

Recruits of the stingless bee *Scaptotrigona pectoralis* learn food odors from the nest atmosphere

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Received: 13 January 2010 / Revised: 26 February 2010 / Accepted: 27 February 2010 / Published online: 1 April 2010
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Abstract The ability to learn food odors inside the nest and to associate them with food sources in the field is of essential importance for the recruitment of nestmates in social bees. We investigated odor learning by workers within the hive and the influence of these odors on their food choice in the field in the stingless bee *Scaptotrigona pectoralis*. During the experiments, recruited bees had to choose between two feeders, one with an odor that was present inside the nest during the recruitment process, and one with an unknown odor. In all experiments with different odor combinations (linalool/phenylacetaldehyde, geraniol/eugenol) a significant majority of bees visited the feeder with the odor they had experienced in their nest (χ^2 -tests; $p < 0.05$). By contrast, the bees showed no preference for one of two feeders when they were either baited with the same odor (linalool) or contained no odor. Our results clearly show that naïve workers of *S. pectoralis* can learn the odor of a food source during the recruitment process from the nest atmosphere and that their subsequent food search in the field is influenced by the learned odor.

Keywords *Scaptotrigona pectoralis* · Stingless bee · Recruitment · Food odor · Odor learning

Introduction

Social insects have developed various kinds of communication mechanisms, which allow them to effectively allocate workers to the various tasks they need to carry out. This is especially true for species living in large colonies, such as stingless bees, the nests of which can contain from a few dozen to 100,000 or more workers (Michener 2000). The exchange of information about the availability or location of food sources, or both, between the individuals of a colony is essential for an effective food collection, which is needed to guarantee a sufficient supply of nourishment for all of its members (Wilson 1971). Workers of stingless bees inform their nestmates about the presence of food and, in several species, about its location, using different modes of communication, such as thorax vibrations and jostling exhibited within the nest, or footprint secretions and pheromone marks deposited in the field (Lindauer and Kerr 1958, 1960; Kerr et al. 1963; Kerr 1969; Jarau et al. 2003; Nieh 2004; Barth et al. 2008; Hrcir 2009; Jarau 2009). In addition, the ability of workers of social bees to learn food odors plays an important role for a quick location of food sources in the field. Most flowers emit a particular odor bouquet, which allows their visitors, such as bees, to find them and to distinguish between different plant species, as well as to locate nectar or pollen within a flower (Barth 1991). Flower visitors, on the other hand, have a well-developed olfactory sense. The honey bee, for example, which is the best studied of all social bees, can discriminate between hundreds of components with exquisite sensitivity and specificity (Reinhard and Srinivasan 2009).

Workers of honey bees (von Frisch 1965), bumble bees (Dornhaus and Chittka 1999, 2004), and stingless bees (Lindauer 1956; Aguilar 2004) learn the odor of a food

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source, which clings to returned foragers, during the recruitment process within the hive and use this information when subsequently searching for food in the field. In *Apis mellifera*, food-receiving bees can associate an odor with food after a single food exchange with a foraging bee, which demonstrates the importance of trophallaxis for the transfer of information about food availability and quality (Gil and De Marco 2005; Grüter et al. 2006; Farina et al. 2007; Farina and Grüter 2009). Recently, Mc Cabe et al. (2007) found that the stingless bee *Melipona quadrifasciata* and the Africanized honey bee (*A. mellifera*) can associatively learn odors under laboratory conditions applying the proboscis extension response (PER) paradigm (see, e.g., Menzel and Müller 1996), whereas another stingless bee, *Scaptotrigona* aff. *depilis*, cannot. These differences are probably due to the fact that the PER protocol for *Apis* was not perfectly suited for small stingless bees, as was recently shown for large bumble bees (Toda et al. 2009). However, the ability to learn and to distinguish between different flower odors may also depend on the foraging and recruitment strategy of a particular bee species (Mc Cabe et al. 2007). For example, foragers of *Scaptotrigona* aff. *depilis* use scent marks to guide nest mates to new food sources (Schmidt et al. 2003). Therefore, the communication of food odors may be less important to them as compared to species that do not guide newly recruited bees to a certain location, such as *Melipona* spp. Nevertheless, for short-range orientation towards a food source, most likely all bees use their olfactory sense.

