Enantioselectivity of Odor Perception in Honeybees (*Apis mellifera carnica*)

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ABSTRACT

The authors tested the ability of 60 free-flying honeybees (*Apis mellifera carnica*) to discriminate a conditioning odor from an array of 26 simultaneously presented substances. The stimuli included 10 pairs of enantiomers and 6 essential oils. The bees (a) significantly distinguished between 98% of the 540 odor pairs tested, thus showing an excellent overall discrimination performance, and (b) were able to discriminate between the optical isomers of limonene, ? -pinene, ? -citronellol, menthol, and carvone but failed to distinguish between the (+)- and (—)- forms of ? -terpineol, camphor, rose oxide, fenchone, and 2-butanol. The findings support the assumptions that enantioselective molecular odor receptors may exist only for some volatile enantiomers and that insects and mammals may share common principles of odor quality perception, irrespective of their completely differing repertoires of olfactory receptors.

Chirality is a ubiquitous phenomenon in nature, ranging from the level of quanta and the parity violation in all weak interactions to an apparent excess of left-handed galaxies ([Janoschek, 1991](#)). At the molecular level, chirality occurs when four different ligands are attached to the same carbon atom, leading to two different structural arrangements that are nonsuperimposable but that are mirror images of each other. The recognition of such molecules, called *enantiomers* , has been shown to play an important role in many interactions with biological sites such as drug response ([Caldwell & Hutt, 1996](#)), enzyme specificity ([Faber & Griengl, 1991](#)), taste perception ([Shallenberger, 1993](#)), and insect chemical communication ([Mori, 1996, 1998](#)). For the last, insect chemical communication, there are numerous reports of asymmetric (i.e., chiral) sex pheromones being involved in mate attraction ([Mayer & McLaughlin, 1991](#)). Chirality of the pheromone molecules is often crucial for species specificity, including a case of reciprocal behavioral agonist—antagonist activities of enantiomeric pheromones in two scarab beetles ([Leal, 1996](#) ; [Wojtasek, Hansson, & Leal, 1998](#)). The pheromone receptor neurons in insects have generally been shown to be extremely sensitive and highly selective for specific compounds, including single enantiomers ([Larsson, Leal, & Hansson, 1999](#)). In fact, no study to date has reported the failure of an insect to discriminate between the enantiomers of its sex pheromone. This should not be surprising given that theory predicts olfactory

receptors to be enantioselective because they are proteins, that is, chiral structures themselves ([Buck & Axel, 1991](#)), and thus they should react differently with the two enantiomeric forms of an odorant leading to differences in perceived odor quality and intensity ([Pickenhagen, 1989](#)). However, at least in vertebrate species such as humans ([Laska & Teubner, 1999](#)) and squirrel monkeys ([Laska, Liesen, & Teubner, 1999](#)), there are reports of enantiomeric odor pairs that could not be discriminated, which seems inconsistent with the assumption that olfactory receptors should be enantioselective per se ([Theimer, Yoshida, & Klaiber, 1977](#)). Furthermore, the discriminative ability of the insect olfactory system, although generally impressive, is not perfect and has its limits. Honeybees, for example, have been shown to confuse structurally related nonenantiomeric odorants such as aliphatic ketones and aldehydes of similar carbon chain length ([Laska, Galizia, Giurfa, & Menzel, 1999](#)).

In contrast to the wealth of data on enantioselectivity of pheromone perception, there is only sparse information as to chiral recognition of nonpheromonal odorants in insects. However, numerous optical isomers have been identified as volatile products of plants, many of which are important floral indicators ([Knudsen, Tollsten, & Bergström, 1993](#)). Despite the obvious biological significance of flower odors for pollinating insects such as honeybees, only a few studies so far have addressed this question. [Lensky and Blum \(1974\)](#) reported that honeybee workers are able to distinguish between the optical isomers of carvone and 2-octanol; [Kafka, Ohloff, Schneider, and Vareschi \(1973\)](#) showed that migratory locusts and honeybee drones discriminated between the antipodes of 4-methyl-hexanoic acid; and [Wibe, Borg Karlson, Persson, Norin, and Mustaparta \(1998\)](#) reported that the pine weevil is capable of distinguishing the prevailing enantiomeric monoterpenes of its host plant.

