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Dynamics of odour learning in *Leptopilina boulardi*, a hymenopterous parasitoid

L. KAISER*, R. PÉREZ-MALUF*†, J. C. SANDOZ*‡ & M. H. PHAM-DELÈGUE*
*Laboratoire de Neurobiologie Comparée des Invertébrés, INRA
†DNC/UESB, University of Bahia, Brazil
‡Centre de Recherches sur la Cognition Animale, Université Paul Sabatier

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We investigated the dynamics of odour learning involved in host location by a parasitoid insect, *Leptopilina boulardi* (Hymenoptera: Figitidae). Females of this species find their *Drosophila* host larvae by probing fruits with their ovipositor. They can be conditioned to respond to an odour when the odour exposure is associated with oviposition. We investigated the effect of the number of conditioning trials, sensitization tests and extinction tests on the retention of the conditioned response. Results showed that: (1) a single odour–oviposition association produced a strong short-term memory (1–2 h), which rapidly decayed over 24 h; (2) multiple odour–oviposition associations produced a memory trace that was strong in both the short term and the longer term (24 h); (3) sensitization to the odour through mere oviposition experience (without odour) was low after a single trial and high after multiple trials, but was only observed for a short period; (4) all memory traces were erased by three successive extinction tests, regardless of the intertest interval. We conclude that the probing behaviour of a *Drosophila* parasitoid is characterized by great plasticity shaped in the short term by sensitization, and in the longer term by associative learning. We compare olfactory plasticity in this parasitoid foraging for hosts and that of the honeybee foraging for food, suggesting common underlying processes in the central nervous system. Finally our results may relate to the dynamics of the foraging activity of *L. boulardi*.

Many parasitoid species can learn olfactory cues in the host-searching process (reviews in Turlings et al. 1993; Vet et al. 1995). The induction of preferences for host or substrate odours, learned either during an oviposition experience or from the rearing or emergence environment has been well studied to understand mechanisms allowing adaptation to available host resources. So far the dynamics of the learning and forgetting processes have received relatively little attention in parasitoids. Studies have examined how long a learned stimulus can be remembered (Wardle & Borden 1985; Kester & Barbosa 1991; Poolman-Simons et al. 1992; Cortesero et al. 1995; Bjorksten & Hofmann 1998). However, in studies on associative learning in a number of animal models, several parameters (e.g. number of training trials, time intervals between trials, repeated unrewarded presentations of the stimulus) influenced both acquisition and retention of memory (Carew & Sahley 1986; Rescorla 1988; Menzel et al. 1993, 2001). In particular, in the honeybee, *Apis mellifera*, another hymenopteran, behavioural studies of learning have been based on the classical conditioning of the proboscis extension, and they have been associated with neurophysiological investigations. Classical (or Pavlovian) conditioning is a process allowing an animal to memorize a stimulus (conditioned stimulus: CS, e.g. an odour) when it is associated with an unconditioned stimulus (US, e.g. food). The conditioned stimulus then becomes predictive of the reward and will elicit a conditioned response identical to the response normally elicited by the unconditioned stimulus (unconditioned response, UR, e.g. proboscis extension in bees). The acquisition and retention of the odour–food association is influenced primarily by parameters such as the temporal contingency between the presentation of the odour and the food intake, the number of rewarded odour presentations (conditioning trials) and nonrewarded odour presentations (extinction trials), and the temporal pattern of these presentations (Gerber et al. 1998; Sandoz 1998;

Correspondence: L. Kaiser, DEPSN, CNRS, 91198 Gif-sur-Yvette, France (email: kaiser@jouy.inra.fr). R. Perez-Maluf is at DCN/UESB (University of Bahia), Estrado do Bem Querer, Km04, 45000-000, Vitoria da Conquista, Bahia, Brazil. J.-C. Sandoz is at the Centre de Recherche sur la Cognition Animale, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse Cedex 4, France. M.-H. Pham-Delègue is at the CNRS Relations Internationales, 3 rue Michel Ange, 75794 Paris Cedex 16, France.

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Menzel et al. 2001). Bees' responses also vary in the short term, through sensitization to odours after food intake (Hammer et al. 1994).

