

Introduction

The dance of the honeybee *Apis mellifera* had been described several times by attentive naturalists dating back to Aristotle, by the time Karl von Frisch dedicated himself to elucidating its enormous complexity and to finally uncovering the meaning of several different components of what he termed the dance language (von Frisch 1965). Ever since then, an unremittingly growing community of scientists has described more and more astounding abilities of this animal. Studies of honeybee communication, foraging and social behaviour as well as their co-evolution with flowering plants have not lost their topicality, but rather provide a basis for combined studies of evolution, ecology, behaviour and neurobiology – to name just those fields that are addressed in my dissertation.

Entomophilous flowers have also been studied extensively since Christian Konrad Sprengel first published his “Uncovered Mystery of Nature in the Construction and Fertilisation of Blossoms” in 1793. He clarified that the two most prominent flower properties – colour and scent – are not presented to please humans, but to attract a great variety of pollinators. Many additional aspects of pollination have since been discovered (Faegri & van der Pijl 1979, Jones and Little 1983, Paulus 1988, Waser and Ollerton 2006). The mutual influences in the plant-pollinator system also roused my interest, especially with regard to floral colours and bee pollinators.

The honeybee has been found exceedingly expedient to the study of many aspects of learning and memory (Menzel and Erber 1978, Gould and Towne 1987, for reviews see: Gould 1984, Menzel 1985, 1990, 2003). Questions that I approached in my dissertation deal with their learning abilities during foraging, i.e. learning about floral colours, reward properties and reliabilities. It is known for a long time that honeybees are able to perceive colours, to discriminate different colours and associate them with a reward (von Frisch 1914, Kühn 1923, Daumer 1956, Menzel 1967). They belong to the group of Apoidea, which are the most important pollinators. They also have the best-studied receiver colour vision system of all insects and thus fulfil an indispensable requirement for an adequate description of colour signals (Menzel and Backhaus 1991, Peitsch *et al.* 1992). Bees differ strongly from humans in their colour perception, because the wavelength range to which they are sensitive is shifted towards the shorter wavelengths. Similarly to humans, they also have three photoreceptor types, but their maximum sensitivities are in the ultraviolet, blue and green parts of the visual

spectrum. Therefore, one has to design behavioural experiments to examine the bees' colour perception and its influence on foraging and communication behaviour or one has to use adequate models of bee colour vision to quantify perceptual similarities of colours (Vorobyev *et al.* 2001). I applied both approaches during my studies.

During foraging, bees develop a reward expectation, which is predicted through the related signal (Menzel 1985). Associations between different colour and reward properties can be learned and compared in terms of efficiency and profitability, which usually leads to a temporary preference for one signal. This phenomenon applied to the conditions in the bee's natural environment is called floral constancy (von Frisch 1927, Grant 1950, Goulson 1999). But foraging decisions are based on many additional aspects, e.g. distance to the food source, abundance of single sources, competition and requirements of the colony (Heinrich *et al.* 1977, Hill *et al.* 1997, for a review see: Chittka *et al.* 1999). Profitable food sources are communicated to nestmates inside the hive via the dance behaviour (von Frisch 1965, Waddington 1982, 2001, Seeley 1995, 2000), which shows that bees are able to estimate, among other things, the flow rate of the reward as well as the time invested by the forager to obtain the reward (Varjú and Núñez 1991). Fülöp and Menzel (2000) showed that honeybees show risk-indifferent foraging behaviour, but so far it could not be shown that this behavioural trait affects dance communication.

Following up on these ideas, I addressed the question of whether honeybees are able to estimate reward reliability independently of energy gain or loss during their foraging bout, and whether they are able to adapt their dance behaviour according to this evaluation of perceived profitability (Chapter I). This experimental work was performed by means of a flight arena containing artificial coloured targets whose actual and perceived profitabilities could be manipulated under controlled conditions. The advantage of these laboratory conditions compared to field conditions is that the motivational status of the animal can be held constant and sensory cues can be substantially reduced to focus on only one aspect of profitability. The results would play a role in the comprehension of bee behaviour, especially with respect to foraging strategies, dance communication and cognitive abilities of honeybees. On the other hand, I was interested in the effects that floral constancy may have on flower colour distribution in the natural environment. Flowering plants require their pollinators' floral constancy to obtain efficient pollination. It is believed that during the process of co-evolution, plants adapted their floral colouration to the visual properties of the pollinators, while many pollinators' colour vision has indeed evolved such that they are able to discriminate flowers well (Chittka *et al.* 1994, Vorobyev and Menzel 1999). Bees, moreover,

