Differential olfactory perception of enantiomeric compounds by blind subterranean mole rats (*Spalax ehrenbergi*)

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Abstract. The behavior of mole rats (*Spalax ehrenbergi*) near pairs of enantiomeric compounds was examined in 901 two-choice experimental tests. Positioning of the nest and food store and the preferred location of the tested animal were used to assess attraction or aversion to the tested odorants. The results indicated that mole rats respond differentially to odors of stereoisomers (enantiomers of carvone, citronellol, and fechone). They responded to one enantiomer of each tested pair but were indifferent to or did not smell the other. Both sexes were attracted to the odor of R-(−)-carvone and repelled by the odor of (+)-citronellol. Females were attracted to the odor of (−)-fenchone while males had no preference. By contrast, all animals were indifferent to or did not smell the odor of S-(+)-carvone, (−)-citronellol, and (+)-fenchone. Further research to distinguish between these alternatives (indifference vs hyposmia/anosmia) is suggested.
Key words. Olfactory perception; enantiomers; subterranean mole rats; *Spalax ehrenbergi.*

Few studies have investigated chemoreception in subterranean mammals[1]. *Spalax ehrenbergi* mole rats are especially suitable for studying chemoreceptive adaptation in subterranean mammals, as well as the contribution of olfaction to reproductive isolation and speciation processes in nature, because the *S. ehrenbergi* superspecies in Israel comprises four actively speciating and adaptively radiating chromosomal species: 2 n = 52, 54, 58, and 60[2]. Mole rats are territorial[3], solitary, and aggressive[4] rodents that live in subterranean tunnel systems[3,5] and feed on the underground parts of plants[3,6]. While mole rats are blind[7], their auditory and seismic sensitivities are adapted to life underground[8,9]. Several studies have suggested that olfaction may be important in mole rats' reproductive isolation and speciation[10-13] as well as in spacing individuals and populations[13,14].
Studies of the ability of various organisms (insects[15-19], mammals[20], and human beings[21-26]) to discriminate between enantiomeric compounds (which differ only in their optical rotation and the speed of their reaction with other chiral molecules) have contributed to understanding olfactory reception/perception processes and the biological activity of these compounds. The purpose of our study was to assess the discrimination between pure enantiomers in a subterranean rodent (*S. ehrenbergi*), in order to obtain more information on mole rats' olfactory perception. Here we report on mole rats' differential responses to the odor of carvone, citronellol, and fenchone enantiomers.

Materials and methods

140 adult experimental animals (79 males and 61 females ranging from 1 to 3 years old) belonging to the Anza population of the 2 n = 60 chromosomal species (see fig. 2 in Nevo[2]) were trapped in the field and brought to the laboratory. They were kept for several months prior to experimentation under constant conditions (21 ± 1 °C; rel. humidity 70 ± 5%; 12L:12D h). They were caged individually and fed a vegetarian diet ad libitum. When animals were used in experiments more than once, at least 10 days intervened between tests.
Odor preference tests were done out of the natural breeding season[3] from April through August of 1989 and 1990. The tests were conducted between 10.00 h and 16.00 h. The experimental apparatus was a two-choice, modular olfactorium made of transparent perspex and comprising two square boxes (20 × 20 × 10 cm) attached to opposite sides of a connecting tunnel (80 × 7 × 7 cm). Each box had a removable top and a perforated (0.5 cm diameter holes) inner partition (0.5 cm thick) situated 2 cm in front of the outer back wall of the box. Filter paper wicks containing 100 µl of test or control substances were placed behind the partition, and the test animal was exposed to the odorants that diffused through the holes. Eight wafers of carrot and 8 strips of laboratory tissue, which simulated food and bedding material, were placed in both boxes before the test. The location of the odor stimuli and the box into which the animal was introduced were chosen randomly. After each test the experimental apparatus was dismantled and thoroughly washed with hot water and detergent.
The tested substances were pairs of enantiomeric compounds which can be isolated from natural plants: R-(−)- and S-(+)-carvone; (+)- and (−)-citronellol; and (+)- and (−)-fenchone. Enantiomeric substances were commercially obtained (Fluka, Switzerland), and solutions (diluted in methylene chloride) in $\log_{10}$ steps (10^{-2}–10^{-5}) were prepared. In several cases the racemic solution (1:1 mixture of the original (−)- and (+)-solutions) was tested as well. 100 µl of methylene chloride

