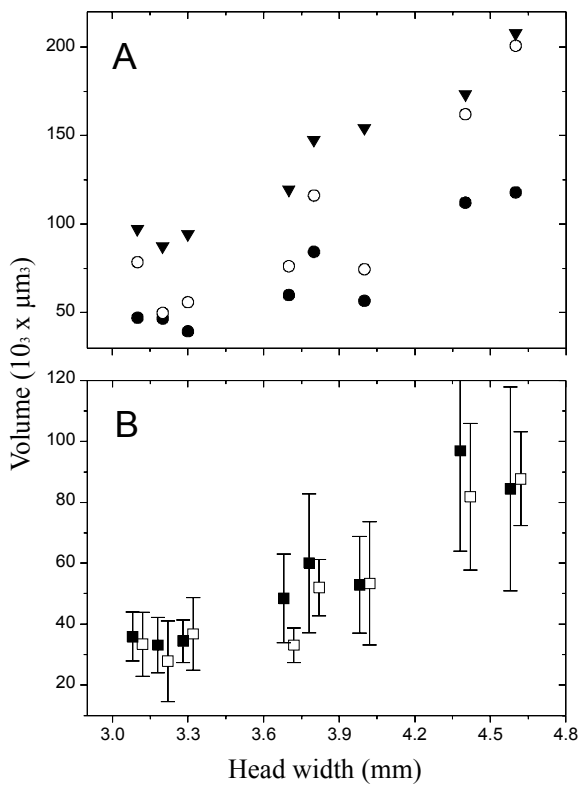
Fig. 6.
Head width plotted against **A:** volume of individually identified glomeruli and **B:** mean volume (Γ S.D.) of the T1-(b-g) and T1-(h-j) groups. All measured glomeruli except T3-a correlate significantly with head width (see table 1). Triangle, T4-a; open circle, T3-a; filled circle, T1-a; open square, T1-(h-j) group; filled square, T1 (b-g) group.

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Non-isometrical scaling of sensory and central neuropils

To examine scaling effects on higher order neuropils in the bumblebee brain, I additionally determined volume of the central body (upper and lower part). In contrast to the mushroom bodies, the central body does not receive olfactory afferents and only few visual afferents from optic neuropils (Homberg, 1987). In bumblebees, the central body increases with increasing head width (N = 8, r_s = 0.81, p < 0.05; Fig. 5B and table 1). Central body volume between largest and smallest bumblebees differs by a factor of 1,7.

Table 1. Correlation between neuropil volumes and head width

	N	r_s	P-value	Significance
Antennal Lobe Neuropil	8	0.86	0.0065	*
Glomerular Neuropil	8	0.86	0.0065	*
Coarse Neuropil	8	0.86	0.0065	*
Central Body	8	0.81	0.0149	*
Glomerulus T1-a	8	0.83	0.0102	*
Glomerulus T3-a	8	0.67	0.0710	n.s.
Glomerulus T4-a	8	0.93	0.0009	*
Glomeruli T1-(b-d)	8	0.88	0.0039	*
Glomeruli T1-(e-j)	8	0.88	0.0039	*
Neuropil / Central body	8	0.79	0.0208	*

Statistics on volume measurements. All neuropil volumes shown in the left column were tested for possible correlation with head width. N, worker number; r_s, Spearman's rank correlation coefficient; *, positive correlation at a table-wide ∇ = 0.05 after sequential Bonferroni correction (Rice, 1989).

Plotting the ratio of antennal lobe volume divided by central body volume as a function of head width revealed that antennal lobe increases disproportionately to central body (Fig. 7 and table 1). In other words in larger bees the antennal lobe neuropil takes up a higher proportion of the bumblebee brain in comparison to the central body.

DISCUSSION

The data clearly show that in bumblebees the olfactory system undergoes considerable quantitative changes with scaling. Sensilla number, volume of the antennal lobe neuropil as well as volume of single glomeruli increase with body size. Furthermore, the antennal lobe increases disproportionately compared to the central body neuropil with increasing brain size, indicating a stronger impact of scaling on sensory neuropils.

Scaling of the olfactory system

The scaling of bumblebee workers comes along with an increase in poreplate number which indicates an increased number of sensory neurons. As a consequence, larger workers possess an enlarged olfactory epithelia. Antennal lobe neuropil and glomerulus volume also increase with worker size. Enlargement of glomeruli volume in large bumblebees is a result of the increased
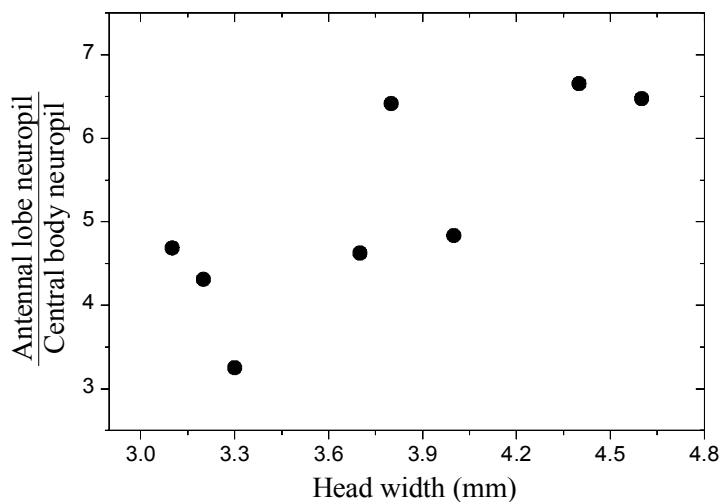
Fig. 7.
Ratio of antennal lobe neuropil and central body neuropil plotted against head width. Both variables correlate significantly (see Table 1). An increased ratio with worker size indicates that in larger bees the antennal lobe neuropil takes up a higher proportion of the brain compared to the central body.

number of sensory neurons converging in each glomerulus. Up to three times more sensory neurons terminate in each glomerulus of the largest compare to the smallest investigated worker. However, an increased number of interneurons, indicated by an enlargement of the coarse neuropil, may also contribute to larger glomerular volumes. Additional factors like the increase of fiber diameter, the increase of collateral ramifications and the multiplication of synapses may also effect glomerular volume, but this has to be surveyed in an electron microscopy investigation.
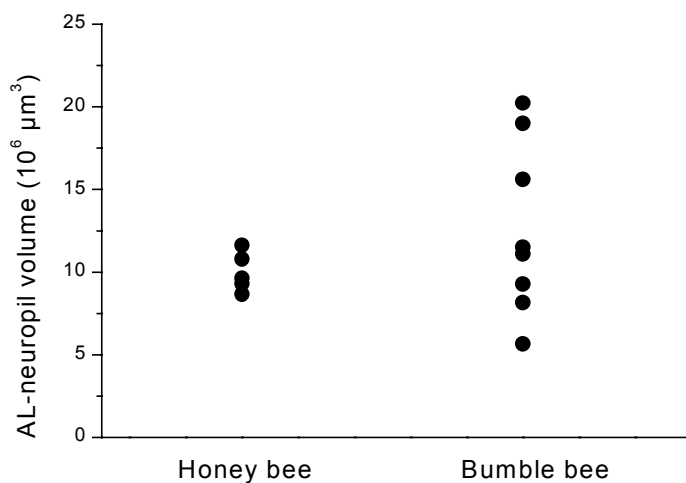
Fig. 8.
Antennal lobe volumes in honey bees (N = 5; data from Arnold, 1985 *et al.*) and bumble bees (N = 8; this study). Absolute volume measurements between species can vary due to different fixation techniques.

