



Population differences in spatial learning in three-spined sticklebacks

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In a changing environment, learning and memory are essential for an animal's survival and reproduction. The role played by the environment in shaping learning and memory is now attracting considerable attention. Until now, studies have tended to compare the behaviour of two, or at best a few species, but interspecific comparisons can be misleading as many life history variables other than environment may differ between species. Here we report on an experiment designed to determine how learning varies between different populations of the same species, the three-spined stickleback. We found differences between the populations in their ability to solve a spatial task and also in the spatial strategies they used. A second simple learning task showed that these differences were not the result of gross differences in learning ability or adaptation to laboratory conditions. We discuss these results and suggest that the behavioural differences may relate to features of the respective habitats from which the fish were sampled.

Keywords: three-spined sticklebacks; spatial memory; discrimination learning

1. INTRODUCTION

The way in which ecology influences learning and memory has recently received considerable attention (Yoerg 1991; Giraldeau 1997). Learning enables an animal to adapt to changing circumstances, and thereby modify its behaviour to suit the characteristics of the local environment. For example, Potting *et al.* (1997) showed that only parasitic wasp species living in relatively heterogeneous environments learned odour cues to locate suitable host microhabitats. Species from homogeneous environments did not use learned odour cues, but instead relied on innate responses to specific stimuli. In addition, foraging efficiency and memory retention has been shown to vary between closely related species of stickleback (*Gasterosteidae*) from marine, estuarine, and freshwater habitats (Mackney & Hughes 1995). Those forms inhabiting more variable environments, and therefore experiencing greater prey diversity (i.e. marine and estuarine forms), exhibited a shorter memory for foraging skills associated with particular prey types than the freshwater population. This was sampled from a landlocked pond which was presumed to contain a smaller diversity of prey species, a circumstance under which the ability to remember particular prey-handling skills for longer would be adaptive. Furthermore, Micheli (1997) found that the feeding behaviour of two species of predatory crab was influenced by their local environments. The crab species that ranged over a large area, encountering a variety of prey, was better able to learn and remember

a new foraging task compared with the other less mobile species, which usually fed on one type of prey.

Although the studies outlined above imply that the environment has an effect on information learned and remembered, most of the evidence that ecology may influence learning and memory typically comes from interspecific comparisons employing two, or at most a few species. Many characteristics other than learning and memory, however, will differ between species, and so it may be difficult to attribute interspecific behavioural differences to any particular cause. To overcome this problem, either comparisons between large numbers of different species should be made or, alternatively, comparisons should be made within one species. An ideal species for an intraspecific comparison investigating the effects of ecology on learning and memory is the three-spined stickleback (*Gasterosteus aculeatus*) because these fish naturally occupy a wide range of aquatic habitats, such as small ponds, larger lakes and rivers (Bell & Foster 1994).

We investigated two types of learning task in four populations of three-spined stickleback selected from different habitats. The first, a spatial task, tested the ability of the different populations to solve two types of maze by using various orientation cues. Spatial ability has been related to ecology in several studies involving closely related species of birds and mammals (Hampton & Shettleworth 1996; Jacobs *et al.* 1990; Sherry *et al.* 1992). Other studies have shown that a hierarchy of spatial cue systems can be employed for orientation (see Able (1993) for a review; Braithwaite *et al.* 1996), and the preferred spatial strategy can be influenced by the environment experienced during development (Wiltschko *et al.* 1987; Braithwaite & Guilford 1995). The hypothesis we wanted