Given the paucity of data on the olfactory discrimination performance of insects for nonpheromonal enantiomers and the possible importance of enantioselectivity for our understanding of the mechanisms underlying the interaction between odor stimulus and olfactory receptor, we decided to test the ability of honeybees to distinguish between 10 pairs of enantiomers. The method we used is based on simultaneous presentation of an array of odors to free-flying honeybees that are conditioned to one of the stimuli ([Laska, Galizia, et al., 1999](#) ; [von Frisch, 1919](#)). This experimental design represents the most naturalistic and probably a more challenging situation for the bees compared with the proboscis extension reflex paradigm that has frequently been used with honeybees, particularly to assess mechanisms of odor learning ([Menzel & Müller, 1996](#)). It allowed us to test the discriminability of enantiomeric odor pairs as well as that of a large number of other odor pairs with differing degrees of structural similarity.

Thus, the aims of this study were threefold: (a) to provide data on the olfactory discrimination ability of free-flying honeybees for an array of nonpheromonal enantiomeric odorants, (b) to assess whether any correlations exist between discrimination performance and structural features other than chirality of the odorants under investigation, and (c) by comparing the honeybees' performance with that of human participants and squirrel monkeys tested previously with the same enantiomeric odor pairs, to assess whether insects and mammals share common principles of odor quality perception.

Method

Subjects

A total of 60 free-flying honeybee workers (*Apis mellifera carnica*) from one of the colonies maintained at the apiary of the Department of Neurobiology at the Free University of Berlin (Berlin, Germany) was used. The hive was 50 m from the room in which the experiments were conducted.

Odorants

A total of 26 odorants, including 10 pairs of enantiomers and six etheric oils, was used (see [Table 1](#) and [Figure 1](#)). The rationale for choosing the substances was to present the bees with compounds of varying degrees of structural similarity to any conditioning odorant. Thus, the bees were presented with odorants that differed in chirality (e.g., (+)-?-pinene vs. (—)-?-pinene), in isomery (e.g., fenchone vs. camphor), in a functional group (e.g., limonene vs. carvone), in cyclicity (e.g., ?-terpineol vs. 2-butanol), or in complexity (e.g., menthol vs. peppermint oil).

Furthermore, the enantiomers were chosen on the basis of earlier studies that (a) reported qualitative attributes of antipodes to range from "identical" to "very different" ([Ohloff, 1994](#)) and (b) used the same substances with human participants ([Laska & Teubner, 1999](#)) and squirrel monkeys ([Laska, Liesen, & Teubner, 1999](#)). The etheric oils were chosen on the basis of earlier studies that showed each of them to contain one of the enantiomers used as a quantitatively prevailing constituent ([Bauer, Garbe, & Surburg, 1990](#); see [Table 1](#)). Most of the substances are known to be components of floral scents ([Knudsen et al., 1993](#)).

Test Procedure

The odor stimuli were presented in opaque 1-L glass bottles. Each bottle contained a circular filter paper with a diameter of 6 cm that was impregnated with 4 ?l of an odorant (see [Table 1](#)). The neck of each bottle was equipped with a tightly fitting Plexiglas tube of 3 cm length and an inner diameter of 1.6 cm with a wire mesh at its inner end to allow an odor stimulus to emanate from the bottle but to prevent an insect from crawling or flying into the distal part of a bottle.

A total of 48 bottles was arranged horizontally in a 70 × 80 cm rack of six rows of 8 bottles each. To prevent the odors from intermingling in front of the bottles, we built the back side of the rack conical with the center connected to a suction pump, which provided a constant and approximately laminar airstream around each bottle neck.

Foraging worker honeybees were trained to approach a feeder baited with a 20% (wt/wt) sucrose solution situated in proximity to the test apparatus. Regularly returning bees were individually marked, collected from the feeder when approaching it by use of a glass vial, and put to one of the bottles of the apparatus that contained the conditioning odor (the conditioned stimulus, S+) and was baited with 60 ?l of a 30% (wt/wt) sucrose solution placed in the neck of the bottle. Usually the bee entered the neck of the bottle voluntarily, ingested the food reward while perceiving the conditioning odor, and then returned to its hive. We conducted five such training trials to allow a bee to build a robust association between food reward and conditioned odor while taking care to change the position of the S+ bottle in the array between each

trial to prevent the occurrence of positional preferences. Care was also taken to prevent the bees from using scent marks for locating the S+ bottle by thoroughly cleaning each bottle neck that a bee had entered after each trial. Following the fifth training trial, the feeder was removed, the position of the bottle containing the conditioned odor was changed again, and on returning from its hive the bee approached the apparatus and started to search for the S+ by hovering in front of the bottle necks. The behavior of the bee was recorded both on videotape and on protocol sheets that showed the position of each stimulus.