Arnold et al. (1985) presented volume measurements of the antennal lobe neuropil of five *Apis mellifera* workers, ranging from 8.6 to 11.6 x 10^6 µm^3, with a maximum volume difference of 34% between smallest and largest antennal lobe (Arnold *et al*., 1985; Fig. 8). In my study, individual bumblebees differ up to 357% in antennal lobe volume and 403% in glomerular volume (T3-a), i.e. an almost 10 times higher variation in comparison to *A. mellifera* workers. *A. mellifera* workers possess about 2600 poreplate sensilla per antenna, and variation in poreplate number is very low (Esslen and Kaissling, 1976). However, workers of the dwarf honey bee, *Apis florea*, bear about 600 poreplates per antenna (Gupta, 1992), being slightly lower than the lowest number of poreplates I determined in bumblebees. Additionally, data on the drone antennal lobe in *A. florea* indicate that the volume of isomorphic glomeruli is half as large as in *A. mellifera* (Brockmann and Brückner, 2001). Thus, the variation found in the olfactory system of *B. terrestris* resembles inter-species differences in the genus *Apis*.

Does the antennal lobe neuropil increase isometrically with size?

In order to answer this question I compared the ratio of the antennal lobe and central body neuropil of different sized workers. Early studies on brain size variation in social hymenopterans had demonstrated that, though sensory neuropils vary between castes, the central neuropils are almost invariant and size differences are closely related to body size (Howse, 1974). For that reason I used the central body volume as a conservative measure for general changes in brain size due to scaling.

Assuming that the antennal lobe and central body change proportionately, the ratio of both neuropil volumes should stay constant among individuals of different size. In this case, the brain of a large bumblebee worker could be produced by simply magnifying the brain of a small worker by means of a 3-d copier. However, the data do not support the idea of such a simple isometrical growth of the brain neuropils. In contrast, the data indicate that in bumblebees the olfactory sensory neuropil increases disproportionately with scaling.

What are the proximate mechanisms underlying this unequal volume change? First order sensory neuropils, like the antennal lobes, are formed by the interaction of sensory neurons and interneurons of the brain. Assuming that the antennae and the brain represent distinct ontogenetic modules (imaginal disc and neuroblasts, respectively; for review see Emlen and Nijhout, 2000), which differ in time and rate of mitotic activity during ontogeny, antennal lobe volume should be affected by both modules. As a consequence interaction of growth rates of both modules might result in a disproportionate growth of antennal lobe compared to higher order brain neuropils with scaling.

Functional significance of scaling of the olfactory system

Odor perception must meet two requirements, the existence of sensory neurons sensitive to odor molecules and a sufficient number of excited sensory neurons enabling the neuronal system to discriminate odor-induced excitation from noise. Thus a higher sensilla number, i.e. a higher

number of sensory neurons, increases odor sensitivity due to a higher probability to perceive the sufficient number of odor molecules (Ochieng and Hansson, 1999).

Assuming that in bumblebees the number of differently tuned sensory neurons does not change among workers, the overall increase in sensilla and sensory neurons number results in an increased odor sensitivity in larger bumblebees, i.e. larger workers are able to detect lower odor concentrations than their smaller nestmates. However, no behavioral investigation in insects exists so far which clearly demonstrates the causality between number of sensory neurons and sensitivity of odor perception among individuals of the same species. Thus, bumblebee workers would be a suitable system to reveal the link between sensilla number and odor sensitivity.

Brain size polymorphism – a factor influencing division of labor in social insects?

The ability to detect flowers and to discriminate between flowers of different species strongly influences foraging efficiency in pollinating insects (Chittka *et al.*, 1999a). Flowers serve as carbohydrate and protein sources, and optimal foraging theory predicts that pollinators strive to exploit these resources efficiently, i.e. to maximize their foraging rate for nectar and pollen. Flowers display several olfactory and optical features which pollinators can use to detect and identify a certain flower type. Thus, the olfactory and visual system of pollinators are important sensory modalities which may limit foraging efficiency. Within bumblebee colonies a size-related division of labor occurs, larger bumblebees tend to forage for nectar and pollen, whereas smaller workers tend to stay in the nest and fulfill nest duties (Heinrich, 1979; Michener, 1974). Several authors suggested that this size-related division of labor is due to improved physiological properties like thermoregulation and flight speed (Heinrich, 1979), which enable larger bumblebee workers to forage at lower temperature and to fly faster, and thus to forage more efficiently. However, large and small workers also differ in their sensory capabilities. Assuming that larger bumblebee workers are more sensitive to flower odors their foraging rate might increase due to an improved flower detection and identification capability.

To summarize, the data clearly reveal a strong impact of body size on the olfactory system of workers of the size polymorphic bumblebee, *Bombus terrestris*. My results predict pronounced differences in the sensitivity of odor perception among different sized workers. Thus bumblebees present a ideal system for testing the influence of neuro-physiological features of the olfactory system on behavior.

CHAPTER IV

Size variation and foraging rate in bumblebees
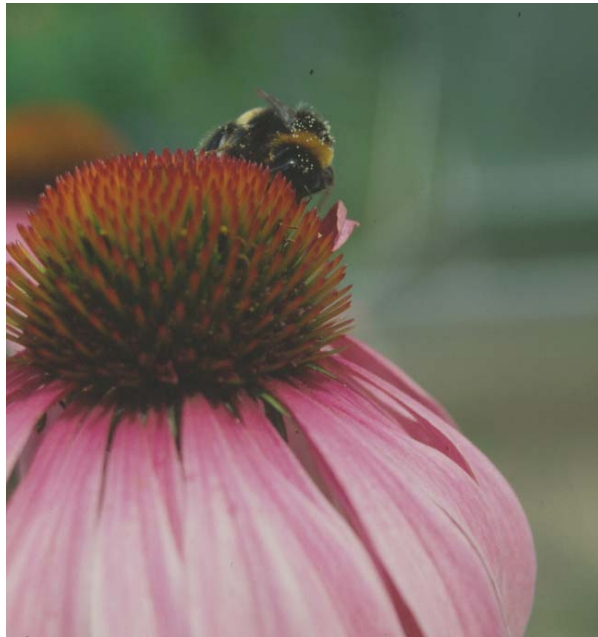
B. terrestris worker foraging on *Echinacea*