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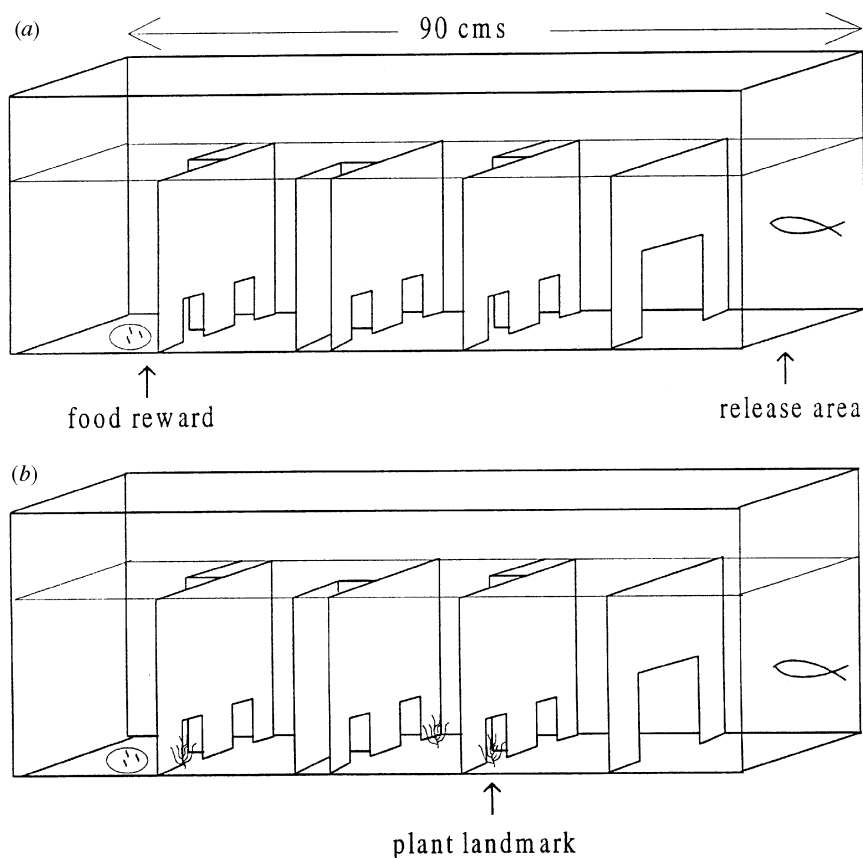


Figure 1. Experimental fish tank set-up for experiment 1 viewed from the side, (a) without plant landmarks (plain maze) and (b) with plant landmarks.

to address was whether different types of habitat influence the spatial strategy used by three-spined sticklebacks to solve two types of maze task. One maze was designed to determine whether sticklebacks used a learned sequence of turns, or an algorithm to negotiate their way through the maze, and the second type, to see if they could rely on using visual cues acting as beacons marking the desired route. Fish from pond populations might be expected to perform better in the presence of visual landmarks. Within a pond, the visual surroundings can be considered as relatively stable, and visual cues might therefore provide reliable orientation information as fish move around their environment. River populations, however, might not be expected to rely so much on visual landmarks as their natural surroundings could be made visually unstable by water movements. It would therefore be less adaptive to remember information about the position of visual landmarks in a river environment.

The second task aimed to examine the ability of the four populations to adapt to laboratory conditions, and to perform a simple learning task in artificial surroundings. We wanted to ensure that any behavioural differences we found in experiment 1 were not simply a result of gross differences in cognitive ability, or in ability to perform a task under laboratory conditions. To investigate this, fish were required to discriminate between two colours, and associate one of them with a food reward. We did not expect this task to be difficult for the sticklebacks, as it is widely known that they have good colour vision and rely heavily on this sense to recognize and locate prey (Fitzgerald & Wootton 1993).

2. METHODS

(a) *Experiment 1*

This experiment was designed to determine whether fish from different populations use the same spatial cue systems to solve a maze task in the presence and absence of visual landmarks.

Twelve three-spined sticklebacks were collected from each of four populations. These were located in Inverleith Pond, Edinburgh; Balmaha Pond, Loch Lomond; River Kelvin, Glasgow; and River Endrick, Loch Lomondside. Fish were sampled outside the breeding season to prevent reproductive behaviour affecting the results. Each population was held in a different 30 cm × 40 cm × 30 cm holding tank, on a diet of chironomid larvae for a four-week settling period. The temperature in the laboratory was constant at 11 °C, with a constant light:dark cycle of 10:14. All four samples were of similar mean body length (Inverleith, 4.40 cm; Balmaha, 5.03 cm; Endrick, 4.59 cm; Kelvin, 4.77 cm). We used this mean body length as an approximate measure of age, estimating the fish to be between 8 and 12 months.