Correct choices consisted of bees both landing on the neck of a bottle containing the S+ and failing to land on a bottle containing an S— after hovering in front of the bottle neck for a minimum of 0.5 s. Conversely, errors consisted of landing on the neck of a bottle containing an S— and failing to land on an S+ bottle after hovering in front of the bottle neck for a minimum of 0.5 s.

To obtain a sufficient number of decisions, we ensured that each bee received a total of 10 extinction trials without food reward and 10 food-rewarded test trials that were alternated with the former. In the extinction trials, the array was composed of 44 bottles containing one S— each (with the 19 enantiomers used as S— in 2 bottles each, and the six etheric oils in 1 bottle each), 1 bottle containing the S+ (without a food reward), and 3 blank bottles without any odor. In the rewarded test trials, the array was again composed of the 44 bottles containing one S— each, 3 bottles containing the S+ (with a food reward), and 1 blank bottle. To maximize the number of decisions per bout and to prevent bees from developing a position preference, we restricted the food reward in the test trials to 10 μ l of sugar solution (full crop load of a honeybee is approximately 60 μ l), and an S+ bottle that already had been visited by a bee was only baited again during the same bout when the bee had landed at 1 of the other 2 S+ bottles and consumed the food reward provided there. To prevent demotivation of the bee, we restricted the time allotted to Extinction Trials 2—10 to 3 min. The first extinction trial, that is, the very first trial following the five (food-rewarded) conditioning trials, lasted only 2 min because a longer duration occasionally caused bees to quit searching and reliably returning to the apparatus. Between any of the 20 trials, the arrangement of the bottles was changed according to a pseudorandomized scheme.

With the exception of the six etheric oils, all odorants were used as S+, and data from 3 bees per S+ were recorded. Usually we tested 2 bees per day, while taking care to renew the odor stimuli and to thoroughly clean the Plexiglas tubes in the neck of the bottles for each subject.

Data Analysis

In assessing performance of the bees, we scored only unequivocal decisions (see above). The minimum number of decisions per bee was 1,012 and averaged 1,508. We determined significance levels separately for extinction and test trials by calculating binomial z scores corrected for continuity ([Siegel & Castellan, 1988](#)) from the number of correct and false responses for each individual and stimulus.

Comparisons across tasks were made with the Friedman two-way analysis of variance (ANOVA). When the ANOVA detected differences between tasks, this was then

followed by pairwise Wilcoxon signed-rank tests for related samples to identify which tasks were involved.

Correlations between the across-task pattern of discrimination performance and frequency of occurrence of substances in flower odors were evaluated using the Spearman rank correlation coefficient and were tested for significance by computing *t* values. All tests were two-tailed, and the alpha level was set at .05. All data are reported as mean plus or minus standard deviation.

Results

General Discrimination Performance

[Figure 2](#) summarizes the discrimination performance of the 60 honeybees tested in the extinction trials. Of the 540 odor pairs, the bees were able to significantly discriminate 530 (i.e., 98%). Only 9 odor pairs (i.e., 2% of all odor pairs) were not distinguished by all 3 bees trained to a given S+ (see filled circles in [Figure 2](#)), and 1 odor pair each was confused by 1 ((+)-carvone vs. (—)-carvone) or by 2 ((+)-camphor vs. (—)-camphor) out of 3 bees.

When used as the S+, 10 out of 20 substances were significantly discriminated from all other stimuli, and the remaining 10 substances were confused with only 1 of the 25 S— each, which in all cases was the optical antipode of the S+. Thus, all 20 substances used as S+ were significantly distinguished from >95% of the other stimuli.

Overall discrimination scores in test trials and extinction trials with a given bee were usually very similar, and differences between the two never exceeded 3%. Interindividual variability in overall discrimination scores was remarkably low, with all 60 bees scoring >91% correct choices in both the extinction trials and the test trials, and the majority of bees even scoring >95% correct. None of the bees failed to correctly identify its conditioning odor at a significant level (diagonal in [Figure 2](#)), and none of the bees confused >1 of the 26 odor pairs.