Such dynamics of odour memory are also expected in parasitoids searching for hosts because, like floral resources in the case of honeybees, hosts are resources that vary in time and space. Furthermore, analogies between the learning of food odours in honeybees and that of host or host habitat odours in parasitic wasps have already been observed (Arthur 1966; Kaiser et al. 1995; Kerguelen & Cardé 1996). Evidence shows that associative conditioning is the mechanism underlying memory of host-associated odours, where host larva cues are an unconditioned stimulus, and ovipositing or contact with such US is a reinforcement (Lewis & Tumlinson 1988; De Jong & Kaiser 1991; Kerguelen & Cardé 1996).

Our aim in the present study was to investigate the dynamics of odour memory involved in host-searching behaviour by the *Drosophila* parasitoid *Leptopilina boulardi* Barb. et al. (Hymenoptera, Figitidae). In this species, it is possible to condition the probing behaviour to an odour. Females maintained in an agar caplet placed in an airflow are conditioned to probe the agar in response to a fruit odour (CS), when odour exposure has been previously reinforced by an oviposition into a host larva (US) (Kaiser et al. 1995). Under natural conditions, females find the digging *Drosophila* host larva by probing wounded areas of fruits with their ovipositor (Vet & Bakker 1985). This behaviour is triggered initially by the perception of larval products and vibrations (Vet & Bakker 1985; Vet et al. 1993).

Based on the odour conditioning of ovipositor probing, we analysed how parameters of the conditioning and test procedures influenced odour memory in *L. boulardi*. We evaluated how probing responses are affected by (1) the number of conditioning trials, (2) sensitization trials through oviposition experience only and (3) the interval between repeated test trials.

METHODS

Parasitoids

Leptopilina boulardi females (strain G464 'Nasrallah', Dr Yves Carton, PGE-CNRS, Gif-sur-Yvette, France) were allowed to infest larvae of the Rosy 295 mutant of *D. melanogaster* (a mutant selected for a high production of *L. boulardi*; Y. Carton & F. Frey, personal communication) which had been fed on an axenic diet (corn meal, dead powdery yeast, sugar and a fungicide). To minimize the olfactory experience of parasitoids before the experiments and to prevent the development of fungi, pupae of infested *Drosophila* were collected, washed in 5% bleach, rinsed in water and stored at 25°C on a 12:12 h light:dark photoperiod in tubes containing agar-agar and honey. Females mated as soon as they emerged. After emergence of the parasitoids, tubes were stored at 17°C and distributed into two photoperiods, 0900–2100 hours and 1300–0100 hours, to homogenize the levels of activity of females observed in the morning and in the afternoon. We conducted experiments with 5–7-day-old, mated females, within 4 h of the beginning of the light cycle, at 20–22°C.

Conditioning and Test Device

We introduced a single female into a device that allowed simultaneous or separated presentation of larvae and an odour. It consisted of a ring of agar-agar (internal diameter: 6 mm) placed on a nylon disc in a plastic caplet pierced in its centre, and with an acetate lid perforated with pin holes. The female was kept in the centre of the ring of agar, of which she could probe the inner side. A continuous airflow (520 ml/min), which could be odorized, arrived from underneath the device, with a gap of 2 mm separating the air outlet and the caplet. Thus, the actual flow through the caplet was lower than 520 ml/min and did not disturb the wasp (Kaiser et al. 1995). Part of the flow could be shifted into a secondary airflow including an odour source, and which flowed into the main flow through a Pasteur pipette just beneath the caplet.

Odour Source

Two glass capillaries (1 cm long, 1.56 mm internal diameter) with one extremity sealed with wax were filled with banana extract (a commercial aroma for food industry, Haarman and Reimer, St-Ouen-l'Aumone, France). They were placed in a glass vial connected to the secondary airflow.