A line maze was set up in a 90 cm × 30 cm × 30 cm tank in 20 cm depth of water. To reduce the use of extra-maze cues, the tank was surrounded by black card. On one side, the card was raised 10 cm from the tank thus enabling the observer to watch the fish through a small slit in the card without disturbing the fish. Lighting was provided by two fluorescent lights, which ran across the plane of the experimental tank but could not be seen from it. The tank was divided across its width by three plain walls made from plastic-coated white card, 15 cm apart, each of which had two evenly spaced doors (6 cm × 3 cm). At one end of the tank there was a 15 cm × 30 cm release site with a trap-door

which could be raised using a pulley system with minimum disturbance to the fish (see figure 1). To begin a trial, a single fish was carefully dip-netted from its holding tank and allowed to settle in the release area for 5 min after which the trap-door was raised. Timing did not begin until the fish chose to swim out through the trap-door. At the other end of the tank, a food reward of three bloodworms, secured in a vaseline-filled Petri dish, was used to encourage the fish to swim through the maze. Each population was divided into two groups: one group was tested in a plain maze, the second group in a maze containing visual landmarks (see figure 1). The fish were individually tagged with coloured plastic rings attached to their spines during the fourth week of the settling period.

(i) *Pre-training*

During the pre-training period, fish were familiarized with the set-up by transferring single fish from the holding tank to the release area, and after the 5-min settling period, releasing them and allowing them to swim the length of the tank to obtain a food reward once a day. At this stage, all the doors were open. For group 1, the maze had no landmarks present (NoLM). For group 2, landmarks (small identical plants) were placed next to all the doors (LM). Fish were returned to their holding tank 5 min after their first contact with the food reward. Fish were not fed in their holding tanks during the experiment, so their only opportunity to feed was to consume the maze food reward. After seven pre-training runs, the fish had learned to swim through the doors and directly obtain the food reward. The maze was then modified for the training phase.

(ii) *Training phase*

During training, one door on each wall was modified to lead into a dead-end, so that the fish now had to swim a particular route to pass through the maze efficiently. Group 1 (NoLM) remained without landmarks (figure 1a), whereas for group 2 (LM) a landmark was positioned next to the open doors only (figure 1b). Fish learned to negotiate the series of open and closed doors until they reached a criterion performance level of three consecutive trials where food was located in less than 150 s. This time represented a direct swim through the maze, with few, if any mistakes being made, and the doors leading to dead-ends being ignored. During the first trial of the training phase, the mean time for a group to complete the task was between three and eight times longer than 150 s, so performance greatly improved during this phase. Trials were between 36 and 48 h apart to maintain a reasonable level of hunger motivation in all subjects. We recorded the number of trials taken to reach the criterion performance, the time fish took from leaving the release area until first contacting the food, and the number of times an incorrect choice was made upon first approaching a wall. When the criterion was reached, the maze was subjected to one final modification for a single test trial.

(iii) *Test trial*

The open and closed doors were now reversed, such that previously open doors now led to dead-ends, and vice versa. For group 2 (LM), the landmarks were moved to the new open doors, thereby remaining reliable indicators of the route through the maze. Fish were given only one test trial in the modified maze. Again, the time from leaving the release area until first contact with the food reward, and the number of times an incorrect choice was made on the first approach to a wall were recorded for comparison with performance in the last trial of the training phase.

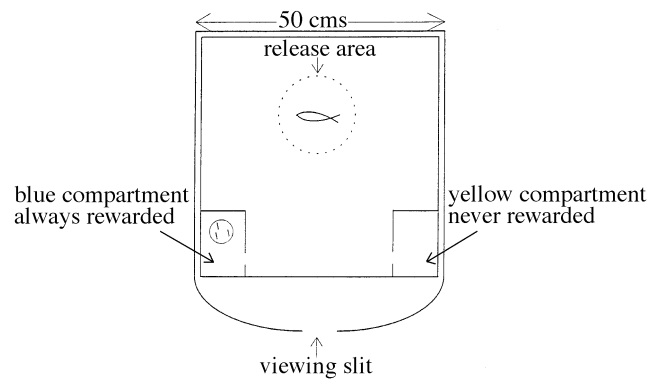


Figure 2. Plan view of experimental tank set-up for discrimination experiment.

(b) *Experiment 2*

Experiment 2 investigated discrimination learning in the same four populations of sticklebacks used in experiment 1. Fish had to discriminate between two colours to locate a hidden food reward. A control test was also done with one of the populations to verify that the fish were using visual cues and not olfactory information to locate the food reward.