Abstract   Size polymorphism is an important life history trait in bumblebees with strong impact on individual behavior and colony organization. Within a colony larger workers tend to serve as foragers, while smaller workers fulfill in-hive tasks. It is often assumed that size-dependent division of labor relates to differences in task performance. In this study I examined size-dependent interindividual variability in foraging, i.e. whether foraging behavior and foraging capability of bumblebee workers is affected by their size. I observed two freely foraging *B. terrestris* colonies and measured i) trip number, ii) trip time, iii) proportion of nectar trips, and iv) nectar foraging rate of different sized foragers. In all observation periods large foragers exhibited a significantly higher foraging rate than small foragers. None of the other three foraging parameters was affected by workers' size. Thus, large foragers contributed disproportionately more to the current nectar influx of their colony. I provide a detailed discussion of the possible proximate mechanisms underlying the differences in foraging rate.

INTRODUCTION

Intra-colonial worker size variation is weak in honeybees and most stingless bee species, but pronounced in bumblebees (Garófalo, 1978; Inouye and Kato, 1992; Knee and Medler, 1965). *Bombus* workers of various sizes are produced throughout the colony life cycle, and body weight differences between small and large workers can reach up to a factor of seven (Cumber, 1949).

The size of a bumblebee worker affects her physiological abilities like thermoregulation (Bishop and Armbruster, 1999; Heinrich and Heinrich, 1983), flight speed (Spaethe, unpublished data; Pyke, 1978), or nectar ingestion rate (Harder, 1983b). Comparing large and small individuals, most investigations reveal a superior performance of larger bees. Larger individuals, for instance, are better adjusted to regulate their body temperature and are therefore able to forage at lower ambient temperatures (Heinrich and Heinrich, 1983).

Body size is also known to affect task preferences of workers. While in bumblebee colonies only weak age-related division of labor occurs (Cameron, 1989), several studies have revealed a correlation between a worker's size and the probability of performing a certain task. Large workers were found to have a higher probability of foraging for nectar and pollen, whereas small workers tended to stay inside the nest and attend to nest duties (Cumber, 1949; Free, 1955b; Garófalo, 1978).

As the survival, growth and reproduction of a colony depend strongly upon the influx of nectar and pollen, selection is expected to maximize energy influx into the colony and result in an efficient allocation of the available workforce to the necessary tasks. This assumption together with the finding that larger bumblebees perform better in several physiological demands during foraging provoked some authors to suggest that size-related division of labor between within-nest tasks and foraging is caused by a superior foraging performance of large workers: "...it is doubtless more economical that larger individuals should forage, and that the smaller ones should do the 'housework'" (Cumber, 1949, p. 16). Although this assertion appears intuitively convincing, I do not know of any investigation which clearly shows that larger workers are more efficient in collecting nectar or pollen, or that smaller workers are better in nursing the brood. Here, I address the question whether workers of different size differ in their foraging success. Although several authors found a positive correlation between a forager's body size and the amount of nectar or pollen she brought back to the nest (Allen *et al.*, 1978; Free, 1955a), no one could rule out that a small forager counterbalanced her smaller loads by completing more shorter collecting trips per time. Thus, in order to evaluate the relative contribution of small and large workers to the colony's food influx, one needs to compare foraging rates (amount of pollen or nectar per time) rather than absolute load sizes of foragers. This is the approach I have taken in the present study.

MATERIAL AND METHODS

Study animals

I studied two colonies of *Bombus terrestris* (Colony A: approximately 100 individuals; colony B: approximately 200 individuals). Both colonies were housed in small wooden boxes (15 * 28 * 11 cm) inside a lab at the bee station of the University of Würzburg, Germany. A Plexiglas tunnel (7 * 45 * 4 cm) between the nest and the outside allowed the bees free outdoor access for foraging.

Data collecting

In each colony, I randomly marked 100 workers with numbered 'Ophalitplättchen'. I collected data during four observation periods, one six-day period in colony A in 1999 (A1, six intermitted days between 11 August and 3 September) and three three-day periods in colony B in 2000 (B2: 07-09 June; B3: 14-16 June; B4: 19-21 June).

In 1999 daily observations lasted from 10:00 to 17:00, in 2000 from 05:00 to 15:00 and from 19:00 to dusk, resulting in a total of 155 hours of observation. For all marked workers I recorded i) departure time, ii) weight at departure, iii) arrival time, iv) weight at arrival, v) and presence of pollen loads. In 1999 I measured the weight of a worker by catching her at the entrance of the tunnel with a small plastic cap while leaving or returning, weighing her on an electric scale (Sartorius BA 61, Göttingen, Germany) and releasing her at the entrance. However, this method did not allow me to record the weights of all marked bees during times of high flight activity. In 2000 I therefore refined the method. I placed the electric scale under a small opening (5 * 3 cm) in the floor of the entrance tunnel, so that all leaving and returning bees walked on the scale while passing through the tunnel. This allowed me to take weight data of every exiting and entering worker while leaving the colony completely undisturbed.

Foraging parameters

All bees returning to the nest without pollen loads were defined as nectar foragers. Bees returning with pollen loads were classified as pollen forager. This group contained individuals which collected pollen only or both, pollen and nectar.

I analyzed the following foraging parameters: The net nectar load of a worker was calculated as the difference between departure weight and arrival weight. The foraging rate of a worker was then computed as the quotient of net nectar load divided by trip time. For each observation period I calculated the mean foraging rate of each worker, including only foragers that performed at least three nectar foraging trips during the observation period. Only trips longer than 10 minutes were considered in order to exclude orientation or dejection flights (Capaldi and Dyer, 1999; Capaldi *et al*., 2000).

For colony B, I also calculated the mean daily trip number and trip time of each worker that performed at least three nectar trips per observation period. Furthermore, for each worker with a minimum of three foraging trips per observation period (irrespective of the kind of collected food) I analyzed the proportion of nectar trips by dividing the number of nectar trips through the number of total foraging trips. The value ranged from 0 (only trips with pollen) to 1 (only nectar trips).

As a measure of size I used the bees' mean body weight at departure. Empty body weight correlates very well with a multitude of morphometric measures like forewing length, head capsule width or intertegular span (Spaethe, unpublished data; Bullock, 1999). Further weight measured at departure seems to be a good estimate of empty body weight because foragers take only very small nectar provisions with them when leaving their colony (Allen *et al.*, 1978).