In the present study we investigated (1) whether workers of *Scaptotrigona pectoralis*, a species that applies scent trails to recruit workers to food (Hemmeter 2008, cited in Jarau 2009), learn floral odors within the hive and (2) whether they use learned odor information to find rewarding food sources in the field. To answer these questions, recruited bees had to choose between two feeders, one with an odor that was present in the nest atmosphere during the recruitment process and the other with an unknown odor.

Material and methods

Study site and bee colonies All experiments were carried out between October 2007 and May 2008 in the bee garden of the *Center for Tropical Bee Research* (CINAT) of the National University in Heredia, Costa Rica with two colonies of *S. pectoralis* (Dalla Torre 1896), which were housed in wooden boxes. A small wooden box (10×10×4 cm; henceforth named “recruitment box”) was installed in front of the entrance to each colony. The exchange of food between foraging bees and bees within the hive mainly took place in these boxes, where the different odors tested in the experiments were presented, too (see below). The experi-

ments were always carried out between 9 and 12 am, because in the afternoon the recruitment behavior usually decreased considerably. The entrance of the colony that was not used for an experiment on a given day was always closed on the preceding evening to ensure that no foragers other than from the tested colony reached the feeders.

Training period For each experiment, three color-marked bees were trained to a training feeder containing unscented 2 M sucrose solution following the training procedure described in Jarau et al. (2000). As feeders, we used laboratory glass dishes (25 mm high, 40 mm in diameter) placed upside down on hexagonal plexiglass plates (75 mm in diameter) with 16 radially arranged grooves allowing the bees to take up the sugar water. The training food source was presented on a wooden platform (10×7.5×2.5 cm) mounted on an iron rack (height, 62.5 cm) and placed at a distance of 5 m from the hive.

Test period During the test periods, which always lasted for 40 min, the training feeder was replaced by two identical test feeders, which both contained the same, unscented 2 M sucrose solution. The two feeders were placed 15 cm to the right and to the left of the training feeder’s original location (total distance between the test feeders =30 cm). Each of these feeders was baited with a different odor by placing a 1 cm² filter paper impregnated with 2 µl pure synthetic compounds on top of it. One of the two odors was simultaneously presented within the recruitment box. The following pairs of odors were tested: linalool/phenylacetaldehyde and eugenol/geraniol (Sigma-Aldrich®). We choose these odors because they are well known from natural flower fragrances (Knudsen et al. 1993). Seven experiments per odor combination and colony were carried out with each of the odors presented in the recruitment box (56 experiments in total). As controls we conducted (1) six experiments per colony with both test feeders bearing the same odor (linalool) and (2) six experiments per colony with two unscented feeders. During the control experiments, (1) linalool or (2) no scent was presented in the recruitment box, respectively.

As soon as all three trained bees arrived at the training feeder, the test odor was presented in the recruitment box (1 µl synthetic compound on a 1 cm² filter paper, left in the box during the entire trial). Five minutes after its application, the experiment was started by replacing the training feeder by the test feeders. During the subsequent test, every newly recruited bee that landed and took up sugar solution at one of the two feeders was immediately captured, marked with green color (water based) on the thorax and released only after an experiment was finished. The number of new bees at each feeder was registered. Every 5 min, the test feeders were replaced by clean ones and the sides of the odors were exchanged in order to

prevent a potential influence of side bias. Bees marked from previous experiments were captured but not counted during later trials to ensure that only the decisions of feeder inexperienced bees were analyzed. After an experiment ended we waited for at least 1 h (much longer for most of the experiments) to allow the test odor to evaporate before starting another experiment.

Statistics In sum, 488 individual bees were tested. The data obtained for the experiments with a particular odor combination did not differ between the two nests (2×2 χ^2 -analysis of contingency tables; all p values >0.05). We therefore pooled them for subsequent analyses. We checked for differences in the distribution of newly recruited bees (actual numbers of participating bees) at the two test feeders by means of χ^2 -tests for each odor combination and for the control experiments, respectively.

Results

During an experiment, the trained foragers regularly switched between the test feeders and never showed a noticeable preference for one of the two odors. We observed that most of the recruited bees were guided by an experienced worker to the feeders' general location by means of piloting. However, in contrast to the trained workers, which quickly landed on one of the test feeders, the newly recruited bees hovered between the two feeders for several seconds before they chose one of them. They then landed on a feeder irrespective of whether an experienced worker was present or not. Rather, their choice was influenced by the feeders' odors, which is reflected by the fact that the recruited bees clearly preferred the feeder with the odor that was simultaneously presented in the recruitment box in the tests with both odor combinations.