Conditioning and Test Protocols

Conditioning phase

About 20 second-instar *Drosophila* larvae were deposited on the ring of agar just before the insertion of a female *L. boulardi* to be conditioned. We performed two types of conditioning procedures, as follows. (1) Conditioning trials (CS/US groups): the odour was delivered during the oviposition, that is, while the wasp had its ovipositor inserted into a larva. This constituted one conditioning trial. In case of several conditioning trials, there was no odour delivery between the ovipositions, when females were searching for larvae. During the period between conditioning and the test, females were placed on clean agar, either in a clean caplet when the test occurred the same day, or in a small 2-ml plastic vial with agar in the bottom when the test occurred 24 or 48 h after the conditioning. (2) Sensitization trials (US groups): females were allowed to perform ovipositions without odour delivery.

With or without odour delivery during oviposition, the mean time ± SE elapsed between the end of an oviposition and the initiation of the next one, corresponding to an intertrial interval (ITI), was 40 ± 3 s when 20 host larvae were present on the agar ring (from Kaiser et al. 1995 and confirmed in the present study). The standard error of the ITI comes from the fact that oviposition occurs when the parasitoid finds a larva. During this ITI, 72% of the time is spent probing, that is,

searching for the next larva; other occasional activities are standing still, walking without probing and preening (Kaiser et al. 1995).

Other control procedures, that is, CS alone and backward US/CS groups, were tested in a previous study and had no effect on probing responses to the CS compared with a group of naïve females (Kaiser et al. 1995), so we did not use them here.

Test phase

The female was placed into a ring of clean agar (without larvae). For the first minute the airflow was not odorized, so that the insect could become familiar with the device. Banana odour was then delivered for 20 s (the mean duration of an oviposition). We recorded the occurrence of a probing response in the minute following the initiation of odour delivery. If the female did not probe the agar within this time, it was counted as not responding to the odour (mean latency of response was about 15 s after the initiation of odour delivery).

Experimental protocols

In experiment 1 we investigated the effect of the number of conditioning trials on probing responses. There were either one, five or 10 conditioning trials and three tests at 12 min, 2 h and 24 h or one to five conditioning trials and two tests at 1 and 24 h.

In experiment 2 we investigated the effect of sensitization trials on probing responses. There were one or five sensitization trials versus one or five conditioning trials, and a single test, 2 or 24 h after the conditioning phase.

In experiment 3 we investigated the effect of the interval between repeated test trials. There were either one or five conditioning trials, and three grouped tests at 2 h, 2 h 5 min and 2 h 10 min after the conditioning phase with a fourth test 24 h later to look for spontaneous recovery of conditioned responses, or one or five conditioning trials, and three spaced tests at 2, 24 and 48 h after the conditioning phase. We added a fourth test because a rapid decrease in conditioned responses between tests may correspond to a temporary extinction of responses without real destruction of memory, allowing a possible spontaneous recovery after a delay (Rescorla 1997a, b).

Data Analyses

To compare frequencies of responding females we used the chi-square test, at a corrected threshold of significance when needed, depending on the number of comparisons (k) made on the same data sample: $\alpha' = 1 - (1 - \alpha)^{1/k}$ (Dunn–Sidak correction, in Sokal & Rohlf 1995). With $\alpha = 0.05$ and $k = 2$ for example, $\alpha' = 0.025$. When comparing the responses of the same wasps between repeated test trials, we used the McNemar test (two test trials) or Cochran's Q test (three tests). When differences were significant, Cochran's Q test was complemented with pairwise comparisons using the Marascuilo and McSweeney method (Zar 1999).