are able to perceive many additional colours besides those found in floral displays (Vorobyev and Menzel 1999). The extent to which pollinators influenced flower colour evolution, is still a controversial discussion. Conclusions from different authors range from opinions that pollinators' preferences are too broad to effectuate floral characteristics (Waser *et al.* 1996, Chittka *et al.* 1999) to likelihoods for the adaptive divergence of floral traits through pollinator-mediated selection (Schemske and Bradshaw 1999, Gumbert and Kunze 2001, Gigord *et al.* 2002). In Chapter II and III, I present my contribution to the ongoing discussion. I looked at the distribution of flower colours and reward properties in simultaneously flowering species. I investigate whether a non-random distribution of colours in highly rewarding plant species in the natural environment might be the reason for the formation of innate colour preferences and/or for differences in learning speed of different colours in honeybees and bumblebees (Menzel 1967, 1968, 1969, Giurfa *et al.* 1995, Gumbert 2000). I also compared plant species that display similar or indistinguishable colours on the basis of their reward properties to find out whether generalisation of colours during foraging is a rewarding and reliable foraging strategy. Special emphasis was placed on the colour analysis as perceived by honeybees by using the Receptor Noise Limited Model of honeybee colour vision (Vorobyev *et al.* 2001). Additionally, I investigated influences of further floral properties, e.g. floral abundance, display size and functional flower shape, on the foraging decisions of bee pollinators to achieve an integrated picture of the complex interactions in undisturbed plant-pollinator communities.

Efficient pollination, as required by entomophilous plants, is facilitated, when flowers are not only reliable, but also easily detected by the pollinator. Most studies thus far analysed the relationship between floral display size and detection or visitation rates (individual level: Robertson and Macnair 1995, Giurfa *et al.* 1996, Stout 2000, Spaethe *et al.* 2001; species level: Biernaskie and Cartar 2004, Grindeland *et al.* 2005, Stang *et al.* 2006). But the enlargement of the single floral display during co-evolution of plants and pollinators is not the only possibility. Alternatively, some species evolved inflorescences that consist of several single flowers, thereby also enlarging the coloured area. As a result, they may be more conspicuous to an insect. However, it is not known how spatial patterning of flower and background colours in complex plant displays affects the insect's perception of targets.

The visual system of the honeybee consists of two different parts coding chromatic and achromatic aspects of visual information (Brandt and Vorobyev 1996, Giurfa *et al.* 1996, 1997, Giurfa and Vorobyev 1998, Hempel de Ibarra *et al.* 2001, 2002, Niggebrügge and Hempel de Ibarra 2003). Chromatic vision is achieved by colour-opponent computation of

receptor signals and is used by honeybees for the detection and discrimination of coloured targets which are close to the bee. Achromatic vision is mediated only by one single receptor type or the summation of receptor responses. The detectability of targets with small angular subtenses depends on the Long wavelength sensitive (L)-receptor contrast between target and background or within the target (Lehrer & Bischoff 1995, Hempel de Ibarra et al. 2001). The proposed processing of L-receptor mediated achromatic information by neurons that have a centre-surround organisation (Hempel de Ibarra et al. 2001) satisfactorily explain the fact that the detectability of targets is enhanced when stimuli have enhanced edges while it is impaired for targets with blurred edges.

I addressed the question of how spatial cues interact with target detection if single-coloured targets are grouped. I aimed to find out whether the detectability of patterns is related to the amount and density of borders, rather than to the area of the target (Chapter IV). The experiment was carried out under laboratory conditions with honeybees and bumblebees that were presented with targets with either an L-receptor contrast to the background or not. The obtained results were then considered when designing measurements of display sizes of flowers presented in Chapter II.

Yet another way, in which plants attract pollinators, is via the sharing of signals with other plants that are successfully visited owing to their profitability – a phenomenon known as flower mimicry of Müllerian type (Dafni 1984, 1986, Roy and Widmer 1999). Müllerian mimicry is mutualistic and involves two or more species with rewarding flowers, which may benefit from sharing a common advertising display. In this case, improper pollen transfer might be avoided by reaching a higher combined flower density (Rathcke 1983, Waser 1983). Müllerian mimicry occurs in contrast to the strategy of diversifying as widely as possible from co-flowering plants in order to ensure correct pollination through bees' floral constancy (Waser 1983). In Chapter V, the adaptiveness of Müllerian similarity between species is demonstrated for the first time by means of two species that belong to families in which flower mimicry had not been suspected before: *Sphaeralcea cordobensis* (Malvaceae) and *Turnera sidoides* ssp. *pinnatifida* (Turneraceae).

The diversified approaches combined in my dissertation range from controlled behavioural experiments to observational studies under natural conditions. I integrated physical characterisations of signal properties into my interpretation of the sensory physiology of the bee pollinators to be able to judge their behavioural impact on the appearance and ecology of plants. In this way, I hope to give the reader a holistic insight into the complexity of the evolution of the plant-pollinator system.