Table 1. Carvone enantiomers

Experimental set No.	Odorants	Description	Dilution (with MC)	Number of tests Total	Male	Female	Results[a,b] Male						Female						Combined					
							R-(−)-	S-(+)-	No preference				R-(−)-	S-(+)-	No preference				R-(−)-	S-(+)-	No preference			
1.	R-(−)- vs S-(+)-carvone	Different animals	10^{-2}	78	44	34	28	12	4				18	6	10				46	18	14			
2.	R-(−)- vs S-(+)-carvone	2 repetitions; same 20 animals	10^{-3}	40	20	20	5[b]	2	3				3	1	6				8	3	9			
							R-(−)- vs MC: R-(−)-	MC	NP	S-(+)- vs MC: S-(+)-	MC	NP	R-(−)- vs MC: R-(−)-	MC	NP	S-(+)- vs MC: S-(+)-	MC	NP	R-(−)- vs MC: R-(−)-	MC	NP	S-(+)- vs MC: S-(+)-	MC	NP
3.	R-(−)- or S-(+)-carvone vs methylene chloride (MC)	Different dilutions; same 20 animals	10^{-2}	20	10	10	4	1	–	2	2	1	4	1	–	2	3	–	8	2	–	4	5	1
			10^{-3}	20	10	10	3	2	–	2	3	–	4	1	–	3	2	–	7	3	–	5	5	–
			10^{-4}	20	10	10	4	1	–	2	3	–	3	2	–	3	2	–	7	3	–	5	5	–
			10^{-5}	20	10	10	1	4	–	2	2	1	0	5	–	2	3	–	1	9	–	4	5	1
							R-(−)- vs Racemate: R-(−)-	Rac	NP	S-(+)- vs Racemate: S-(+)-	Rac	NP	R-(−)- vs Racemate: R-(−)-	Rac	NP	S-(+)- vs Racemate: S-(+)-	Rac	NP	R-(−)- vs Racemate: R-(−)-	Rac	NP	S-(+)- vs Racemate: S-(+)-	Rac	NP
4.	R-(−)- or S-(+)-carvone vs racemate	Different animals	10^{-2}	66	49	17	15	4	5	10	9	6	6	1	2	3	5	0	21	5	7	13	14	6

[a] The results describe the number of tests which were completed with 'preference' or 'no preference'/'NP' (see definitions in Materials and methods) for the tested odors. [b] The results of experimental set 2 represent the combined results of two tests done by each animal (see Results and discussion).

(which evaporates quickly and leaves no odor) served as a control substance.

The odorants used in the experimental sets were: pairs of enantiomeric compounds; one of the enantiomeric pairs against methylene chloride or a racemic solution; and a racemic solution against methylene chloride (see table 2, experimental set No. 7).

The animal's location and movement of food and bedding in the apparatus were recorded at 10-min intervals during each 1-h test. Attraction to or preference for a particular odor was determined by the animal's most frequently chosen location and by whether the animal moved food and/or bedding to the preferred box. In cases of odor vs no odor control, movement of food and bedding away from the box with the odorant was considered avoidance behavior. 'No preference' was recorded when food and bedding were spread throughout the apparatus or were moved from both boxes into the connecting tunnel, and in the rare cases when the animal did not move any food and bedding and was inactive during the test.

The statistical analyses were based on the number of tests in which the mole rats showed a preference. Binomial tests[27] were used to assess the significance of differences.