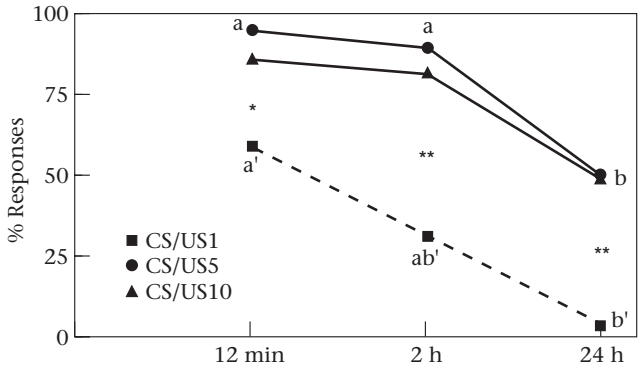

Figure 1. Odour memory after one, five or 10 conditioning trials. Percentages of conditioned responses to banana odour in associative conditioning groups (CS/US) with one, five or 10 conditioning trials, at three repeated tests. CS/US1: $N=22$; CS/US10: $N=20$; CS/US5: $N=20$. Asterisks indicate significant difference between one and five conditioning trials, at the 5% level, i.e. at the corrected $\alpha'=0.025$ (*), or at the 1% level, i.e. at the corrected $\alpha'=0.005$ (**) level. Within each conditioning group, different letters indicate a significant decrease in the proportion of conditioned responses along the repeated tests (pairwise comparisons using the Marascuilo & McSweeney method, after the Cochran Q test, at the 5% error level).

RESULTS

Experiment 1: Number of Conditioning Trials

One, five and 10 conditioning trials

We first compared groups with one, five and 10 conditioning trials, subjected to three repeated tests (Fig. 1). At the first test 12 min after conditioning, probing responses to banana odour were expressed by 59% of females after one conditioning trial and 95% after five trials (chi-squared test: $\chi^2_1=7.36$, $P=0.006$), and 10 conditioning trials did not induce more responses (86%; $\chi^2_1=0.88$, $P=0.34$). At the second test 2 h after conditioning, 32 and 90% of responses were observed after one and five conditionings, respectively ($\chi^2_1=11.2$, $P<0.001$), and 82% after 10 conditionings (5 versus 10 conditionings: $\chi^2_1=0.45$, $P=0.449$). At the third test at 24 h, groups with one and five conditionings produced, respectively, 4.5 and 50% of responses to banana odour ($\chi^2_1=11.46$, $P<0.001$), and 50% of responses was also observed in the group with 10 conditionings.

The percentage of responses after one conditioning trial decreased rapidly with repeated tests: it decreased by half at the second test 2 h after conditioning, and only one of 20 females still responded at the third test. This progressive decrease was significant (Cochran's Q test: $Q_2=16.62$, $P<0.0003$; Marascuilo & Sweeney pairwise comparisons in Fig. 1). However, the percentage of responses after five or 10 conditioning trials decreased only at the third test, where 50% of females still responded in both groups (CS/US5: $Q_2=16.22$, $P<0.0003$; CS/US10: $Q_2=10.36$, $P<0.0056$; see Fig. 1 for pairwise comparisons). Thus, compared to one conditioning trial,

Table 1. Odour memory after one to five conditioning trials

	Number of conditioning trials				
	1	2	3	4	5
1st test (at 1 h)	77.4	90.4	83.9	83.9	83.9
2nd test (at 24 h)	29.0	38.7	35.5	35.5	58.1
McNemar χ^2_1	9.39*	14.06**	8.52*	10.32*	3.5

Percentages of conditioned responses to banana odour in associative conditioning groups (CS/US) with one to five conditioning trials, tested twice, at 1 and 24 h after conditioning. McNemar test shows comparisons between both repeated tests, within each group (N=31 per group).
*P<0.01; **P<0.001.

memory induced by five or 10 conditioning trials lasted longer and resisted better extinction by repeated tests.

One to five conditioning trials

When tested 1 h after conditioning, the percentages of females exhibiting a conditioned probing response to banana odour were high without any significant difference caused by the number of conditioning trials (χ^2_4=1.9, NS; Table 1). When tested again 1 day later, conditioned responses appeared less frequent when females had one to four conditioning trials than when they had five conditioning trials (Table 1). Although the overall increase in conditioned responses after one to five conditioning trials was not significant (χ^2_4=6.3, NS), the values for the two extreme groups differed significantly (χ^2_1=5.31, P<0.021). Responses during the second test were significantly lower than those recorded at the first test in groups conditioned with one to four trials, but not in the case of five conditioning trials (Table 1). Thus, even after a single conditioning trial, wasps showed high responses to the odour in the short-term test (1 h), but only five conditioning trials led to higher responses in the long-term test (24 h).