Ten new fish were sampled from each of the four populations in experiment 1, and allowed to settle for four weeks in the laboratory under the same conditions. Each sample was of similar mean body length (Inverleith, 4.48 cm; Balmaha, 5.31 cm; Endrick, 4.47 cm; Kelvin, 4.80 cm). During the settling period, fish were maintained on a diet of bloodworms, and were individually tagged as before.

A square-based test tank (50 cm × 50 cm × 30 cm) contained two compartments (3 cm × 4 cm × 15 cm) built from plastic building bricks (Lego). These were positioned in the two front corners of the tank (see figure 2). One compartment was blue, and always contained a food reward consisting of three bloodworms secured in a Petri dish of vaseline (diameter 5 cm). The other compartment was yellow, and never rewarded. The two compartments were randomly switched between the sides of the tank between trials to prevent the fish associating a position with the food reward. The test tank was screened using black card in the same way as in experiment 1. A pre-training period involved groups of ten fish from single populations experiencing a 24 h period in the test tank, at the start of which the blue compartment was baited copiously. This pre-training was repeated five times to enable fish to learn which compartment was rewarded. By the end of this phase, the fish were used to the experimental set-up, and were calm and eager to locate food when transferred into the test tank.

The test phase consisted of ten trials, one trial per fish per day (fish rested for at least 36 h between trials to maintain a high level of motivation to find the food reward). After being netted individually from the holding tank to the release area (see figure 2), and allowed a settling period of 5 min, fish were released into the experimental tank, and the order in which the compartments were visited was recorded. Fish were left in the test tank for 5 min after their first contact with the food reward to reduce the chance of an association arising between completion of the task and being netted from the experimental set-up.

(i) *Control test*

Three-spined sticklebacks are classed as a microsmatic species, and use vision to locate food (Wootton 1976) rather than olfaction. However, to check that fish were not able to find the

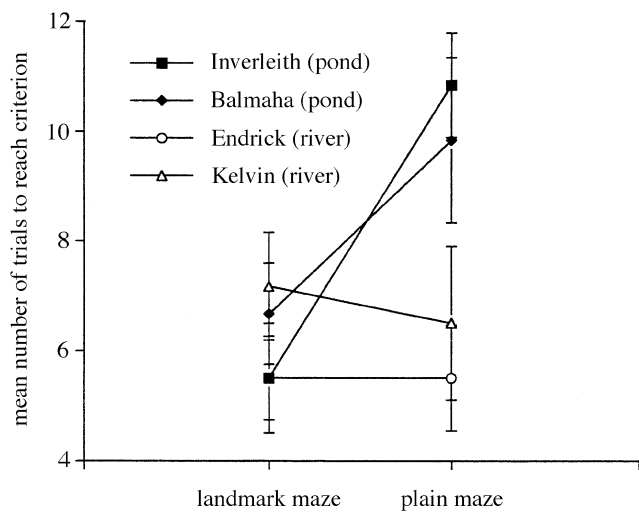


Figure 3. Mean number of trials to reach criterion performance for each population, for the plain maze and the landmark maze.

food reward using olfactory cues we included a control test. This was done with ten naive fish from one of the populations (Inverleith Pond) using two white compartments, one of which was baited with a food reward. Apart from the colour of the compartments, the control test was carried out according to the same criteria described in the previous paragraph, and under the same laboratory conditions.

3. RESULTS

(a) Experiment 1

We examined whether the number of trials required to reach the criterion varied between populations. A two-way analysis of variance (ANOVA), with the number of trials to reach the criterion as the dependent variable, revealed a significant main effect of population ($F_{3,40}=2.85$, $p=0.049$). The type of maze (i.e. LM or NoLM) also affected performance ($F_{1,40}=6.5$, $p=0.015$) and there was an interaction between population and maze type ($F_{3,40}=3.3$, $p=0.03$). Figure 3 shows that Inverleith Pond and Balmaha Pond populations took longer to learn the task when the plant landmarks were absent from the maze than when they were present. Both the River Endrick and Kelvin populations, however, were equally efficient at learning the task with and without plant landmarks in the maze.