Enantiomers

[Figure 3](#) summarizes the mean performance of the honeybees in discriminating between the 10 enantiomeric odor pairs. As a group, the bees were only able to discriminate between the optical isomers of limonene, ?-pinene, ?-citronellol, menthol, and carvone, whereas they failed to distinguish between the (+)- and (—)-forms of ?-terpineol, camphor, rose oxide, fenchone, and 2-butanol.

In the majority of tasks, all 6 bees either succeeded (limonene, ?-pinene, ?-citronellol, and menthol) or failed (?-terpineol, rose oxide, 2-butanol, and fenchone) to significantly discriminate between the antipodes of a given substance, and thus interindividual variability in these tasks was generally low and <20% between the highest- and lowest-scoring bee (see standard deviations in [Figure 3](#)). In the remaining tasks, 1 out of 6 bees failed (carvone) or succeeded (camphor) to distinguish between the optical isomers of a given substance, and accordingly interindividual variability in these tasks was high. However, the ANOVA detected

significant differences in the bees' performance between tasks (Friedman, $p < .001$), and subsequent pairwise tests revealed that the enantiomers of limonene, α -pinene, α -citronellol, and menthol were significantly less difficult to discriminate compared with the other tasks (Wilcoxon, $p < .05$). The scores of the latter three substances did not differ significantly from each other (Wilcoxon, $p > .05$). Similarly, the five enantiomeric odor pairs that were not discriminated at the group level did not differ significantly from each other in their degree of discriminability (Wilcoxon, $p > .05$).

Nonenantiomeric Isomers

Fenchone and camphor are nonenantiomeric structural isomers; that is, they share the same molecular formula and functional groups but differ in structure in a nonchiral manner (see [Figure 1](#)). The discrimination of these substances presented little difficulty to the bees. Overall scores of correct choices were not significantly different from that of other, nonisomeric odor pairs that involved one of these substances (Wilcoxon, $p > .05$).

Type of Oxygen Moiety

Carvone, fenchone, and camphor share the same type of functional group, that is, a keto group. Similarly, α -citronellol, α -terpineol, menthol, and 2-butanol share an alcohol group as a common structural feature (see [Figure 1](#)). Substances sharing the same type of oxygen moiety were never confused, and overall scores for such odor pairs did not differ significantly from that of odor pairs that involved one of these substances but with different functional groups (Wilcoxon, $p > .05$). The same is true for α -pinene and limonene, which both share a lack of an oxygen moiety.

Type of Alkyl Rest at the Chiral Carbon Atom

Carvone and limonene share a propenyl group at the chiral carbon atom. Similarly, menthol, rose oxide, α -citronellol, fenchone, and camphor share a methyl group at the chiral center (see [Figure 1](#)). The discrimination of substances sharing the same type of alkyl rest at the chiral carbon atom again did not present much difficulty to the bees and was not significantly different from that of odor pairs that involved one of these substances but with different types of ligands at the chiral center (Wilcoxon, $p > .05$).

Number of Chiral Carbon Atoms

Menthol has three independent chiral centers, whereas the other monomolecular substances used have only one (see [Figure 1](#)). The discrimination of substances sharing the same number of chiral carbon atoms did not present much difficulty to the bees and did not differ significantly from that of odor pairs that involved menthol, that is, a substance with a different number of chiral centers (Wilcoxon, $p > .05$).

Cyclicity of Substances

The discrimination of monocyclic terpenes, such as carvone, limonene, α -terpineol, menthol, and rose oxide, or of bicyclic terpenes, such as α -pinene, fenchone, and camphor, did not lead to any confusions and did not differ significantly from that of odor pairs that involved one of these substances but differed in cyclicity (Wilcoxon, p

> .05). The same is true for the discrimination of ?-citronellol and 2-butanol, which both are acyclic substances.

Complexity of Stimuli

The discrimination of monomolecular substances from essential oils, that is, of odor pairs that differ in complexity, usually presented little difficulty to the bees. However, the discrimination of (+)-carvone from caraway oil yielded overall scores that were significantly lower than those of odor pairs that involved (+)-carvone and other monomolecular substances or other essential oils (Wilcoxon, $p < .05$). Similarly, the discrimination of (—)-menthol from peppermint oil resulted in overall scores that were significantly lower than those of odor pairs that involved (—)-menthol and other monomolecular substances or other complex stimuli (Wilcoxon, $p < .05$).