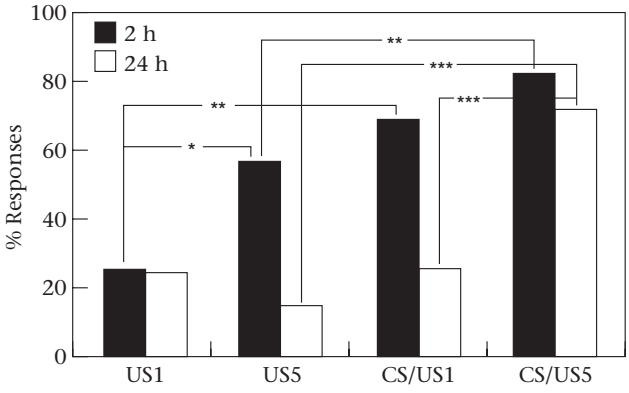

Figure 2. Influence of sensitization trials. Percentages of responses to banana odour in associative conditioning (CS/US) or sensitisation (US) groups with one or five ovipositions. Independent groups were tested at 2 and 24 h. Test at 2 h: CS/US1: N=22; CS/US5: N=22; US1: N=30; US5: N=30; test at 24 h: CS/US1: N=48, CS/US5: N=48; US1: N=28, US5: N=28. Chi-square test: significant differences, according to type of conditioning (CS/US versus US), or number of ovipositions (one or five) indicated by asterisks. Level of significance of number of asterisks as in Fig. 1. α=0.0005 (***).

Experiment 2: Effect of Sensitization Trials

Single test 2 h after conditioning

We first compared the frequencies of responses between CS/US and US groups. When insects were tested for the first time 2 h after conditioning, we observed 68% of probing responses after one associative conditioning and 26% after one sensitization trial (chi-square test: χ^2_1=9.4, P<0.01). Similarily, after five trials, 81% of responses arose from associative conditioning and 57% from sensitization trials (χ^2_1=7.2, P<0.01). Second, we compared the frequencies of probing responses after different numbers of ovipositions (Fig. 2). After sensitization trials, the group with five ovipositions showed 56% of responses, the group with one oviposition showed 25% (χ^2_1=6.0, P<0.025). In the case of associative conditioning, 81% of responses were observed after five trials, and 68% after a single trial, but this difference was not significant at the 2-h test (χ^2_1=3.5, NS). One sensitization trial did not increase the percentage of probing responses to banana odour, compared to the value of ca. 20% obtained with naïve females (Kaiser et al. 1995), whereas five sensitization trials and one or five associative conditioning trials significantly increased the frequency of responses 2 h after conditioning.

Single test 24 h after conditioning

Compared to data measured 2 h after conditioning, frequencies of responses after 24 h were much higher in the group that had five associative conditioning trials (71%) than in the corresponding sensitization control (14%; χ^2_1=22.6, P<0.001) and in the group with one associative conditioning trial (25%; χ^2_1=20.2, P<0.001). The frequencies of responses measured after one associative conditioning trial or one sensitization trial were equally low (25%) and close to the frequency of responses known for naïve females. The frequency of responses did not change from one to five sensitization trials (25% and 14%, respectively, N=28 in both groups; χ^2_1=1.7, NS; Fig. 2).