A second ANOVA examined the effect of the maze reversal on each group, with the difference in time to complete the task before and after the reversal as the dependent variable. There was a significant main effect of population ($F_{3,40}=3.16$, $p=0.035$), but no effect of maze type on the change in performance, and no interaction between population and maze type ($F_{1,40}=3.2$, $p=0.08$ and $F_{3,40}=0.78$, $p=0.5$, respectively). The River Kelvin population performed differently from the other three populations by taking longer to solve the task after the reversal when no landmarks were present (figure 4).

Before the maze was reversed, there was no difference in the number of mistakes made by each population ($F_{3,40}=0.29$, $p=0.83$), but after the reversal, there was a significant main effect of population ($F_{3,40}=2.93$, $p=0.045$). Again, the River Kelvin population performed

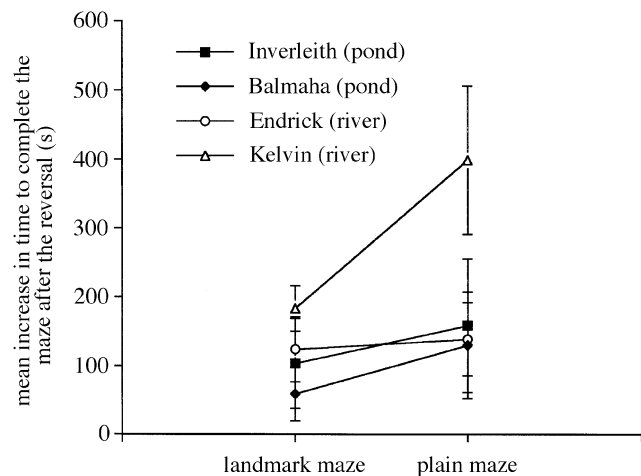


Figure 4. Mean increase in time to complete the maze after reversal for each group (s).

differently from the other three populations by making the most mistakes under both maze conditions.

(b) Experiment 2

In experiment 2, all four populations entered the correct compartment first in significantly more test trials than would be expected if the fish were selecting the compartments at random. A Cochran test was used to test this (Armitage & Berry 1987), and the following results were obtained; Inverleith $\chi^2=11.3$, $p<0.01$; Balmaha $\chi^2=4.34$, $p<0.05$; Kelvin $\chi^2=5.21$, $p<0.05$; Endrick $\chi^2=14.22$, $p<0.01$. When the four populations were compared with one another, no significant difference in the mean number of correct choices made by each population was found (Kruskal–Wallis test; $K=0.25$; $p=0.969$).

In the control test, the ten Inverleith fish did not choose the rewarded compartment any more often than expected by random choice (Cochran test: $\chi^2=0.083$, $p>0.05$).

4. DISCUSSION

We found clear differences in ability to learn a spatial task among the four populations tested. In experiment 1, fish from each population were trained to solve a maze. Some fish were tested in a plain maze, whereas others were tested in a maze with small plastic plants acting as landmarks, or beacons, to guide the fish towards the open doors. Fish from the Inverleith Pond and Balmaha Pond populations took fewer trials to reach the criterion when the landmarks were present. This suggests that these two populations were using the conspicuous plant landmarks to guide them through the maze. Without the visual landmarks, they were still able to learn the correct route to the food reward, but it took them longer to do so. It would appear then that a hierarchy of spatial strategies may be employed by the Balmaha and Inverleith fish, with visual landmarks being preferred, and some alternative method being used in their absence.

The Kelvin and Endrick populations took the same amount of time to learn the maze regardless of the presence or absence of landmarks. This suggests that they did not rely on the plant landmarks to the same extent as

the other populations, but may have used an alternative strategy. As the sides of the tank containing the maze were covered by black card, and the tanks were under a uniformly white ceiling, the fish were unlikely to have been using global, extramaze cues to learn the correct route. One alternative explanation is that the fish were developing a behavioural algorithm, i.e. a learned series of turns (turn left at first wall, right at the second wall, and so on). Taken together, these results support our prediction that fish from a pond habitat would be better able to rely on visual landmarks than those from a river habitat.