Results and discussion

The results of the 4 experimental sets using the carvones are given in table 1. Mole rats of both sexes significantly preferred ($p = 0.05$, for males and females, and $p < 0.001$ for both sexes) to build their nests and food stores and to stay in the box with the R-(−)-carvone odor when tested against S-(+)-carvone. In the second experimental set, 20 mole rats participated twice in preference tests between carvone enantiomers. Eight mole rats preferred the R-(−)-carvone, 3 preferred the S-(+)-carvone, 8 preferred the (R)-(−)-carvones once and the S-(+)-carvone once, and 1 mole rat did not show a preference in either test. Only animals that chose the same enantiomer twice were included in the binomial test. The result was statistically insignificant but corroborated the significant result in set 1. The results of experimental set 3 (table 1) revealed that both males and females significantly ($p = 0.05$) preferred the odor of R-(−)-carvone when it was tested against methylene chloride (see also fig. 1). The mole rats were neither attracted to nor avoided S-(+)-carvone when it was tested against methylene chloride. This response pattern (see fig. 1) was found for all the dilutions tested (10^{-2}–10^{-5}), and suggests that they are either indifferent to or are unable to smell the S-(+)-enantiomer. The attraction pattern towards the R-(−)-carvone was observed in dilutions 10^{-2}–10^{-4} but was not evident in the 10^{-5} dilution. Because it is unlikely that an aversion would appear to a weak dilution, this is probably a random result and the 10^{-4} dilution may be the lowest detectable solution of the R-(−)-carvone. The results of experimental set 4 revealed that mole rats significantly ($p < 0.05$) prefer the

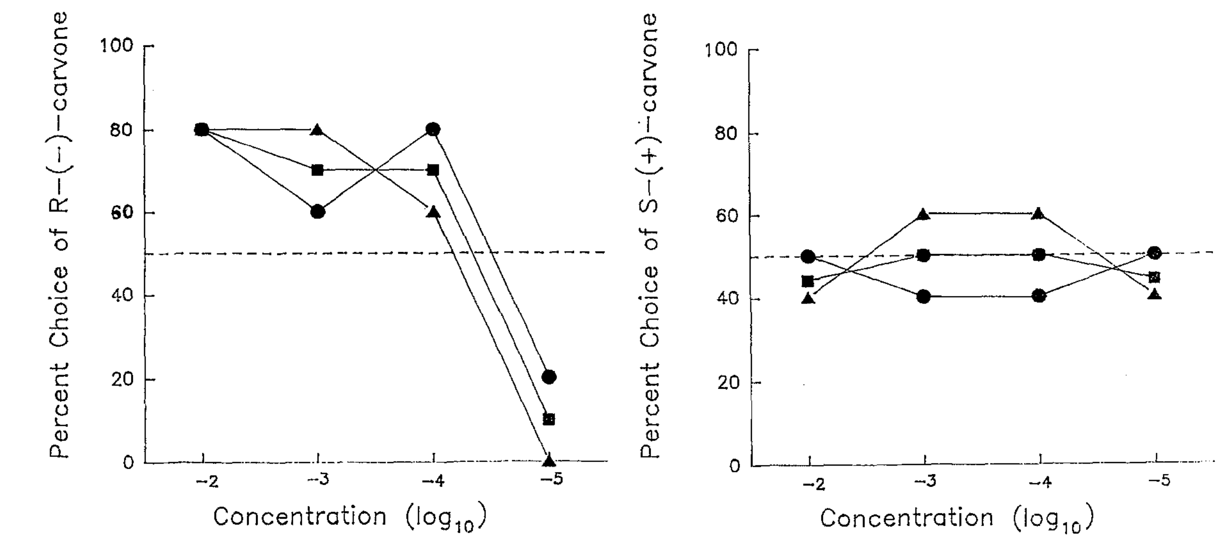

Figure 1. Percent choice of R-(−) and S-(+)-carvone solutions (at different dilutions) when tested against methylene chloride (see experimental set 3 in table 1).
● – males; ▲ – females; ■ – both sexes combined.