When linalool was present in the nest atmosphere, a significant majority (69.6%) of the recruited bees chose the test feeder with this odor over the phenylacetaldehyde scented feeder ($\chi^2=19.21$, $df=1$, $p<0,001$, $n=125$; Fig. 1). An even higher percentage of recruits chose the feeder scented with phenylacetaldehyde (84.0 %) when this odor was present in the recruitment box ($\chi^2=14$, $df=1$, $p<0,001$, $n=56$; Fig. 1). During the presentations of geraniol and eugenol in the nest atmosphere, recruited bees preferred the feeder with the same scent as that presented in the nest, too (geraniol, 71.4%, $\chi^2=12.19$, $df=1$, $p<0.001$, $n=69$; eugenol, 65%, $\chi^2=9.51$, $df=1$, $p<0.01$, $n=100$; Fig. 2). By contrast, no significant differences could be found in regard to the distribution of newly recruited bees at the test feeders during the control experiments (Fig. 3). When no odor was used for the tests 48.1% and 51.9% of the recruits visited one of the two feeders ($\chi^2=1.33$, $df=1$, $p>0.05$, $n=108$).

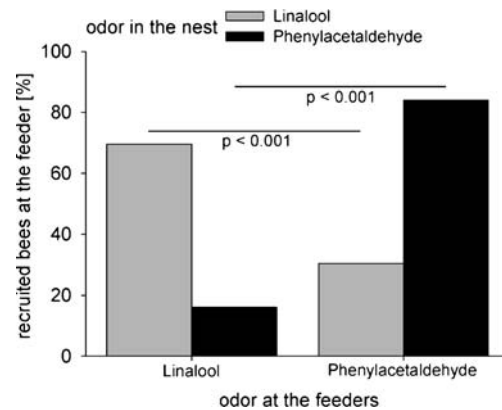


Fig. 1 Relative distribution of the recruited bees that had to choose between a pair of feeders, one baited with linalool and the other with phenylacetaldehyde. One of the odors was simultaneously present in the nest atmosphere. Significance levels (p values) for differences in the distribution of bees were calculated by means of χ^2 -tests

The distribution of bees that landed on the test feeders when both were scented with linalool was 53.3% and 46.7% ($\chi^2=0.26$, $df=1$, $p>0.05$, $n=30$).

Discussion

Our experiments clearly show (1) that recruited bees of *S. pectoralis* learn food odors inside the hive during the recruitment process and (2) that odor learning inside the nest subsequently influences their foraging decisions in the field. These results are in accord with findings in honey bees (Gil and De Marco 2005; Arenas et al. 2007; Farina et al. 2007), bumble bees (Dornhaus and Chittka 1999, 2004; Molet et al. 2009), and other species of stingless bees (Lindauer and Kerr 1960; Aguilar et al. 2005). Lindauer and Kerr (1960) had

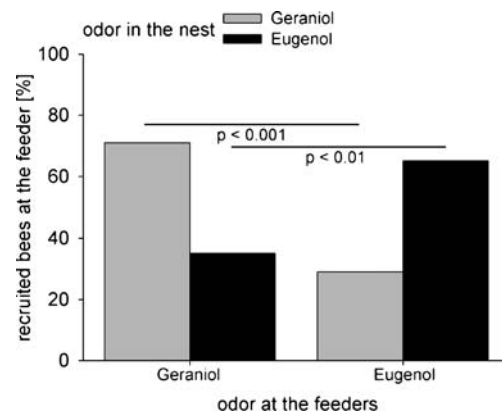


Fig. 2 Relative distribution of the recruited bees that had to choose between a pair of feeders, one baited with geraniol and the other with eugenol. One of the odors was simultaneously present in the nest atmosphere. Significance levels (p values) for differences in the distribution of bees were calculated by means of χ^2 -tests