Discussion

The results of this study demonstrate that (a) honeybees have an excellent olfactory discrimination ability when tested in a free-flying situation with an array of structurally related aromatic substances and (b) the ability of honeybees to discriminate between enantiomeric odor pairs is substance specific and thus not a generalizable phenomenon. The excellent performance of *Apis mellifera carnica* found here is in agreement with earlier studies on odor discrimination using free-flying honeybees ([Laska, Galizia, et al., 1999](#) ; [von Frisch, 1919](#)) or conditioning of the proboscis extension reflex ([Smith & Menzel, 1989](#) ; [Vareschi, 1971](#)). However, whereas von Frisch's landmark study mainly used etheric oils (i.e., complex odor mixtures of high biological significance) and the other studies mentioned mainly used monomolecular aliphatic substances, we could show that honeybees are also able to clearly distinguish between a large number of aromatic substances in a paradigm designed to simulate odor-guided foraging behavior. Despite the presumably more challenging conditions of a free-flying test situation with simultaneous presentation of stimuli compared with the testing of restrained bees with sequential presentation of odorants, the honeybees in our study scored an average of >95% correct choices and thus showed the same high level of performance as bees used in studies that used the proboscis extension reflex paradigm. This is remarkable considering that our experimental setup required a bee to perform approach flights and spatial orientation under environmentally realistic conditions in addition to the actual discrimination task.

Our finding that the honeybees were only able to distinguish 5 of the 10 enantiomeric odor pairs tested, and thus of substance specificity in chiral odor discrimination, is in line with the few studies so far that used discrimination procedures to assess the ability of vertebrate species to detect differences between optical isomers. Human participants ([Laska & Teubner, 1999](#)) and squirrel monkeys ([Laska, Liesen, & Teubner, 1999](#)) tested with the same 10 pairs of antipodes as the honeybees failed with 7 and 6 of the 10 tasks, respectively. Interestingly, the three species display some similarities in their across-task patterns of performance. [Table 2](#) shows a comparison of the ability of honeybees, human participants, and squirrel monkeys to discriminate between the 10 enantiomeric odor pairs. The two primate species correspond in their discriminative abilities in 9 out of 10 tasks, which might not be too surprising given that they are phylogenetically closely related and have recently been shown to share a

large proportion of molecular odor receptors ([Isse!-Tarver & Rine, 1997](#) ; [Rouquier, Blancher, & Giorgi, 2000](#)). However, the performance of the honeybees still coincides with that of the human participants and the squirrel monkeys in 8 and 7 out of 10 cases, respectively, which is remarkable given their phylogenetic distance and given that insects and mammals are believed to differ completely in their repertoires of olfactory receptors ([Clyne et al., 1999](#) ; [Freitag, Ludwig, Andreini, Rossler, & Breer, 1998](#)).

This finding is in accordance with recent studies of olfactory discrimination performance using homologous series of aliphatic alcohols, aldehydes, and ketones with human and nonhuman primates ([Laska, Trolp, & Teubner, 1999](#)) as well as with honeybees ([Laska, Galizia, Giurfa, & Menzel, 1999](#)). Furthermore, it demonstrates that the similarities found in the structural organization of the olfactory systems of insects and vertebrates ([Boeckh, Distler, Ernst, Hosl, & Malun, 1990](#)) are paralleled by similarities in relative discrimination abilities, thus supporting the assumption that similar mechanisms of odor coding and discrimination may underlie olfaction in both taxa ([Hildebrand & Shepherd, 1997](#)).

The question arises as to possible reasons why the honeybees succeeded in discriminating between the antipodes of limonene, ? -pinene, ? -citronellol, menthol, and carvone but failed to distinguish between the (+)- and (—)- forms of ? -terpineol, camphor, rose oxide, fenchone, and 2-butanol. The possibility that an inability to perceive an odorant might have contributed to the failure of the honeybees in discriminating between the enantiomers of the latter substances can be excluded because all bees were clearly able to both correctly identify their conditioning odor at a significant level (see diagonal in [Figure 2](#)) and correctly discriminate it from odorless blanks (see last column in [Figure 2](#)). The possibility that molecular features other than chirality might account for the observed substance specificity in discrimination performance is also unlikely because, for example, ? -citronellol, menthol, ? -terpineol, and 2-butanol all share the same type of oxygen moiety (i.e., a functional alcohol group). Nevertheless, the antipodes of the former two substances were discriminated, whereas the optical isomers of the latter two were not. Similarly, carvone, fenchone, and camphor share a functional keto group, but the bees were only able to distinguish the enantiomers of carvone.