R-(−)-carvone when tested against the carvone racemate, but show no particular response toward S-(+)-carvone or the racemate. The results support the previous conclusion that mole rats are attracted to the odor of R-(−)-carvone and are indifferent to or do not smell the odor of S-(+)-carvone or the racemate, which is treated like the methylene chloride control. Interestingly, they also suggest that mole rats are indifferent to or do not smell the carvone racemate in spite of being able to respond to at least one (the R-(−)-) of its individual components.

Four experimental sets were conducted with the (−)- and (+)-citronellol enantiomers (see results in table 2). In experimental set 5 each animal participated in 4 repeated tests and chose between (−)- and (+)-citronellol. Twelve mole rats preferred the (−)-citronellol, 4 (+)-citronellol and 4 showed no preference. The binomial test included only the animals that showed a preference. The results suggest a preference (though insignificant, $p < 0.06$, in binomial test) for the (−)-citronellol box; however, in the light of the results of experimental sets 6–8, it seems that this result reflects an aversion to (+)-citronellol rather than an attraction to the (−)-citronellol. In 2 different experimental sets (6 and 7), mole rats were indifferent to or did not smell the odor of (−)-citronellol when it was tested against the odor of methylene chloride, and this was the result when (−)-citronellol was tested against the citronellol racemate (set 8). A clear aversion tendency to (+)-citronellol was found (in the 10^{-2}–10^{-4} dilutions) when it was tested against methylene chloride (set 6; see fig. 2). Female mole rats (but not males) showed significant ($p < 0.01$, set 7) aversion to the racemic solution when it was tested against methylene chloride, perhaps as a response to its (+)-citronellol component. However, this pattern of response was not found when the racemate was tested against (−)-citronellol (set 8). Further investigation is needed to clarify this discrepancy.

The results of the 2 experimental sets (9 and 10) for fenchone enantiomers are presented in table 3. In both sets, [(−)- against (+)-fenchone, and each fenchone enantiomer against methylene chloride], females preferred the odor of (−)-fenchone (for set 10: $p < 0.05$ in binomial test) and were indifferent to or unable to smell the (+)-fenchone. A similar pattern of response toward (+)-fenchone was found for the males; however, their responses toward (−)-fenchone in the different sets were ambiguous. Again the tendencies were most apparent in the stronger dilutions.

Current theories of olfaction (see reviews by Lancet[28] and Reed[29]), and the recent discovery of genes that are likely to encode odorant receptor proteins[30], emphasize the important role of neurons of the olfactory epithelium and their protein receptors in the process of discriminating among odors. Perception of enantiomeric compounds may serve to demonstrate the recognition and resolution capability of the olfactory system in mammals[28,29]. The results of our study suggest that mole rats have a differential perception of enantiomers and they respond to only one of each enantiomeric pair of carvone, citronellol, and fenchone. Based on these results, it is impossible to determine whether mole rats can smell (i.e. detect and perceive) both enantiomers but are indifferent to one member of each pair or whether they are unable to smell (specific hyposmia/anosmia) one member of each pair. This lack of response was consistently observed in all the dilutions of the 3 pairs of tested stereoisomers and suggests that the differential perception of carvone, citronellol, and fenchone enantiomers may be a characteristic pattern in subterranean mole rats. If further experiments demonstrate this to be true, the phenomenon could