Data analysis

I tested correlations between each of the measured foraging parameters and body size by a nonparametric test for association (Spearman's rank order test). All p-values above 0.05 were considered as not statistically significant. I treated each group of data obtained from each observation period in colony B independently because only two individuals (2.3 %) foraged in all three periods and more than 80 % of all bees in period B2 and B3 were not observed in any other observation period. Since I performed multiple significance tests on data from colony B I adjusted p-values using the sequential Bonferroni procedure to control for Type I error (Rice, 1989).

RESULTS

Trip time, number of trips and probability of nectar foraging

The first workers started foraging between 05:00 and 05:42 in the morning. Foraging trips of bees which spent the night outside the nest and brought back nectar or pollen very early were excluded from the analysis. The last foragers entered the hive between 20:47 and 21:42, when daylight began to decline. The mean trip number (nectar and pollen trips) in each observation period ranged from 3.3 to 5.8 trips per daily observation time, the mean trip time from 57 to 75 minutes (Table 1).

In all observation periods roughly one-fourth of the observed foragers collected nectar and were never seen with pollen loads (B2: N = 10 (15 %); B3: N = 14 (26 %); B4: N = 14 (29 %)). On average, two thirds of all trips were nectar trips (B2: 59 %; B3: N = 69 %; B4: 71 %; Table 1). Between 9 and 18 % of all foragers in each period collected pollen on more than 75 % of their trips (B2: N = 12 (18 %); B3: N = 5 (9 %); B4: N = 5 (10 %)), but only one or no forager always carried pollen when returning to the colony (B2: N = 1 (2 %); B3: N = 1 (2 %); B4: N =

0). For none of the three parameters (trip time, trip number, and proportion of nectar trips) did I find a significant correlation with body weight.

Table 1. Foraging parameter

Observation period	N	Trip number			Trip time			N	Proportion of nectar trips		
		Mean daily trip number [max]	r_s	p - value	Mean trip time [min; max]	r_s	p - value		Ratio nectar trips / all foraging trips [min; max]	r_s	p - value
B2 (07. - 09.06.)	34	4.8 [10]	-0.10	> 0.05	57 [24; 103]	-0.22	> 0.05	66	0.59 [0;1]	0.17	> 0.05
B3 (14. – 16.06.)	38	3.3 [8]	-0.03	> 0.05	75 [29; 129]	-0.10	> 0.05	53	0.69 [0;1]	-0.10	> 0.05
B4 (19. – 21.06.)	37	5.8 [12]	-0.16	> 0.05	72 [27; 139]	0.04	> 0.05	48	0.71 [0.07;1]	-0.01	> 0.05

Mean daily trip number, mean trip time and proportion of nectar foraging trips of all foragers of colony B with a minimum of three nectar foraging trips per observation period (for the proportion of nectar foraging trips we used foragers with a minimum of three foraging trips). None of the three parameters showed a significant correlation with body size (r_s, Spearman-rank correlation coefficient, all p-values > 0.05).
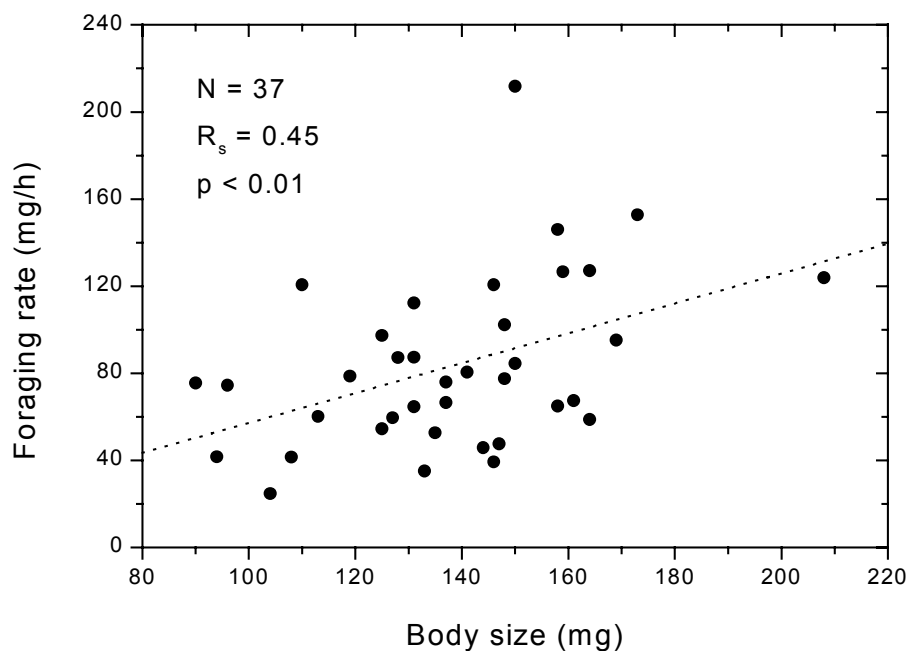
Fig. 1.
Nectar foraging rate as a function of body size (observation period: B4 (19 – 21 June)). Equation for the regression line:
$y = -11.4 + 0.69 * x$.

Nectar foraging rate

In each observation period I analyzed between 30 and 38 foragers with at least 3 nectar foraging trips. The mean foraging rate ranged from 54.9 to 86.9 mg nectar per hour. The mean empty body weight ranged from 138.1 to 149.3 mg (Table 2), the smallest and largest forager differed more than two-fold in weight. In all four observation periods I found a significant correlation between body weight and nectar foraging rate (Fig. 1 and Table 2). Larger foragers had higher nectar returns to the colony per time compared to their smaller nestmates.

Table 2. Foraging parameter

Colony	Observation period	Mean foraging rate (mg*h^{-1}) [min;max]	Mean body weight (mg) [min;max]	N	r_s	p - Value
A	A1 (11.8.-3.9.1999; 6 observation days)	54.9 [1.4; 222.9]	140.4 [98; 210]	30	0.41	**0.02**
B	B2 (7.-9.6.2000)	86.9 [22.6; 215.3]	149.3 [104; 245]	34	0.36	**0.03** *
B	B3 (14.-16.6.2000)	58.3 [16.6; 149.3]	142.1 [96; 213]	38	0.38	**0.02** *
B	B4 (19.-21.6.2000)	83.3 [24.8; 211.8]	138.1 [90; 208]	37	0.45	**0.006** *

Mean nectar foraging rate and mean empty body weight of the foragers in the four observation periods; N, number of observed nectar foragers; r_s, Spearman's rank correlation coefficient for nectar foraging rate and body weight; *, significant correlation after sequential Bonferroni correction for a table-wide ∇-level of 0.05 in colony B.