References

- Biernaskie JM, Cartar RV** (2004) Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Functional Ecology* 18: 125-129
- Brandt R, Vorobyev M** (1996) Metric Analysis of Threshold Spectral Sensitivity in the Honeybee. *Vision Research* 37:425-439
- Chittka L, Vorobyev M, Shmida A, Menzel R** (1994) Bee colour vision – the optimal system for the discrimination of flower colours with three spectral photoreceptor types? In: *Sensory Systems of Arthropods* (eds. Wiese K, Gribakin SG, Popov AV, Renninger G) Birkhauser Verlag, Berlin pp. 211-218
- Chittka L, Thompson JD, Waser NM** (1999) Flower Constancy, Insect Psychology and Plant Evolution. *Naturwissenschaften* 86: 361-377
- Dafni A** (1984) Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* 15: 259-278
- Dafni A** (1986) Floral mimicry – mutualism and unidirectional exploitation of insects by plants. In: *The Plant Surface and Insects* (eds. Southwood TRE, Juniper BE), Edward Arnold, London, pp. 81-90
- Daumer K** (1956) Reizmetrische Untersuchung des Farbensehens der Bienen. *Zeitschrift für vergleichende Physiologie* 38: 413-478
- Faegri K, van der Pijl L** (1979) The principles of pollination ecology. Pergamon Press, Oxford, UK
- Fülöp A, Menzel R** (2000) Risk-indifferent foraging behaviour in honeybees. *Animal Behaviour* 60: 657-666
- Giurfa M, Vorobyev M** (1998) The angular range of achromatic target detection by honey bees. *Journal of Comparative Physiology A* 183: 101-110
- Giurfa M, Núñez J, Chittka L, Menzel R** (1995) Colour preferences of flower-naive honeybees. *Journal of Comparative Physiology A* 177: 247-259
- Giurfa M, Vorobyev M, Kevan P, Menzel R** (1996) Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *Journal of Comparative Physiology A* 178: 699-709
- Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R** (1997) Discrimination of coloured stimuli in honeybees: alternative use of achromatic and chromatic signals. *Journal of Comparative Physiology A* 180: 253-243.
- Grindeland JM, Sletvold N, Ims RA** (2005) Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Functional Ecology* 19: 383-390
- Gould JL** (1984) Natural history of honey bee learning. In: *The Biology of Learning* (ed. Marler P, Terrace HS) Springer Verlag, Berlin, pp. 149-180
- Gould JL, Towne WF** (1987) Honey bee learning. *Advances in Insect Physiology* 20: 55-75
- Goulson V** (1999) Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. *Perspectives in Plant Ecology, Evolution and Systematics* 2(2): 185-209
- Grant V** (1950) The flower constancy of bees. *The Botanical Review* 16: 379-398
- Gumbert A** (2000) Color choices by bumblebees (*Bombus terrestris*): innate preferences and generalization after learning. *Behavioural Ecology and Sociobiology* 48: 36-43
- Heinrich B, Mudge PR, Deringis PG** (1977) Laboratory analysis of flower constancy in foraging bumblebees: *Bombus ternarius* and *B. terricola*. *Behavioral Ecology and Sociobiology* 2(3): 247-265