The type of alkyl rest at the chiral carbon atom also does not explain why some of the enantiomers were discriminable for the honeybees whereas others were not. Although five of the substances used share a methyl group at their chiral center, the bees distinguished only the (+)- and (—)- forms of menthol and ? -citronellol but not those of rose oxide, fenchone, and camphor. However, carvone and limonene, whose antipodes were both discriminable for the honeybees (as well as for the two primate species tested previously; see [Table 2](#)), both have a propenyl group as a ligand at the asymmetric carbon atom. Thus, it seems worthwhile to include other enantiomeric odor pairs that show this molecular feature in future studies of olfactory discrimination performance to elucidate whether this particular alkyl rest might play a role in enantioselectivity of odor receptors. Other molecular features such as the number of chiral carbon atoms or the cyclicity of substances also do not suffice to draw generalizable conclusions with regard to odor structure—activity relationships that would allow us to predict whether a given pair of enantiomers can be olfactorily discriminated.

A more biological approach in trying to explain the observed substance specificity in the ability to distinguish optical isomers is that enantioselectivity of the honeybee or perhaps even all olfactory systems may be restricted to substances for which both antipodes are widely present in a species' natural odor world. In a meta-analysis of 118 published articles, [Knudsen et al. \(1993\)](#) listed a total of more than 700 floral scent compounds retrieved by head-space gas chromatography and mass spectrometry from 441 species of plants. Using this comprehensive database, we performed a correlational analysis to assess whether the frequency of occurrence of the 10 chiral substances used here in the odors of insect-pollinated flowers is connected with the discrimination performance of the honeybees. We found a significant correlation between the two parameters (Spearman $r = .82$, $p < .02$), suggesting that the discriminative abilities of the honeybees for chiral flower odor compounds may reflect an evolutionary adaptation to their ecological niche. In contrast, the discrimination performance of humans and squirrel monkeys—which obviously are not flower-visiting species and thus live in an odor world that is different from that of the bees—for the same 10 chiral substances did not correlate with their frequency of occurrence in the odors of insect-pollinated inflorescences (Spearman $r = .49$ and $.32$, $p > .05$, for humans and squirrel monkeys, respectively). To further corroborate the hypothesis that substance specificity of enantioselectivity is correlated with the natural occurrence and distribution of substances in a species' odorous environment, one must include other species and other enantiomeric odor pairs in future studies of olfactory discrimination performance.

A final aspect of the present study is the finding that the discrimination of monomolecular aromatic substances from essential oils yielded mixed results, with the majority of such odor pairs presenting little difficulty to the bees, whereas two of them were distinguished at significantly lower scores than the rest. It is commonly agreed that the olfactory systems of both vertebrates and insects are particularly adjusted to process complex odor mixtures as the vast majority of naturally occurring odors comprise numerous compounds ([Laing, Cain, McBride, & Ache, 1989](#)). However, the fact that (+)-carvone was at least partially confused with caraway oil, and (—)-menthol with peppermint oil, suggests that honeybees—similar to mammals ([Laska & Hudson, 1993](#))—may at least partially rely on key compounds (i.e., monomolecular substances that characterize a *Geruchsgestalt*) to recognize odor mixtures. This supposition is supported by behavioral studies coupled with chemical analyses of floral volatiles that have shown honeybees capable of relying on only a portion of the volatiles to recognize flower species such as alfalfa ([Waller, Loper, & Berdel, 1974](#)), of discriminating between different genotypes of sunflower ([Pham-Delegue, Etievant, Guichard, & Masson, 1989](#)), and of generalizing between oilseed rape flower odor and some of its constituents ([Le Metayer et al., 1997](#)).

It is interesting to note, however, that the relative amount of a substance in a complex flower odor does not allow us to predict whether it will be used as a key compound by the honeybee or not. Whereas (+)-carvone and (—)-menthol constitute approximately 50%—60% of the total amount of volatiles in caraway oil and peppermint oil, respectively, (—)- α -pinene and (+)-limonene even account for about 90% of turpentine oil and bitter orange oil (see [Table 1](#)). However, the former two substances were partially confused with the corresponding essential oils, whereas the latter two were not.