Table 2. Citronellol enantiomers

Experimental set No.	Odorants	Description	Dilution (with MC)	Number of tests Total	Male	Female	Results[a,b] Male						Female						Combined					
							(−)-Cit	(+)-Cit	No Preference				(−)-Cit	(+)-Cit	No Preference				(−)-Cit	(+)-Cit	No Preference			
5.	(−)- vs (+)- Citronellol	4 repetitions; same 20 animals	10^{-2}	80	40	40	6[b]	2	2				6	2	2				12	4	4			
							(−)-Cit vs MC			(+)-Cit vs MC			(−)-Cit vs MC			(+)-Cit vs MC			(−)-Cit vs MC			(+)-Cit vs MC		
							(−)-Cit	MC	NP	(+)-Cit	MC	NP	(−)-Cit	MC	NP	(+)-Cit	MC	NP	(−)-Cit	MC	NP	(+)-Cit	MC	NP
6.	(−)- or (+)- Citronellol vs methylene chloride (MC)	Different dilutions; same 20 animals	10^{-2}	20	10	10	4	1	–	1	2	2	2	3	–	2	3	–	6	4	–	3	5	2
			10^{-3}	20	10	10	3	2	–	1	3	1	1	4	–	2	3	–	4	6	–	3	6	1
			10^{-4}	20	10	10	3	2	–	1	3	1	4	1	–	2	3	–	7	3	–	3	6	1
			10^{-5}	20	10	10	1	4	–	2	3	–	3	2	–	3	2	–	4	6	–	5	5	–
			total	80	40	40	11	9	–	5	11	4	10	10	–	9	11	–	21	19	–	14	22	4
							(−)-Cit vs MC			Rac. vs MC			(−)-Cit vs MC			Rac. vs MC			(−)-Cit vs MC			Rac. vs MC		
							(−)-Cit	MC	NP	Rac.	MC	NP	(−)-Cit	MC	NP	Rac.	MC	NP	(−)-Cit	MC	NP	Rac.	MC	NP
7.	(−)-Citronellol or racemate vs methylene chloride (MC)	Different dilutions; same 32 animals	10^{-2}	70	44	26	11	9	1	11	8	4	4	8	2	2	10	–	15	17	3	13	18	4
			10^{-3}	52	25	27	8	3	2	5	4	3	7	3	2	5	9	1	15	6	4	10	13	4
			10^{-4}	23	11	12	2	2	1	3	3	–	2	3	1	–	5	1	4	5	2	3	8	1
			total	145	80	65	21	14	4	19	15	7	13	14	5	7	24	2	34	28	9	26	39	9
							(−)-Citronellol vs Racemate						(−)-Citronellol vs Racemate						(−)-Citronellol vs Racemate					
							(−)-Cit	Rac.	NP				(−)-Cit	Rac.	NP				(−)-Cit	Rac.	NP			
8.	(−)-Citronellol vs racemate	Same 29 animals	10^{-2}	36	24	12	12	11	1				6	4	2				18	15	3			
			10^{-3}	49	37	12	14	18	5				3	5	4				17	23	9			
			10^{-4}	36	24	12	13	9	2				3	7	2				16	16	4			
			total	121	85	36	39	38	8				12	16	8				51	54	16			

[a] The results of experimental sets 6–8 describe the number of tests which were completed with 'preference' or 'no preference'/'NP' (see definitions in Material and methods) for the tested odors. [b] The results of experimental set 5 describe the combined results of 4 tests done by each animal (see Results and discussion).

Table 3. Fenchone enantiomers

Experimental set No.	Odorants	Description	Dilution (with MC)	Number of tests Total	Male	Female	Results[a,b] Male						Female						Combined					
							(−)-Fen	(+)-Fen	No Preference				(−)-Fen	(+)-Fen	No Preference				(−)-Fen	(+)-Fen	No Preference			
9.	(−)- vs (+)- fenchone	Different animals	10^{-2}	20	10	10	4	5	1				7	3	–				11	8	1			
			10^{-3}	29	17	12	8	7	2				5	5	2				13	12	4			
			10^{-4}	20	10	10	3	6	1				8	2	–				11	8	1			
			10^{-3}(b)	19	15	4	10	5	–				2	2	–				12	7	–			
			total	88	52	36	25	23	4				22	12	2				47	35	6			
							(−)-Fen vs MC			(+)-Fen vs MC			(−)-Fen vs MC			(+)-Fen vs MC			(−)-Fen vs MC			(+)-Fen vs MC		
							(−)-Fen	MC	NP	(+)-Fen	MC	NP	(−)-Fen	MC	NP	(+)-Fen	MC	NP	(−)-Fen	MC	NP	(+)-Fen	MC	NP
10.	(−)-or (+)- fenchone vs methylene chloride (MC)	Different animals	10^{-3}	43	33	10	7	5	3	8	9	1	4	1	–	3	2	–	11	6	3	11	11	1
			10^{-4}	60	30	30	9	6	–	6	9	–	11	4	–	9	5	1	20	10	–	15	14	1
			10^{-5}	20	10	10	3	2	–	2	3	–	3	2	–	2	3	–	6	4	–	4	6	–
			total	123	73	50	19	13	3	16	21	1	18	7	–	14	10	1	37	20	3	30	31	2

[a] The results describe the number of tests which were completed with 'preference' or 'no preference'/'NP' (see definitions in Material and methods) for the tested odors. [b] These tests were done in 1989 while other tests in this experimental set were done in 1990.