DISCUSSION

The aim of this study was to test whether bumblebee workers of different size differ in their foraging success. The data reveal a clear size effect on nectar foraging rate. Larger foragers not only return to their colony with larger loads, as has been previously reported (Allen *et al*., 1978; Fisher, 1987), but collect their loads in the same amount of time as their smaller nestmates and thus contribute disproportionately to the total nectar influx of their colony. Thus, the data support the hypothesis that larger foragers are superior in nectar foraging. The results are consistent with findings published in a methodological paper on *B. griseocollis* (Fisher, 1987), the only other study that reports foraging rates of differently sized workers.

Trip time, trip number and probability of pollen foraging

In contrast to foraging rate, the other foraging parameters measured, trip time, trip number and probability of collecting pollen, respectively, were unaffected by forager size. Mean foraging trip times lasted between 57 and 75 minutes (Table 1) and were longer compared to results of most earlier studies (Cartar, 1992: 25 to 30 minutes; Brian, 1952: 18 minutes). This might be due to differences in species and habitats and in applied methods. For example, the observation periods in Brian's study lasted no more than one hour, thus excluding trips longer then one hour (Brian, 1952). Brian also included trip times shorter than 10 minutes which are usually none-foraging flights (see above). Both factors may lead to an underestimation of foraging times. The only other study in which observational methods are comparable to my data reports very similar trip times (Allen *et al.*, 1978). On average, foragers made between 3 and 6 trips per daily observation time (Table 1). Number of trips varied substantially among foragers, but was not affected by forager size. Likewise, I found no correlation between proportion of pollen trips and size (Table 2). This finding is in contrast to earlier observations, which showed that the proportion of pollen trips was higher in larger foragers (Brian, 1952; Free, 1955b, Fisher, 1987). Again, different results might arise from fundamental differences in the life history of the observed species (Cartar, 1992; Heinrich, 1979), from varying observational methods or because colonies were observed at different stages of their life cycle (Cartar, 1992).

Why do large foragers have higher nectar foraging rates?

Which factors are responsible for the size-dependent differences in foraging rate? First of all, flight speed presumably has strong impact on foraging rate. Bumblebees visit several patches with hundreds to thousands of flowers during one foraging trip (Michener, 1974), and flight time between flowers and patches constitutes up to 80 % of total foraging time (Heinrich, 1979). Additionally, the distance between the nest and a forage site can reach 1 to 4 kilometers in *B. terrestris* (Hedtke, 1996; Walther-Hellwig and Frankl, 2000). By increasing flight speed only slightly, a forager can substantially economize her trip time expenditure. When foragers increase their flight speed, their fuel expenditure per mileage does not increase, because for an individual bumblebee the costs of flying remain constant over a wide range of its flight speed (Ellington *et al.*, 1990). Thus larger foragers may have higher foraging rates because they spend less time flying to and between flowers (Pyke, 1978).

A second factor which can vary with size is handling time at flowers. In order to exploit the nectar in a flower the bees first have to reach the nectaries, which is sometimes rendered difficult by a long corolla tube or a narrow entrance, and then ingest the nectar. Larger bees may reach the nectaries more easily and extract the nectar faster because of their longer tongues and stronger sucking related muscles (Harder, 1983b; Winston, 1979). Indeed, several authors discovered that bees with longer tongues spend less probing time per flower (Harder, 1983a; Heinrich, 1979). In a study investigating the distribution of long- and short-tongued bumblebee foragers of *B. vagans* among cow vetch (*Vicia cracca*) flowers, Morse (1978) found a correlation between proboscis length and floret depth of visited flowers but no differences in

handling time (Morse, 1978). However, assuming that larger flowers provide more nectar (Cohen and Shmida, 1993, and citations therein) and that larger bees are able to ingest nectar more rapidly (see above), I would expect larger bees to extract more nectar at each flower per time even when handling times do not differ. Thus, the nectar foraging rate of larger workers may be higher because they spend less time per flower for the same amount of nectar compared to their smaller nestmates.

Finally, an often overlooked factor influencing foraging behavior of bumblebee workers is a size-dependent constraint imposed by the bees' sensory system. In chapter one I could show that bumblebees searching for artificial flowers of varying sizes and colors are strongly constrained by their visual abilities. Small flowers evoked a substantial increase in search time because the spatial resolution of a bee's eye is poor and a small decrease of floral size at a critical value considerably lowers the probability of detecting the flower. I predict that a similar increase in search time will occur when small bees with smaller eyes (and lower spatial resolution, see chapter two) search for a certain floral size. Due to their poorer resolving power they can detect a flower only from a shorter distance than larger bees. Thus, larger foragers may have shorter search times than their smaller nestmates due to their superior ability in detecting flowers, again resulting in higher foraging rates.

In summary, various size-dependent factors are known to influence the foraging abilities of bumblebees and may lead to the observed differences in foraging rates between large and small workers of a colony.

Worker size variation – constraint or adaptation?

The data reveal a clear size effect on nectar foraging rate. Larger foragers contribute disproportionately to the total nectar influx of their colony. Clearly, by allocating its largest workers to foraging a colony will maximize its nectar influx. And indeed, others found that larger workers are more prone to adopt foraging tasks than within-nest duties (Cumber, 1949; Free, 1955b; Garófalo, 1978). So why do bumblebee colonies produce small workers at all?

Some of the factors responsible for worker size variation at the proximate level have been identified. The primary factor seems to be unequal food provisioning during the larval stage (Plowright and Pendrel, 1977; Sutcliffe and Plowright, 1988). Bumblebee nests consist of a more or less irregular conglomerate of egg clumps, larval cells and nectar and pollen cells. The larvae positioned at the border and at the bottom of the nest are visited less often by nurse bees and thus receive less food during their development than central larvae (Sladen, 1912, cited in Cumber, 1949). Further factors may be competition among the larvae from one egg clump (Cumber, 1949; Michener, 1974) and differences in temperature conditions depending on position in the nest.

The ultimate reasons, if any, for the production of workers of different sizes are much harder to assess. Is size variation found among workers of a bumblebee colony an adaptive feature or is it caused by some constraint? In other words, does selection promote behavior which leads to unequal food provisioning of the larvae, or does some unknown constraint prevent bumblebees

from building regular layers of larvae cells, which seems to be a prerequisite for producing monomorphic workers? Here I can only offer some speculative thoughts. One important step in answering this question would be to know whether polymorphism in bumblebees arose secondarily from monomorphism or whether it represents the ancestral state. If the pronounced size polymorphism in *Bombus* is indeed a derived feature, it will be interesting to find out more about the functional significance of a size related division of labor, that is, the benefits of having workers of different sizes under different conditions. To this aim, future studies will need to identify the size related capability of workers at within-nest tasks such as brood care and nest climate control.