Taken together, the results of the present study provide evidence that the ability of honeybees to discriminate between nonpheromonal enantiomeric odor pairs is substance specific and thus support the assumptions that enantioselective molecular odor receptors may exist for only some but not all volatile enantiomers and that widespread occurrence of both enantiomeric forms of a substance in a species' odorous environment might be a prerequisite for the development of chiral recognition. Furthermore, the results suggest that insects and mammals may share common principles of odor quality perception, irrespective of their completely differing repertoires of olfactory receptors.

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Table 1. Substances Used

Table 1
Substances Used

Substance	Chemical description	Odor quality
1. (–)-carvone	Monocyclic terpene-ketone	Spearmint
2. (+)-carvone	Monocyclic terpene-ketone	Caraway
3. (–)-limonene	Monocyclic terpene-hydrocarbon	Turpentine
4. (+)-limonene	Monocyclic terpene-hydrocarbon	Orange
5. (1R, 2S, 5R)-(–)-menthol	Monocyclic terpene-alcohol	Peppermint, strong cooling
6. (1S, 2R, 5S)-(+)-menthol	Monocyclic terpene-alcohol	Peppermint, less cooling
7. (–)- α -terpineol	Monocyclic terpene-alcohol	Tarry, cold pipe
8. (+)- α -terpineol	Monocyclic terpene-alcohol	Flowery, lilac
9. (–)-rose oxide	Monocyclic terpene-oxide	Green, herbal
10. (+)-rose oxide	Monocyclic terpene-oxide	Green, sweet
11. (–)- β -citronellol	Acyclic terpene-alcohol	Geranium oil-type
12. (+)- β -citronellol	Acyclic terpene-alcohol	Citronella oil-type
13. (–)-fenchone	Bicyclic terpene-ketone	Camphoraceous, sweet
14. (+)-fenchone	Bicyclic terpene-ketone	Camphoraceous, sweet
15. (–)-camphor	Bicyclic terpene-ketone	Camphoraceous
16. (+)-camphor	Bicyclic terpene-ketone	Camphoraceous
17. (–)- α -pinene	Bicyclic terpene-hydrocarbon	Pinelike
18. (+)- α -pinene	Bicyclic terpene-hydrocarbon	Pinelike
19. (–)-2-butanol	Aliphatic alcohol	Oily-vinous
20. (+)-2-butanol	Aliphatic alcohol	Oily-vinous
21. Caraway oil	50%–60% (+)-carvone, 20%–30% (+)-limonene	
22. Fennel oil	15%–20% (+)-fenchone, 4%–9% limonene, 0.5%–1.0% α -pinene	
23. Turpentine oil	90% (–)- α -pinene	
24. Bitter orange oil	90% (+)-limonene, 1% α -pinene, 1% α -terpineol, 1% camphor	
25. Thuja oil	14% (–)-fenchone, 3% α -pinene, 2% limonene, 2% camphor	
26. Peppermint oil	45%–65% (–)-menthol	

Note. Chemical descriptions are according to Falbe and Regiz (1992) and Boelens (1983); odor qualities are according to Ohloff (1994).

Table 2. Across-Species Comparison of Discrimination Performance for Enantiomeric Odor Pairs

Table 2
Across-Species Comparison of Discrimination Performance for Enantiomeric Odor Pairs

Substance	Honeybees	Human participants	Squirrel monkeys
Limonene	+	+	+
α -pinene	+	+	+
Carvone	+	+	+
Menthol	+	–	–
β -citronellol	+	–	–
α -terpineol	–	–	–
Camphor	–	–	–
Rose oxide	–	–	–
2-butanol	–	–	–
Fenchone	–	–	+

Note. + indicates the ability to significantly discriminate between the antipodes of a given substance; – indicates the failure to significantly discriminate between the antipodes of a given substance. Data for human participants are from Laska and Teubner (1999); data for squirrel monkeys are from Laska, Liesen, and Teubner (1999).

Figure 1. Molecular structures of the 10 pairs of enantiomers used.

Figure 2. Performance of 60 honeybees in discriminating between the 26 odorants used. Each circle represents the mean performance of 3 honeybees conditioned to the same conditioned stimulus. Filled areas indicate the range of performance.

Figure 3. Performance of 60 honeybees in discriminating between 10 pairs of enantiomers. Each data point represents the percentage ($M \pm SD$) of correct choices from 10 extinction trials per bee. Filled symbols indicate odor pairs that were not discriminated above chance at the group level. Figures above the abscissa indicate the number of bees that failed to perform significantly above chance in the corresponding task.