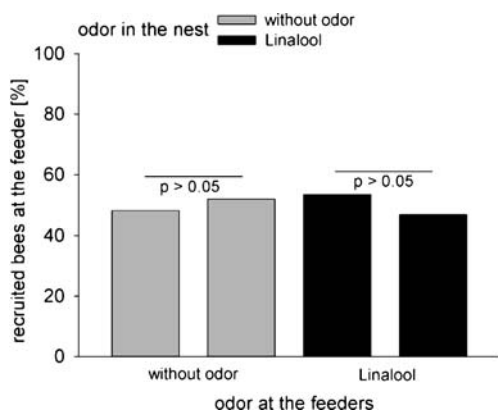


Fig. 3 Relative distribution of the recruited bees that had to choose between two unscented feeders or between two linalool scented feeders. Significance levels (p values) for differences in the distribution of bees were calculated by means of χ^2 -tests

already suggested that foraging bees likely provide information about visited flowers to other workers through the scent adhering to their hairs or mouthparts. Aguilar et al. (2005) found that recruited bees of *Trigona corvina*, *Plebeia tica*, and *Tetragonisca angustula* were less likely to discover feeders that were scented with a different odor than the feeder visited by the recruiting foragers in the field. Importantly, however, the odor presented in the recruitment area in our experiments was not directly associated with the food itself or with the foraging bees, but was only present in the nest atmosphere. Recently, Molet et al. (2009) showed that scented air (anise scent) within the hive was sufficient for odor learning in bumble bees (*Bombus terrestris dalmatinus*). Similar to *S. pectoralis*, bumble bee workers showed a preference for artificial feeders that were baited with the odor they had previously learned. Our experiments and the study by Molet et al. (2009) thus demonstrate that the odor does not need to be transmitted directly from one bee to another.

We suggest two possible ways of how odors in the nest atmosphere could have been learned by the bees in our experiments. (1) Recruited workers probably learned the odor during trophallactic contacts with foragers that returned from the field in an associative manner (see Menzel and Müller 1996; Menzel 1999). If this was the case, the scented air could be considered as conditioned stimulus, the sucrose solution as unconditioned stimulus, and the transferred food samples as reward. (2) Alternatively, preferences for a feeder could have been influenced by “familiarization” (Molet et al. 2009) with the odor present inside the nest, which was, later on, available in a different context in the field. This would mean that the bees were attracted by the odor in the field without “knowing” that the odor indicates food (no direct association between odor and food). It is possible that associative and non-associative processes are involved in odor learning during

the recruitment of nestmates within the nest. To clarify this question, more detailed observations of the interactions between recruiting foragers and recruits have to be carried out. In particular, it would be important to observe whether the recruited bees, which show up at the food sources, have trophallactic contacts with the foragers within the nest and whether they receive food samples.

In our experiments the bees showed a stronger preference for phenylacetaldehyde over linalool and for geraniol over eugenol than the other way round. Recently, Mc Cabe and Farina (2009) found that *M. quadrifasciata*, too, showed a better ability to discriminate between phenylacetaldehyde and linalool in experiments applying classical conditioning of proboscis extension response, when phenylacetaldehyde was used as rewarded stimulus. An interesting question, thus, is where such preferences for a particular odor come from. Phenylacetaldehyde and geraniol may be components that are contained in odor bouquets occurring in the bees’ natural flower market. The workers inside the hive could have been in contact with the odors before our experiments, e.g., from stored honey or pollen, which could have influenced their preferences in the field. To answer this question, however, a more profound knowledge about the plants visited by *S. pectoralis* and about their respective odor bouquets is needed. Unfortunately, this information is not available to date.

An alternative explanation for the bees’ preference for particular odors could be a genetically determined one. For example, several bumble bee species show an innate preference for violet–blue flowers, which indicates a phylogenetically ancient adaptation (Raine and Chittka 2009). This preference appears to be advantageous, since violet and blue flowers contain high nectar rewards in a variety of habitats throughout the distribution range of these bumble bee species (Raine and Chittka 2009). Furthermore, Praz et al. (2008) found that the oligolectic bee species, *Heriades truncorum*, shows a preference for pollen of its host plant even if they never had contact with it before. Therefore, the authors suggest a genetic basis for the host plant recognition in *H. truncorum*. Unfortunately, nothing is known about innate preferences for specific floral odors in stingless bees and their possible relation to the flowers they visit in nature.