Further, it will be important to identify the costs for the colony of producing small vs. large workers and to test whether size polymorphic workers increase colony fitness. The latter can be done by comparing the reproductive output of colonies which are artificially assembled and comprise equally 'expensive' monomorphic and polymorphic worker groups respectively.

In summary, the results show that size affects foraging success in bumblebee workers. How this size dependent interindividual variability relates to colony organization and colony fitness remains to be investigated.

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Summary

Pollinating insects exhibit a complex behavior while foraging for nectar and pollen. Many studies have focused on ultimate mechanisms of this behavior, however, the sensory-perceptual processes that constrain such behavior have rarely been considered. In the present study I used bumblebees (*Bombus terrestris*), an important pollinating insect, to investigate possible sensory constraints on foraging behavior. Additionally, I survey inter-individual variation in the sensory capabilities and behavior of bumblebees caused by the pronounced size polymorphism among members of a single colony.

In the first chapter I have focused on the sensory-perceptual processes that constrain the search for flowers. I measured search time for artificial flowers of various sizes and colors, a key variable defining the value of a prey type in optimal foraging theory. When flowers were large, search times correlate well with the color contrast of the targets with their green foliage-type background, as predicted by a model of color opponent coding using inputs from the bee's UV, blue, and green receptors. Targets which made poor color contrast with their backdrop, such as white, UV-reflecting ones, or red flowers, take longest to detect, even though brightness contrast with the background is pronounced. When searching for small targets, bumblebees change their strategy in several ways. They fly significantly slower and closer to the ground, so increasing the minimum detectable area subtended by an object on the ground. In addition they use a different neuronal channel for flower detection: instead of color contrast, they now employ only the green receptor signal for detection. I related these findings to temporal and spatial limitations of different neuronal channels involved in stimulus detection and recognition.

Bumblebees do not only possess species-specific sensory capacities but they also exhibit inter-individual differences due to size. Therefore, in the next two chapters I have examined size-related effects on the visual and olfactory system of *Bombus terrestris*. Chapter two deals with the effect of scaling on eye architecture and spatial resolving power of workers. Foraging efficiency in bees is strongly affected by proficiency of detecting flowers. Both floral display size and bee spatial vision limit flower detection. In chapter one I have shown that search times for flowers strongly increases with decreasing floral display size. The second factor, bee spatial vision, is mainly limited by two properties of compound eyes: (a) the interommatidial angle $\cap\Sigma$ and (b) the ommatidial acceptance angle $\cap\zeta$. When a pollinator strives to increase the resolving power of its eyes, it is forced to increase both features simultaneously. Bumblebees show a large variation in body size. I found that larger workers with larger eyes possess more ommatidia and larger facet diameters. Large workers with twice the size of small workers (thorax width) have about 50 % more ommatidia, and a 1.5 fold enlarged facet diameter. In a behavioral test, large and small workers were trained to detect the presence of a colored stimulus in a Y-maze apparatus. The stimulus was associated with a sucrose reward and was presented in one arm, the other arm contained neither stimulus nor reward. The minimum visual angle a bee is able to detect was estimated by testing the bee at different stimuli sizes

subtending angles between 30° and 3° on the bee's eye. Minimum visual detection angles range from 3.4° to 7.0° among tested workers. Larger bumblebees are able to detect objects subtending smaller visual angles, i.e. they are able to detect smaller objects than their small conspecifics. Thus morphological and behavioral findings indicate an improved visual system in larger bees.

Beside vision, olfaction is the most important sensory modality while foraging in bees. Bumblebees utilize species-specific odors for detecting and identifying nectar and pollen rich flowers. In chapter three I have investigated the olfactory system of *Bombus terrestris* and the effect of scaling on antennal olfactory sensilla and the first olfactory neuropil in the bumblebee brain, the antennal lobes. I found that the pronounced size polymorphism exhibited by bumblebees also effects their olfactory system. Sensilla number (I measured the most common olfactory sensilla type, s. placodea), sensilla density, volume of antennal lobe neuropil and volume of single identified glomeruli correlate significantly with worker's size. The enlarged volume of the first olfactory neuropil in large individuals is caused by an increase in glomeruli volume and coarse neuropil volume. Additionally, beside an overall increase of brain volume with scaling I found that the olfactory neuropil increases disproportionately compared to a higher order neuropil, the central body. The data predict a higher odor sensitivity in larger bumblebee workers.

In the last chapter I have addressed the question if scaling alters foraging behavior and rate in freely foraging bumblebees. I observed two freely foraging *B. terrestris* colonies and measured i) trip number, ii) trip time, iii) proportion of nectar trips, and iv) nectar foraging rate of different sized foragers. In all observation periods large foragers exhibit a significantly higher foraging rate than small foragers. None of the other three foraging parameters is affected by workers' size. Thus, large foragers contribute disproportionately more to the current nectar influx of their colony.

To summarize, this study shows that understanding the mechanisms of visual information processing and additionally comprising inter-individual differences of sensory capabilities is crucial to interpret foraging behavior of bees.

Zusammenfassung

Blüten bestäubende Insekten zeigen während ihrer Suche nach Nektar und Pollen ein komplexes Sammelverhalten. Bisher wurde eine Vielzahl von Studien durchgeführt um die ultimaten Mechanismen dieses Verhaltens aufzuklären; jedoch die diesem Verhalten zugrundeliegenden sensorischen Leistungen und Limitierungen wurden dabei nur selten berücksichtigt. In der vorliegenden Arbeit habe ich das Sammelverhalten von Hummeln (*Bombus terrestris*) und potentielle, das Verhalten limitierende sensorischen Zwänge untersucht. Zusätzlich konnte ich Unterschiede im sensorischen System individueller Hummeln aufdecken, die durch den ausgeprägten Größenpolymorphismus dieser Tiere verursacht werden.

Im ersten Kapitel habe ich die visuellen Prozesse, die die Suche nach Blüten limitieren betrachtet. Hierfür habe ich die Suchzeiten von Hummeln für künstliche Blüten verschiedener Größe und Farbe in einer Flugarena bestimmt. Bei großen Blüten korrelieren die gemessenen Suchzeiten mit dem Farbkontrast zwischen der Blüte und dem blatt-grünen Hintergrund. Bei Blüten mit geringem Farbkontrast benötigen die Tiere am längsten um sie zu detektieren, obwohl die Blüten einen starken Helligkeitskontrast aufweisen. Diese Ergebnisse stimmen mit den Vorhersagen eines Farbseh-Modells überein, das die Information von den UV-, Blau- und Grünrezeptoren der Hummel verrechnet. Bei der Suche nach kleinen Blüten allerdings ändern die Hummeln ihre Strategie. Sie fliegen jetzt signifikant langsamer und näher am Untergrund um dadurch die Wahrscheinlichkeit zu erhöhen, die Blüten zu detektieren. Zusätzlich benutzen die Hummeln einen anderen neuronalen Kanal für die Blütenerkennung: anstatt des Farbkontrastes nutzen sie jetzt nur noch die Informationen des Grünrezeptors, d.h. den Kontrast zwischen Blüte und Hintergrund, der durch den Grünrezeptor wahrgenommen wird. Ich konnte zeigen, dass der Wechsel zwischen den beiden neuronalen Kanälen durch zeitliche und räumliche Eigenschaften dieser Kanäle verursacht wird.