To investigate further, fish were subjected to one reversal trial where the sequence of open and closed doors was switched. Only fish using a learned series of turns should be affected by this reversal because the landmarks remained reliable cues. The Kelvin population showed the greatest increase in time to complete the maze. This increase was significantly greater than that of the other three populations when no plant landmarks were present (see figure 3). This suggests that Kelvin fish did rely on a learned series of turns to solve the plain (NoLM) maze. When the plant landmarks were present, the increase in time to complete the maze was no greater than that for the other three populations. This suggests that the Kelvin fish were paying at least some attention to the plant landmarks, and perhaps relying on them only when the algorithm was no longer effective. We can conclude that of the four populations, the strongest algorithmic behaviour was developed by the Kelvin population in the absence of landmarks, but when landmarks were present, these were used to some extent.

The Inverleith Pond, Balmaha Pond and River Endrick populations showed no difference in the increase in time to complete the maze after the reversal, regardless of the presence of landmarks. As the Inverleith and Balmaha populations seemed to use the plant landmarks when learning the task, it is likely that when present, these plant landmarks guided them quickly through the maze after reversal. However, performance remained high for these two populations after reversal of the open doors, even in the NoLM maze. How can fish from these two populations still be able to complete the reversed maze quickly without the presence of landmarks? A possible explanation for this is that the fish were able to detect an alternative landmark. The way the maze was constructed meant that the amount of light passing through the open and closed doors differed. A closed door was effectively in shadow, whereas an open door was relatively lighter. Examination of the maze after the experimental work confirmed that doors leading to dead-ends were on average 40% darker than open doors (measured by a Weston Master V light meter). We believe that the Inverleith and Balmaha fish may have used these differences in door light levels as a form of landmark. This explanation implies that these populations preferred to rely on landmark information, either as conspicuous plastic plants or differences in light levels, rather than learning a sequence of turns. The difference in light levels could conceivably be more difficult to distinguish as a reliable landmark than the plants, and this increased difficulty may then have translated to the longer time these populations spent learning the correct route through the plain (NoLM) maze (figure 3).

The Endrick population was equally fast at learning both types of maze (LM and NoLM). It also showed no difference in the effect of the maze reversal for either type of maze. How could these fish be negotiating both types of maze equally well, and yet not be affected by the reversal test? Again, we believe that these fish were responding to the difference in light levels coming through the different doors. Unlike the Inverleith and Balmaha fish, however, the results suggest that the fish are either equally good at using the difference in light levels in the NoLM maze and the plants in the LM maze, or that they use the difference in light levels to solve both mazes. Whichever cue they do actually use, the Endrick fish were clearly better than the Inverleith and Balmaha populations in adopting a successful spatial strategy to solve the NoLM maze (see figure 3). Different populations of the same species, therefore, can exhibit variability in their spatial learning behaviour.

Experiment 2 showed that this variation does not arise through gross differences in learning ability, or in the motivation to learn a task in a laboratory setting. We observed no differences in overall performance between the four populations. Each population was equally able to discriminate between the two coloured compartments, and to associate one colour with a food reward. This showed that the differences found in experiment 1 are likely to result from intrinsic learning differences rather than from differences in boldness, adaptation to laboratory conditions, or gross learning ability.

As three-spined sticklebacks are classed as a micro-matic species, that is, having a poorly developed sense of smell, and vision is known to be the primary sense used in hunting (Wootton 1976), we did not expect olfaction to be involved in experiment 2. The control test was designed to determine whether the discrimination task was solved by the use of olfactory or visual cues. We found no evidence that fish from the population tested were using olfaction, and therefore, we believe it is unlikely that fish from any of the populations were using information other than the colour cues to solve the discrimination task.

The results reported here highlight some interesting differences in spatial behaviour between different populations, possibly related to the nature of the habitat occupied. The Kelvin fish, which apparently preferred to solve the maze task using a sequence of turns, or algorithm, were sampled from a eutrophic river with poor visibility, much aquatic vegetation, and a noticeable level of pollution. Such conditions may not promote the use of visual information for orientation because the visual surroundings are likely to be unstable. Inverleith and Balmaha populations, which apparently rely more on visual information to orientate, were sampled from standing water undisturbed by currents and, therefore, are likely to be visually stable. The river from which the Endrick population was sampled was clear and oligotrophic, with little submerged vegetation and a largely mineral substratum. These conditions might favour the use of visual information for orientation as there is likely to be less variation in the surroundings, and more visual stability. We are currently testing fish from a larger sample of habitats to determine whether the observed differences in spatial memory relate to these features of pond and river environments.

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