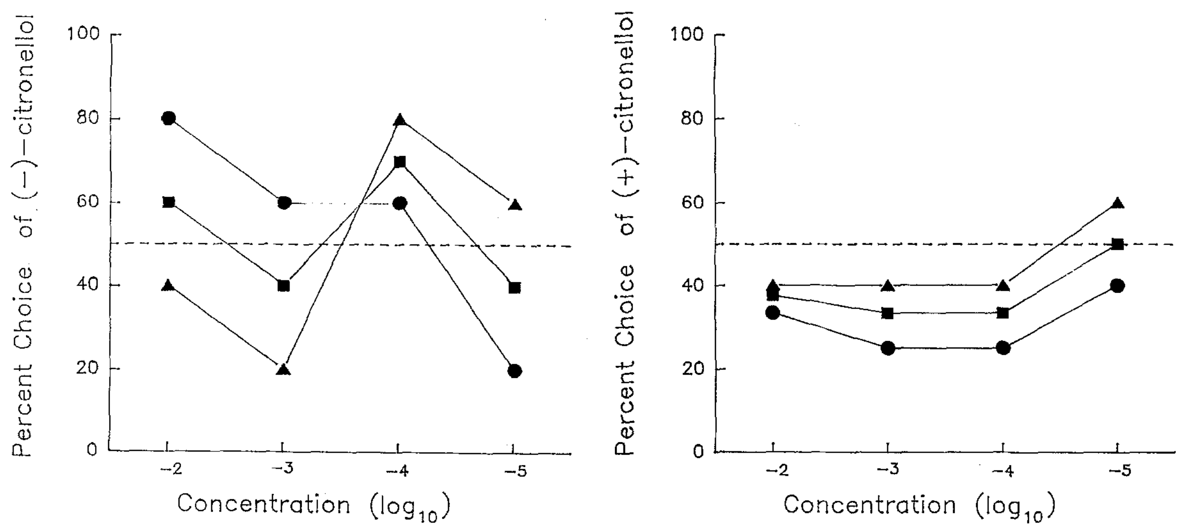

Figure 2. Percent choice of (−)- and (+)-citronellol solutions (at different dilutions) when tested against methylene chloride (see experimental set 6 in table 2).
● – males; ▲ – females; ■ – both sexes combined.

reflect a specific adaptation to life in the underground habitat.

The ability of mammals to discriminate between enantiomers has been investigated almost exclusively in humans (see literature cited in Ohloff et al.[31,32]). To the best of our knowledge, no study has been done with rodent species and only one study[20] with black-tailed deer. In many cases, the odors of enantiomers were discriminated (with varying success) by human observers[31,32], though some, such as 2-alkanone enantiomers[33], were difficult to discriminate and others, such as camphor[34] and fenchone[26], were indiscriminable. Black-tailed deer showed only a slightly stronger response for (−)-lactone, the predominant enantiomer of its pheromone (the 'deer lactone'), than to its enantiomer[20].