Die sensorischen Leistungen einer Hummel sind nicht nur durch ihre Artzugehörigkeit festgelegt, sondern weisen beträchtliche Unterschiede zwischen großen und kleinen Tieren auf. In den nächsten zwei Kapiteln habe ich deshalb Größeneffekte auf das visuelle und olfaktorische System von *Bombus terrestris* untersucht. Im zweiten Kapitel beschäftige ich mich mit den Auswirkungen des Größenpolymorphismus auf die Augenmorphologie und das räumliche Auflösungsvermögen von Hummelarbeiterinnen. Das räumliche Auflösungsvermögen des Hummelauges wird hauptsächlich von zwei Faktoren bestimmt: (a) dem Divergenzwinkel zwischen zwei Ommatidienachsen $\cap\Sigma$, und (b) dem Öffnungswinkel eines Ommatidiums $\cap\zeta$. Beide Faktoren sind von der Zahl und dem Durchmesser der vorhandenen Ommatidien in einem Komplexauge beeinflußt. Ich konnte nachweisen, daß sich große und kleine Hummeln stark in der Zahl und dem Durchmesser ihrer Ommatidien unterscheiden. Große Hummeln mit der doppelten Thoraxbreite im Vergleich zu ihren kleinen Nestgenossinnen weisen 50% mehr Ommatidien und einen 1.5-fachen Linsendurchmesser auf. In einem Verhaltensversuch habe ich den kleinsten Sehwinkel, mit dem ein farbiges Objekt von

einer Hummel noch erkannt werden kann bestimmt. Auch hier zeigte sich ein starker Größeneffekt. Um so größer die Hummel ist, um so kleiner ist der Sehwinkel unter dem sie ein Objekt gerade noch wahrnehmen kann. Sowohl morphologische Daten als auch Verhaltensdaten zeigen deutlich, dass größere Hummeln ein besseres visuelles System besitzen. Neben dem Sehen ist der Duft die wichtigste sensorische Modalität, die Hummeln während des Sammelns nutzen. Im nächsten Kapitel habe ich mich daher mit möglichen Größeneffekten auf das olfaktorische System beschäftigt. Ich konnte zeigen, daß die Zahl der wichtigsten olfaktorischen Sensillen auf der Antenne, *Sensilla placodea*, mit zunehmender Körpergröße ansteigt. Das erste olfaktorische Neuropil im Gehirn, die Antennalloben, skalieren ebenfalls mit der Körpergröße. Die Volumenzunahme des Neuropils ist auf eine Volumenzunahme der einzelnen Glomeruli und der Zahl der Interneurone zurückzuführen. Außerdem konnte ich nachweisen, daß das Volumen des olfaktorische Neuropils im Vergleich zu zentralen Hirnregionen überproportional zunimmt. Die Ergebnisse lassen eine höhere Sensitivität des olfaktorischen Systems bei großen Hummeln erwarten.

Im letzten Kapitel habe ich mögliche Auswirkung der Körpergröße auf das Sammelverhalten von Hummeln unter natürlichen Bedingungen untersucht. Ein überlegenes visuelles und olfaktorisches System bei größeren Hummeln läßt eine bessere Blütenerkennung, und damit auch eine höhere Sammeleffizienz vermuten. Hierfür habe ich Nektarsammelraten von verschieden großen Tieren im Freiland bestimmt. Größere Tiere zeigen dabei eine höhere Sammelrate (Nektareintrag pro Zeit) im Vergleich zu ihren kleineren Nestgenossinnen. Größere Tiere tragen damit überproportional zum täglichen Nektarinflux einer Kolonie bei.

Die Ergebnisse dieser Arbeit zeigen deutlich, dass das Sammelverhalten bei Blüten besuchenden Insekten nur dann richtig verstanden und interpretiert werden kann, wenn man die dem Sammeln zugrundeliegenden sensorischen Prozesse und mögliche individuelle Modifikationen kennt und mit einbezieht.

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Appendix
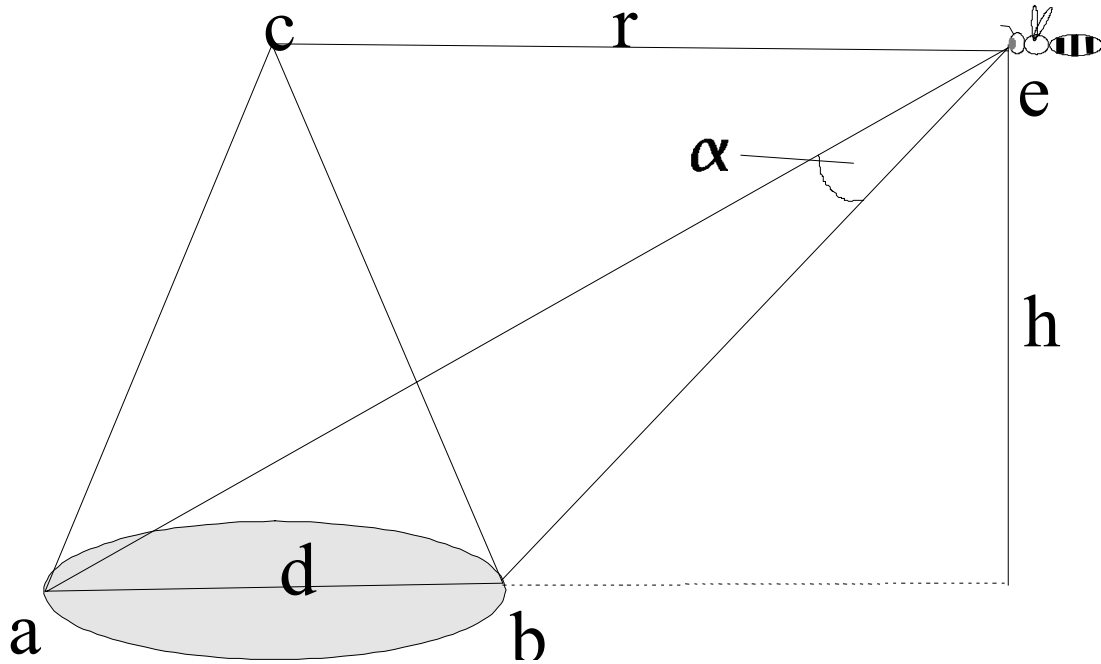
Appendix Figure. This sketch illustrates the geometry necessary to calculate the radius of the *detection area* r, within which a bee flying at a given height h will be able to detect a flower with diameter d, given a resolution of α ≥ 5° (α ≥ 15° for the *color recognition area*).