Apart from following scent trails, “guiding flights”, which are known from several species of stingless bees (Lindauer and Kerr 1958, 1960; Aguilar et al. 2005), seem to play an important role in the recruitment behavior of *S. pectoralis*. During our experiments, we frequently observed that the newly recruited bees followed an experienced (trained) bee to the feeders’ general location. For close-range orientation towards a specific feeder, however, they apparently used the scent they had previously experienced inside their nest rather than being affected by local enhancement or inhibition (Slaa and Hughes 2009) caused

by experienced workers already sitting on a feeder. Sánchez et al. (2007) found that naïve recruits of *S. mexicana*, which were led to a certain food patch by an experienced bee, significantly more often chose the communicated feeder (formerly marked by experienced bees) within the patch, while the experienced workers did not. The authors suggested that experienced foragers may be more likely to shift to other food sources in the proximity of a known one in order to increase the effective exploitation of additional food sources. The frequent switch between the two feeders by the experienced workers in our experiments seems to support this idea.

Our demonstration that learning of food odors within the nest plays an important role in the foraging behavior of a scent trail laying stingless bee sheds new light on the complex interactions between olfactory signals and cues in this specialized communication mechanism. Although recruits can be precisely guided to feeding sites by means of trail pheromones, they still rely on previously learned food odors for short-range orientation. Thus, our experiments and the observations reported in the literature (see [Introduction](#)) indicate that learning of food odors within the hive and the subsequent preference for resources with the same odor in the field are general features in social bee foraging behavior, regardless of the mode of forager activation and recruitment. To provide a more profound picture of this important aspect of bee behavior, however, the learning abilities of additional species, such as primitively social sweat bees (Halictidae), must be studied in the future.

Acknowledgments We would like to express our thanks to Javier Guevara from the Ministerio del Ambiente y Energía (MINAE) of Costa Rica, for his help in obtaining research permits (080-2006-SINAC, 133-2007-SINAC). This work was supported by a scholarship from the German Academic Exchange Service (DAAD) granted to CR.