- Hempel de Ibarra N, Giurfa M, Vorobyev M** (2001) Detection of coloured patterns by honeybees through chromatic and achromatic cues. *Journal of Insect Physiology* A 187: 215-224
- Hill PSM, Wells PH, Wells H** (1997) Spontaneous flower constancy and learning in honey bees as a function of colour. *Animal Behaviour* 54(3): 615-627 (13)
- Jones CE, Little RJ** (1983) Handbook of Experimental Pollination Biology. Scientific and Academic Editions, New York, pp. 558
- Kühn A** (1923) Versuche über das Unterscheidungsvermögen der Bienen und Fische für Spektrallichter. *Nachrichten der Gesellschaft für Wissenschaften* 1: 66-71
- Lehrer M, Bischof S** (1995) Detection of Model Flowers by Honeybees: The Role of Chromatic and Achromatic Contrast. *Naturwissenschaften* 82: 145-147
- Menzel R** (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifera*). *Zeitschrift für vergleichende Physiologie* 56: 22-62.
- Menzel R** (1968) Das Gedächtnis der Honigbiene für Spektralfarben. I. Kurzzeitiges und Langzeitiges Behalten. *Zeitschrift für vergleichende Physiologie* 60: 82-102.
- Menzel R** (1969) Das Gedächtnis der Honigbiene für Spektralfarben. II. Umlernen und Mehrfachlernen. *Zeitschrift für vergleichende Physiologie* 63: 290-309.
- Menzel R** (1979) Spectral sensitivity and colour vision in invertebrates. In: *Handbook of Sensory Physiology, Invertebrate photoreceptors* (ed. Autrum H) Springer Verlag, Berlin, Heidelberg, New York, pp. 503-580
- Menzel R** (1985) Learning in honeybees in an ecological and behavioural context. In: *Experimental Behavioural Ecology* (eds. Hölldobler B, Lindauer M) Fischer, Stuttgart pp. 55-74
- Menzel R** (1990) Learning, memory and 'cognition' in honey bees. In: *Neurobiology of Comparative Cognition* (eds. Kesner RP, Olton DS) Lawrence Erlbaum, Hillsdale, New Jersey, pp. 237-292
- Menzel R** (2003) Invertebrate Learning: Associative Learning and Memory Processing in Bees. In: *Learning & Memory*, The MacMillan Psychology Reference Series, Second Edition (ed. Byrne JH) MacMillan, London, pp. 273-277
- Menzel R, Backhaus W** (1991) Colour vision in insects. In: *Vision and visual dysfunction: The perception of colour* (ed. Gouras P) MacMillan, London, pp. 262-288
- Menzel R, Erber J** (1978) Learning and memory in honey bees. *Scientific American* 239: 102-111
- Paulus HF** (1988) Co-evolution and unilateral adaptations in flower-pollinator systems: pollinators as pacemakers in the evolution of flowers. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 81: 25-46
- Peitsch D, Fietz A, Hertel H, de Souza V, Ventura DF, Menzel R** (1992) The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology* A 170: 23-40
- Rathcke B** (1983) Competition and facilitation among plants for pollination. In: *Pollination Biology* (ed. Real L), Academic Press, New York, pp. 305-338
- Robertson AW, Macnair MR** (1995) The effects of floral display size on pollinator service to individual flowers of *Myosotis* and *Mimulus*. *Oikos* 72: 106-114
- Roy B, Widmer A** (1999) Floral mimicry. A fascinating yet poorly understood phenomenon. *Trends in Plant Science* 418: 325-330
- Schemske DW, Bradshaw HD** (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *PNAS* 96(21): 11910-11915
- Seeley TD** (1995) The Wisdom of the Hive. The Social Physiology of Honey Bee Colonies. Harvard University Press., Cambridge, Massachusetts, London, England

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- Seeley TD** (2000) Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *Journal of Comparative Physiology A* 186: 813-819
- Spaethe J, Chittka L** (2003) Interindividual variation of eye optics and single object resolution in bumblebees. *Journal of Experimental Biology* 206: 3447-3453
- Spaethe J, Tautz J, Chittka L** (2001) Visual constraints in foraging bumblebees: Flower size and colour affect search time and flight behaviour. *PNAS* 98(7): 3898-3903
- Sprengel CK** (1793) Das entdeckte Geheimnis der Natur im Bau und der Befruchtung der Blumen. Friedrich Vieweg, Berlin
- Stang M, Klinkhamer PGL, van der Meijden E** (2006) Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. *Oikos* 112: 111-121
- Stout JC** (2000) Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). *Apidologie* 31: 129-139
- Varjú D, Núñez J** (1991) What do foraging honeybees optimize? *Journal of Comparative Physiology A* 169: 729-736
- von Frisch K** (1914) Der Farben und Formensinn der Bienen. *Zoologische Jahrbücher (Physiologie)* 35: 1-188
- von Frisch K** (1965) Tanzsprache und Orientierung der Bienen. Springer Verlag, Berlin, Heidelberg
- Vorobyev M, Menzel R** (1999) Flower advertisement for insects: Bees, a case study. In: *Adaptive Mechanisms in the Ecology of Vision*. (eds. Archer SN *et al.*) Kluwer Academic Publishers, GB, pp. 537-553
- Vorobyev M, Brandt R, Peitsch D, Laughlin SB, Menzel R** (2001) Colour thresholds and receptor noise: behaviour and physiology compared. *Vision Research* 41: 639-653
- Waddington KD** (1982) Honey Bee Foraging Profitability and Round Dance Correlates. *Journal of Comparative Physiology A* 148: 297-301
- Waddington KD** (2001) Subjective evaluation and choice behaviour by nectar- and pollen-collecting bees. In: *Cognitive ecology of pollination*. (eds. Chittka L, Thompson JD) Cambridge University Press, pp. 41-50
- Waser NM** (1983) Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: *Handbook of Experimental Pollination Biology* (eds. Jones CE, Little RJ) Scientific and Academic Editions, New York, pp. 277-293
- Waser NM, Ollerton J** (2006) Plant-Pollinator Interactions From Specialization to Generalization. University of Chicago Press, pp. 488
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J** (1996) Generalization in pollination systems, and why it matters. *Ecology* 77: 1043-1060