In the first step we calculate the triangle Δ abe by

$$\frac{dh}{2} = \frac{1}{2}\left[\overline{ae}\right]\left[\overline{be}\right]\sin\alpha \qquad\qquad (i)$$

where

$$\left[\overline{ae}\right] = \sqrt{h^2 + \left(r + \frac{d}{2}\right)^2} \qquad\qquad (ii)$$

and

$$\left[\overline{be}\right] = \sqrt{h^2 + \left(r - \frac{d}{2}\right)^2} \qquad (iii).$$

Now we insert equation (ii) and (iii) in (i):

$$\frac{dh}{2} = \frac{1}{2}\sqrt{h^2 + \left(r + \frac{d}{2}\right)^2} \sqrt{h^2 + \left(r - \frac{d}{2}\right)^2} \sin\alpha \qquad (iv).$$

We square both sides

$$\left(\frac{dh}{\sin\alpha}\right)^2 = \left[h^2 + \left(r + \frac{d}{2}\right)^2\right]\left[h^2 + \left(r - \frac{d}{2}\right)^2\right] = h^4 + 2h^2\left(r^2 + \frac{d^2}{4}\right) + r^4 - r^2\frac{d^2}{2} + \frac{d^4}{16} \qquad (v)$$

and replace r^2 by y:

$$\left(\frac{dh}{\sin\alpha}\right)^2 = h^4 + 2h^2\left(y + \frac{d^2}{4}\right) + y^2 - y\frac{d^2}{2} + \frac{d^4}{16} \qquad (vi).$$

In the next step we solve the quadratic equation for y:

$$y_{1/2} = -\frac{2h^2 - \frac{d^2}{2}}{2} \pm \sqrt{\frac{\left(2h^2 - \frac{d^2}{2}\right)^2}{4} - \left(h^4 + h^2\frac{d^2}{2} + \frac{d^4}{16} - \left(\frac{dh}{\sin\alpha}\right)^2\right)} \qquad (vii).$$

Finally, we calculate r by

$$r = \sqrt{|y|} \qquad (viii).$$

Publications

Full papers

Spaethe, J., and A. Brockmann (2001): Size polymorphism in the olfactory system of bumblebees. *In prep.*

Spaethe, J., and A. Weidenmüller (2001): Size polymorphism and foraging rate in bumblebees. *Submitted to Insectes soc.*

Spaethe, J., J. Tautz and L. Chittka (2001): Visual constraints in foraging bumble bees: flower size and color affect search time and flight behavior. *PNAS* 98 (7): 3898-3903.

Chittka L., **J. Spaethe**, A. Schmidt & A. Hickelsberger (2001): Adaptation, constraint, and chance in the evolution of flower color and pollination color system. In: Chittka, L. & J. D. Thomson (eds) *Cognitive Ecology of Pollination*, pp 106-126. Cambridge University Press, Cambridge.

Nieh, J. C., J. Tautz, **J. Spaethe**, and T. Bartareau (2000): The communication of food location by a primitive stingless bee, *Trigona carbonaria. Zoology* 102: 238-246.

Conference participations

Spaethe, J. and L. Chittka (2001): Interindividual variation of visual resolution in a size polymorphic bumblebee species. International Conference on Invertebrate Vision. Lund, Sweden. (Poster)

Wolf, B., R. Ulrich, **J. Spaethe** & J. Tautz (2001): Impact of motivation, age and body size on the response threshold to sucrose in bumblebees (*Bombus terrestris*). *Apidologie, in press*. (Poster)

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Spaethe, J., J. Tautz and L. Chittka (2000): Foraging economics in bumble bee colonies. TMR Network 1996-2000: Workshop Social Insects as Model Systems. Firenze, Italy. (Poster)

Spaethe, **J.**, J. Tautz and L. Chittka (1999): Foraging behaviour in *Bombus terrestris*: the influence of flower size and flower color on foraging efficiency and flight pattern. TMR Network 1996-2000: Workshop Social Insects as Model Systems. Castleton, UK. (Poster)

Spaethe, **J.**, J. Tautz and L. Chittka (1999): Foraging behaviour in bumble bees: the influence of flower size and flower color on foraging efficiency and flight pattern. Rosenkranz, P. & Garrido, C. (eds). IUSSI Tagung Hohenheim 1999; ISBN 3-901864-01-6. (Talk)

Spaethe, **J.**, J. Tautz und L. Chittka (1999): Der Einfluß von Blütenfarbe und Blütengröße auf die Sammeleffizienz von *Bombus terrestris*. *Apidologie* 30: 461-462. (Poster)

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Book review

A. Weidenmüller, J. Schikora, A. Dornhaus, **J. Spaethe**, C. Kleineidam (1999) in *Anim Behav* 58 (2): 455-456; Cognitive Ecology: The Evolutionary Ecology of Information Processing and Decision Making. Edited by R. Dukas. Chicago: University of Chicago Press (1998).

Curriculum Vitae

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01.12.1968	Geburt in Erlangen (Bayern)
1975 – 1979	Grundschule Erlangen - Frauenaurach
1979 – 1988	Gymnasium Herzogenaurch
Juni 1988	Allgemeine Hochschulreife (Abitur) mit der Gesamtnote 1.9
1988 – 1990	Zivildienst im Alten- und Pflegeheim Roncalli Stift in Erlangen
1990 – 1996	Diplomstudium Biologie an der Julius-Maximilians-Universität Würzburg
09.1996	Diplomprüfung mit der Gesamtnote sehr gut
08/1995 – 09/1996	Dilpomarbeit am Lehrstuhl für Verhaltensphysiologie und Soziobiologie (Zoologie II), betreut von Prof. Dr. J. Tautz: "Ethologische Untersuchungen zum Schweresinn der Honigbiene, *Apis mellifera*".
11 bis 12/1996	Forschungsaufenthalt in Sumatra, Indonesien. Mitarbeit bei Prof. Dr. Erdelen an einer demographischen Studie über den Nilwaran, Blutpython und Netzpython.
01 –07/1997	Wissenschaftlicher Mitarbeiter am Lehrstuhl Zoologie II.
08/1997 bis dato	Promotionsstudium am Lehrstuhl Zoologie II: "Sensory Ecology of Foraging in Bumblebees" betreut von PD Dr. L. Chittka.
02/1999	Forschungsaufenthalt in Queensland, Australien; Freilandstudie mit Prof. J. Tautz und Dr. J. Nieh zur Kommunikation bei stachellosen Bienen.