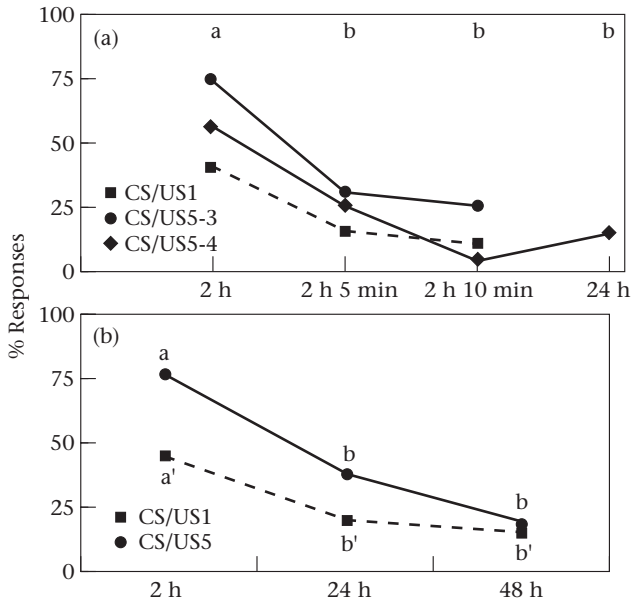

Figure 3. Influence of the interval between repeated tests on the resistance of odour memory to extinction. (a) Grouped tests: percentages of conditioned responses to banana odour at three repeated tests 5 min apart and to a fourth test at 24 h. (b) Spaced tests: three repeated tests 24 h apart. Values with the same letter are not different (pairwise comparisons using Marascuilo & McSweeney method after the Cochran *Q* test, at the 5% error level). In (a) as a statistically similar decrease was observed in the three tested groups, letters are not repeated for each group. (a) CS/US1: *N*=20; CS/US5-3: *N*=20; CS/US5-4: *N*=28. (b) CS/US1: *N*=20; CS/US5: *N*=21.

Experience 3: Effect of Interval Between Test Trials

Three grouped tests

After both one and five associative conditioning trials, the frequencies of conditioned responses decreased by half when insects were tested a second time 5 min after the first test at 2 h, but no decrease was observed between the second and third tests. The decrease in conditioned responses was significant in both conditioning groups (Cochran *Q* test: CS/US1: Q_2=7.75, P<0.05; CS/US5: Q_2=14.0, P<0.001; Fig. 3a). This result points out the negative effect of repeating the odour presentation without the oviposition reinforcement. When we repeated the experiment with the CS/US5 group, adding a fourth test 24 h later (recovery test), we found a general decrease in responses with repeated tests (Q_3=23.6, P<0.001), from 57% at the first test to 3% at the third test. At the recovery test, no significant increase in responses occurred (from 3 to 14%; Marascuilo & McSweeney S=0.91, threshold S=2.76, NS), suggesting that no spontaneous recovery took place after extinction.

Three spaced tests

For both CS/US1 and CS/US5 groups, responses decreased along the three successive tests with 24-h intervals (Cochran's *Q* test: CS/US1: Q_2=8.85, P<0.05; CS/US5: Q_2=16.0, P<0.001; Fig. 3b). Again the frequencies of conditioned responses decreased by half in the second test, from 45 to 20% in the group with one conditioning trial and from 76 to 38% in the group with five conditioning trials. Frequencies of responses did not decrease further at the third test.

DISCUSSION

Our experiments established that: (1) a single odour–oviposition association produced a memory trace that was strong at short term (after 1–2 h), but disappeared over 24 h; (2) multiple odour–oviposition associations produced a memory trace that was strong at both short and long term (24 h); (3) sensitization through mere oviposition experience was low after a single trial and high after multiple trials, but was observed only at short term; (4) all memory traces were highly sensitive to repeated unrewarded odour presentation.

These results allow us, for the first time, to characterize in a parasitic wasp different processes involved in the induction of conditioned olfactory responses at short and long term. At short term, a single odour–oviposition association produced high probing responses, and more associative trials did not always increase the response probability. We found that sensitization through oviposition experience affected responses only at short term. After a single oviposition experience, we observed almost no effect, but after multiple experiences we found strong probing responses. Thus, probing responses at short term were the product of two parallel processes with different properties: first an associative memory trace, which developed at the first associative trial, and nonassociative sensitization, which increased with increasing trial numbers. Such sensitization was, however, short lived, and was not observed after 24 h.

We can thus propose a model for the organization of memory phases in *L. boulardi* (Fig. 4), as has already been developed for other insects, such as the honey bee (Menzel et al. 1993; Menzel 1999) and *Drosophila* (Tully et al. 1994). We postulate two memory phases: (1) a short-term memory (STM) phase which is created by a single conditioning trial, and which can sometimes have an important nonassociative component, through sensitization; (2) long-term memory (LTM) phase, which appears only after multiple conditioning trials. This long-term memory phase does not appear to last a lifetime in *Leptopilina*; preliminary results from 10 females indicate that only a few females probe banana odour 48 h after conditioning (L. Kaiser & R. Perez-Maluf, unpublished data).