Recently, Cowart[26] studied human sensitivity to and ability to discriminate between the enantiomers of carvones and fenchones, and found that there was no difference in thresholds for the fenchone enantiomers (diluted in mineral oil) and that despite significant differences in thresholds for carvone enantiomers, many observers (10 of 12 for fenchones; 7 of 12 for carvones) could not distinguish between the pairs of enantiomers even at high concentrations (50–100%). By contrast, in our study the responses to odors of R-(−)-carvone, (+)-citronellol, and (−)-fenchone were observed in dilutions (in methylene chloride) as low as 10^{-4} (but were absent in the 10^{-5} dilution), while there were no observable responses to S-(+)-carvone, (−)-citronellol, and (+)-fenchone at any on the dilutions tested. In comparison to patterns of perception of these enantiomers in humans, mole rats seem to perceive the carvone, citronellol and fenchone enantiomers differently: either they perceive the enantiomers at similar detection thresholds and show their distinction between them by their preferential responses or the detection thresholds are quite different and they are hyposmic/anosmic to one member of each pair.

It would be worthwile to determine which possibility (indifference vs hyposmia/anosmia) accounts for the mole rat's peculiar perception pattern of these enantiomers and the racemates. Experimental techniques (e.g. learning models) could further examine a mole rat's ability to perceive an odorant, rather than just examining its preference for an odorant. If hyposmia/anosmias are demonstrated, the mole rat could be an appropriate research model to study the mechanisms of anosmia in mammals. Moreover, it would be interesting to assess differences in the ability of other subterranean and aboveground rodent species to discriminate between enantiomers. Such studies could contribute to a better understanding of how the mammalian olfactory system varies and functions in different life environments.

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Odour preference of a parasitic wasp depends on order of learning

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Abstract. Female *Leptopilina boulardi* wasps, larval parasites of *Drosophila melanogaster*, can learn to respond to more than one odour by associating these odours with oviposition experience. These wasps can memorise and respond to at least two different odours, and prefer the last one learnt.
Key words. Leptopilina boulardi; Hymenoptera; Eucoilidae; parasitoid; olfaction; learning; memory; olfactometer.

There is increasing evidence that insects can learn to respond to different environmental stimuli like odours, colours and shapes. Work on olfactory learning showed that classical conditioning is an important mechanism underlying behavioural plasticity in insects. The response to an odour can be changed after an association of this odour with a reward, such as food [1,2], oviposition [3,4], or a kairomone [5-7]. Koltermann [8,9] described a time-linked memory in bees. Bees relate the time of day to the learning of an odour and can be trained to respond to different odours at different times. Koltermann [9] suggested that this type of behaviour may be an example of 'state-dependent learning' [10], in which bees would associate an odour with a certain inherent physiological state. The work of Lewis and Takasu [1] showed that *Microplitis croceipes*, a larval parasitoid, can learn two novel odours associated with separate host and food resources, and make a choice between these odours on the basis of its relative host and food needs. As in bees, the memory for a particular odour seems to depend on the physiological state of the insects, as hungry wasps chose the food-associated odour, and well-fed wasps the oviposition-associated odour. Interestingly, foraging bees cannot learn two kinds of flowers at the same time of day [11]. The question arises whether insects can remember different odours independently of their physiological state. In this paper we analyse this problem by studying the effects of two successive conditionings to different odours on the orientation behaviour of *Leptopilina boulardi* Barb. et al. (Hymenoptera; Eucoilidae), a larval parasitoid of *Drosophila melanogaster* [12,13]. These odours were associated with the same reward, i.e. oviposition, so that influences of competing biological needs were avoided [1].

Materials and methods

Bioassays were performed in a four-armed airflow olfactometer designed by Pettersson [14], similar to the one described in detail by Vet et al. [15] with some modifications [3]. The olfactometer consisted of an exposure chamber connected to four arms through which air flowed (200 ml/min) into the chamber. The air was sucked through a central hole in the bottom of the chamber and created four distinct fields of equal area. Insects were introduced into the centre of the chamber and were observed for 1 min. During that time we measured the time spent in the different fields, of which one or more could be permeated with odour. One day before testing, the female wasps were allowed to oviposit in host larvae during two periods of 30 min with a 1-h interim period. For the purpose of conditioning, the wasps were kept in an airflow which could be permeated with odour, and in the presence of agar-agar infested with host larvae. Artificial strawberry (S) and banana odours (B) (supplied by Haarmann & Reimer, 92000 Nanterre, France, and used in an undilut-