References

- Aguilar I (2004) Communication and recruitment for the collection of food in stingless bees: a behavioral approach. Dissertation, University of Utrecht
- Aguilar I, Fonseca A, Biesmeijer JC (2005) Recruitment and communication of food source location in three species of stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* 36:313–324
- Arenas A, Fernández VM, Farina WM (2007) Floral odor learning within the hive affects honeybees' foraging decisions. *Naturwissenschaften* 94:218–222
- Barth FG (1991) *Insects and flowers. The biology of a partnership*. Princeton University Press, Princeton
- Barth FG, Hrnčir M, Jarau S (2008) Signals and cues in the recruitment behaviour of stingless bees (Meliponini). *J Comp Physiol A* 194:313–327
- Dalla Torre CG de (1896) *Catalogus Hymenopterorum; Hucusque Descriptorum Systematicus et Synonymicus*. Leipzig: Engelmann Vol. 10 viii+1–643 pp
- Dornhaus A, Chittka L (1999) Evolutionary origins of bee dances. *Nature* 401:38
- Dornhaus A, Chittka L (2004) Information flow and regulation of foraging activity in bumble bees (*Bombus* spp.). *Apidologie* 35:183–192
- Farina W, Grüter C (2009) Trophallaxis. A mechanism of information transfer. In: Jarau S, Hrnčir M (eds) *Food exploitation by social insects. Ecological, behavioral, and theoretical approaches*. CRC, Boca Raton, pp 183–197
- Farina WM, Grüter C, Acosta L, Mc Cabe S (2007) Honeybees learn floral odors while receiving nectar from foragers within the hive. *Naturwissenschaften* 94:55–60
- Gil M, De Marco RJ (2005) Olfactory learning by means of trophallaxis in *Apis mellifera*. *J Exp Biol* 208:671–680
- Grüter C, Acosta LE, Farina WM (2006) Propagation of olfactory information within the honeybee hive. *Behav Ecol Sociobiol* 60:707–715
- Hrnčir M (2009) Mobilizing the foraging force. Mechanical signals in stingless bee recruitment. In: Jarau S, Hrnčir M (eds) *Food exploitation by social insects. Ecological, behavioral, and theoretical approaches*. CRC, Boca Raton, pp 199–221
- Jarau S (2009) Chemical communication during food exploitation in stingless bees. In: Jarau S, Hrnčir M (eds) *Food exploitation by social insects. Ecological, behavioral, and theoretical approaches*. CRC, Boca Raton, pp 223–249
- Jarau S, Hrnčir M, Zucchi R, Barth FG (2000) Recruitment behaviour in stingless bees. *Melipona scutellaris* and *M. quadrifasciata*. I. Foraging at food sources differing in direction and distance. *Apidologie* 31:81–91
- Jarau S, Hrnčir M, Schmidt VM, Zucchi R, Barth FG (2003) Effectiveness of recruitment behavior in stingless bees (Apidae, Meliponini). *Insect Soc* 50:365–374
- Kerr WE (1969) Some aspects of the evolution of social bees (Apidae). *Evol Biol* 3:119–175
- Kerr WE, Ferreira A, de Mattos NS (1963) Communication among stingless bees—additional data (Hymenoptera: Apidae). *J NY Entomol Soc* 71:80–90
- Knudsen JT, Tollsten L, Berström LG (1993) Floral scents—a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry* 33:253–280
- Lindauer M (1956) Über die Verständigung bei indischen Bienen. *Z Vergl Physiol* 38:521–557
- Lindauer M, Kerr WE (1958) Die gegenseitige Verständigung bei den stachellosen Bienen. *Z Vergl Physiol* 41:405–434
- Lindauer M, Kerr WE (1960) Communication between the workers of stingless bees. *Bee World* 41:29–41–65–71
- Mc Cabe SI, Farina WM (2009) Odor information transfer in the stingless bee *Melipona quadrifasciata*: effect of in-hive experiences on classical conditioning of proboscis extension. *J Comp Physiol A* 195:113–122
- Mc Cabe SI, Hartfelder K, Santana WC, Farina WM (2007) Odor discrimination in classical conditioning of proboscis extension in two stingless bee species in comparison to Africanized honeybees. *J Comp Physiol A* 193:1089–1099
- Menzel R (1999) Memory dynamics in the honeybee. *J Comp Physiol A* 185:323–340
- Menzel R, Müller U (1996) Learning and memory in honeybees: from behavior to neural substrates. *Annu Rev Neurosci* 19:379–404
- Michener CD (2000) *The bees of the world*. The John Hopkins University Press, Baltimore
- Molet M, Chittka L, Raine NE (2009) How floral odours are learned inside the bumblebee (*Bombus terrestris*) nest. *Naturwissenschaften* 96:213–219
- Nieh JC (2004) Recruitment communication in stingless bees (Hymenoptera, Apidae, Meliponini). *Apidologie* 35:159–182

- Praz CJ, Müller A, Dorn S (2008) Host recognition in a pollen-specialist bee: evidence for a genetic basis. *Apidologie* 39:547–557
- Raine NE, Chittka L (2009) Measuring the adaptiveness of social insect foraging strategies. An empirical approach. In: Jarau S, Hrnir M (eds) Food exploitation by social insects. Ecological, behavioral, and theoretical approaches. CRC, Boca Raton, pp 9–28
- Reinhard J, Srinivasan MV (2009) The role of scents in honey bee foraging and recruitment. In: Jarau S, Hrnir M (eds) Food exploitation by social insects. Ecological, behavioral, and theoretical approaches. CRC, Boca Raton, pp 165–182
- Sánchez D, Kraus FB, de Jesús Hernández M, Vandame R (2007) Experience, but not distance, influences the recruitment precision in the stingless bee *Scaptotrigona mexicana*. *Naturwissenschaften* 94:567–573
- Schmidt VM, Zucchi R, Barth FG (2003) A stingless bee marks the feeding site in addition to the scent path (*Scaptotrigona* aff. *depilis*). *Apidologie* 34:237–248
- Slaa EJ, Hughes WHO (2009) Local enhancement, local inhibition, eavesdropping, and the parasitism of social insect communication. In: Jarau S, Hrnir M (eds) Food exploitation by social insects. Ecological, behavioral, and theoretical approaches. CRC, Boca Raton, pp 147–164
- Toda NRT, Song J, Nieh JC (2009) Bumblebees exhibit the memory spacing effect. *Naturwissenschaften* 96(10): 1185–1191
- von Frisch K (1965) *Tanzsprache und Orientierung der Bienen*. Springer, Berlin
- Wilson EO (1971) *The insect societies*. Belknap Press of Harvard University Press, Cambridge