When studying conditioned orientation to odours learned by associative conditioning in previous studies, we found high percentages of responses 24 h after conditioning (e.g. De Jong & Kaiser 1991; Kaiser & De Jong 1995), but few responding females 48 h after conditioning (L. Kaiser & R. De Jong, unpublished data). Similarly, Poolman-Simons et al. (1992) reported conditioned choices for 24 h in most *L. boulardi* females, for 48 h in a lower proportion, and no further conditioned responses 3 days after the experience. Thus 48 h would be the maximum duration of odour memory involved in different

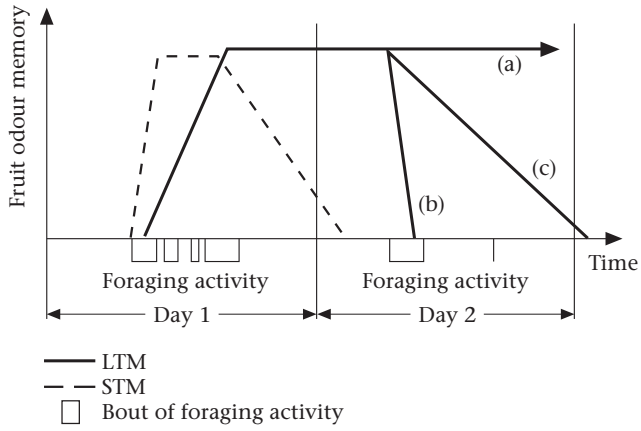

Figure 4. Model of memory course in *L. boulardi* postulating two memory phases: (1) a short-term memory (STM) phase lasting a few hours which is created by a single conditioning trial, and which may have an important nonassociative component, through sensitization; (2) long-term memory (LTM) phase, lasting overnight, which appears only after multiple conditioning trials. LTM could follow three possible processes. It could (a) be renewed by successful ovipositions on the same fruit type; (b) rapidly decline after perception of the fruit odour without larvae, causing females to leave a fruit with few or no host larvae; (c) slowly wane if the female does not forage because of poor atmospheric conditions. See text for further explanation.

host-searching activities in *L. boulardi*, although females would live for about 2 weeks in nature (Carton et al. 1986). However, longer retention duration might be found in *L. boulardi* by changing some parameters of the learning procedures. We subjected wasps to up to 10 conditioning trials, but could not prolong the duration of long-term memory. However, females are likely to experience several dozens of trials a day in nature.

Another parameter that strongly affects both acquisition and retention is the interval between repeated trials (intertrial intervals). In our experiments, intertrial intervals in the conditioning phase were relatively short (40 s) because wasps placed on the agar encountered larvae with a high probability and therefore oviposited with a high frequency. Thus, the conditioning phases were performed in a massed fashion (short intertrial intervals), which in other animal models produces slower acquisition and shorter retention than conditioning with spaced trials (Tully et al. 1994; Menzel et al. 2001). In honeybees, a particular form of long-term memory, which allows higher retention of odour–food associations for 3 days or more, depends on the synthesis of new proteins and is triggered only by multiple trials performed in a spaced schedule (Menzel et al. 2001). Experiments in which intertrial intervals could be precisely controlled, for instance by controlling the availability of larvae (the US) in the apparatus, could help to identify new memory forms in *Leptopilina*.

The odour–oviposition association is highly sensitive to unrewarded presentation of the CS. When we repeated tests 2 h after one or five conditioning trials, responses decreased rapidly with unrewarded presentations of the odour, so that the level of response was reduced by half at the second test and reached the level of naïve females at the third test. This result was obtained when the tests were applied with short (5 min) and long (24 h) intertrial intervals. As we wanted to test whether the decrease in responses observed during extinction could be related to a transient inhibitory process or to a real memory decrement, we explicitly tested for spontaneous recovery in experiment 3. Spontaneous recovery of responses after a period of rest after extinction has already been observed in several species (rats: Rescorla 1997a, b; honeybees: Sandoz 1998; Menzel et al. 2001). In these cases, extinction is interpreted as the occurrence of a transient process that inhibits responses to the CS but does not produce a memory decay; then after a period of rest, this inhibition disappears, and responses to the CS are found again. In *Leptopilina*, we found no evidence for spontaneous recovery, which suggests that the reduction in responses that we observed during extinction was indeed due to a memory decay.

In one case, when the extinction phase started earlier after conditioning (12 min, experiment 1), the second extinction test appeared to have almost no effect on the frequency of probing responses by wasps conditioned in five or 10 trials. This result could mean that spontaneous recovery could have taken place between the first and the second test, so that responding after 24 h (second test) was still very high. This interpretation would fit with results obtained in honeybees, that spontaneous recovery appears only when the interval between conditioning and testing is short (Sandoz 1998).

With regard to the dynamics of learning, the relation between temporal characteristics of associative learning and behavioural activity under natural conditions has been particularly well explored in the honeybee (Menzel et al. 1993; Menzel 1999). One can distinguish the formation of a short-term memory phase that may serve within a foraging bout, and the formation of a long-term memory phase that would serve when foraging is interrupted by a return to the hive, or when bees resume foraging activity from day to day. We can also interpret our results in relation to the foraging behaviour of *L. boulardi*. *Drosophila melanogaster* females aggregate on oviposition sites (wounds on ripe fruits) where tens to hundreds of larvae can then develop (Pearl 1932). *Leptopilina boulardi* females emerge with 200–300 mature eggs (Carton et al. 1986), so they will usually perform more than one oviposition on a patch of host larvae, unless accidentally interrupted. In the studied strain, the daily parasitic activity is limited to a couple of hours and resumes on the next day (Fleury et al. 2000). During the period of activity, females take breaks and visit several host patches, even when their host population is far from being fully parasitized (Mangel 1993; L. Kaiser, unpublished data). This behaviour is consistent with evidence that in the *Leptopilina* genus, females are more time than egg limited, which is usually the case for proovogenic parasitoids. Under time-limited conditions, selection will favour increasing the host encounter rate rather than selecting the better host (which is likely to occur under egg-limited conditions; Mangel 1993; Vet et al. 1995). The dynamics of conditioned responses to fruit odour in

L. boulardi can be viewed as a mechanism enhancing host encounter rate. Short-term memory (highly dependent on sensitization) and long-term memory (highly associative) would be involved, respectively, in two phases of the parasitic activity (Fig. 4). First, throughout the same day, sensitization of the probing activity to fruit odour, lasting for at least 2 h, would be the main process maintaining a high searching activity during a foraging bout on one infested fruit and between foraging bouts (Fig. 4, day 1). Second, on the next day, associative learning would be involved solely in remembering the fruit odour when the insect resumes a period of searching activity (Fig. 4, day 2). Rapid decline of conditioned probing after perception of the fruit odour without larvae would help females to leave a fruit with few or no host larvae. Considering that females renew their associative conditioning by daily ovipositions, a memory lasting more than 24 h would be little used.

In conclusion, our study showed that the probing behaviour of a *Drosophila* parasitoid is characterized by great plasticity shaped in the short term by sensitization, and in a longer term by associative learning. This result is consistent with the general properties of associative learning in different animal models. In particular, we established analogies between olfactory plasticity in this hymenopterous parasitoid foraging for hosts and the honeybee foraging for food, suggesting underlying nervous processes of olfactory plasticity common to both types of Hymenoptera. The characterization of learning dynamics in parasitoids is of interest for two research fields. First, with regard to biological control, manipulation of parasitoids' behaviour through learning has been examined to increase parasitic efficiency on a target host, but with only limited success. A possible explanation for this result is the waning of the conditioned searching responses depending on the frequency of encountering hosts, that is, of reinforcements (Lewis & Martin 1990). Our results confirm this explanation. A related field of investigation is the search for optimality in foraging behaviour in an evolutionary context, but so far the contribution of learning has been considered in terms of host preferences, and not in the exploitation of host patches. Our results show that sensitization and extinction produce highly plastic responses to host cues, and could be key factors in the optimality of patch exploitation.

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