

## COLOUR LEARNING IN THE HAWKMOTH *MACROGLOSSUM STELLATARUM*

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### Summary

Newly eclosed specimens of the day-active hawkmoth *Macroglossum stellatarum* were trained to artificial flowers in dual-colour choice situations. Learning curves were obtained from learning and reversed-learning experiments. Initially, learning behaviour was influenced by innate colour preferences. In comparison with other lepidopterans, *Macroglossum stellatarum* learns well. When

trained in a dual-choice situation, the moths learned not only to visit the rewarded colour but also to avoid the unrewarded colour. These good learning abilities are discussed in the context of the biology of the species.

Key words: Lepidoptera, Sphingidae, *Macroglossum stellatarum*, moth, colour learning, learning performance.

### Introduction

Many insect species rely on flowers for food and must thus be able to distinguish a flower from other non-floral objects. With respect to the learning of flower characteristics, the honeybee has, for many decades, been the best studied insect (e.g. Menzel, 1983). However, learning ability has also been studied in other nectar-feeding insects, mainly hymenopterans. Social insects have been shown to possess a better learning capacity than solitary ones (e.g. Dukas and Real, 1991), which can be explained by the much higher food demands of the colony compared with the individual animal. Flower fidelity of social insects is a direct consequence of the need to optimize the foraging strategy.

Learning capacity has also been studied in several butterfly (Ilse, 1928; Swihart, 1971; Scherer and Kolb, 1987; Lewis, 1986; Goulson and Cory, 1993; M. R. Weiss, unpublished results) and moth (Knoll, 1922) species, and the results again suggest a correlation between the insect's learning performance and its ecological needs. In *Macroglossum stellatarum*, flower fidelity has been reported by several authors (Knoll, 1922; Hasselmann, 1962; M. Pfaff, personal communication), and thus this lepidopteran is expected to possess at least some learning capacity, as has already been suggested by Knoll (1922). In the present study, the learning performance of *M. stellatarum* is analysed in some detail, using the colours of artificial flowers as the cue to be learned.

*Macroglossum stellatarum* is a day-active member of the otherwise crepuscular sphingid family. This hawkmoth, despite its distinct innate preferences for particular colours, shapes and sizes (Kelber *et al.* 1994; Kelber and Varjú, 1995), feeds from a variety of flower species of almost all colours, shapes and sizes (Müller, 1881; Knoll, 1922; Hasselmann, 1962; Herrera, 1992; M. Pfaff, personal communication). Among all day-active flower-visiting insects in Europe,

*M. stellatarum* possesses the longest proboscis (25–28 mm), which enables it to exploit several flower species whose nectar is not available to other anthophilous insects (Müller, 1881; Herrera, 1990, 1992). Sphingid moths take up nectar whilst hovering in front of flowers. They are large insects adapted to high-velocity flight, with small wings and high wing loading (Bartholemew and Casey, 1978). In comparison with other lepidopterans, their energy demand is high, forcing them to visit a large number of flowers on every foraging trip. They perform several foraging trips each day with intermittent returns to the same resting places (M. Pfaff, personal communication).

### Materials and methods

*Macroglossum stellatarum* L. (Lepidoptera, Sphingidae) was bred in the laboratory throughout the year. The day before eclosion, pupae were placed in dark cages in which the newly emerged adults spent their first day inactive and without feeding. During the following days, colour-learning behaviour was studied in dual-choice situations. Experiments were performed with free-flying moths in a 75 cm × 75 cm × 150 cm flight cage illuminated from the side and from above by fluorescent tubes. Only one moth was in the flight cage at a time; one experimental session lasted for as long as the animal was flying and visiting artificial flowers.

Since *M. stellatarum* feeds whilst hovering in front of the nectar source, the time taken for a flower visit is very short. Two identical rewarded and two identical unrewarded artificial paper flowers were used, to encourage the animals to visit the artificial flowers alternately instead of introducing the proboscis into the same flower several times. To obtain a high number of visits during one experimental session, only 5 µl of

20% sugar solution was given each time as reward. The artificial flowers had a diameter of 28 mm and were fixed onto a vertical grey cardboard disk (30 cm in diameter) which could be rotated to avoid position learning. Because naive moths have been shown preferentially to visit blue flowers (Kelber *et al.* 1994), in some of the experiments the artificial flowers were provided with a small central blue disk (diameter 8 mm) that served as a 'nectar guide'. Each insertion of the proboscis into the feeding tube in the centre of an artificial flower was counted as a visit. Prior to choosing a flower, moths could not determine whether or not it contained a reward. Therefore, it was not necessary to conduct unrewarded tests. Thus, each feeding session was a test session, with additional unrewarded tests conducted only sporadically.

On the first day of each experiment, each moth was allowed one foraging trip during which it made between 20 and 60 visits to the artificial flowers. After about 30 min, the moth stopped foraging and alighted on the wall of the flight cage. It was then placed in a small cage and was kept in the dark until the next day, when it was allowed the second feeding session (see Knoll, 1922). This procedure made it possible to train and test several individuals per day. On the basis of the insects' choices between the two colours, learning curves were constructed giving the proportion of correct choices (visits to rewarded flowers) as a function of the number of visits (i.e. the duration of training). Learning curves obtained in this way did not differ significantly from those obtained from animals that were allowed to fly and feed in the cage during the whole day, performing 1–4 foraging flights.

## Results

A preference for a particular, previously rewarded colour over another unrewarded one only indicates a learning capacity if the animal is not guided by innate preferences towards the rewarded colour. Because it has already been demonstrated that *Macroglossum stellatarum* possesses an innate preference for blue (Kelber *et al.* 1994), blue was used as the unrewarded colour in the experiments described below.

### Learning motivation

In the first experiment, the rewarded artificial flowers were yellow. At the beginning of the training period, most of the moths visited only the unrewarded blue flowers, totally ignoring the yellow ones (Fig. 1, visits 1–10). The rejection of the yellow flowers might have been due to lack of motivation, i.e. before having been rewarded even once the moths might not have interpreted the yellow artificial flowers as being predictors for a food reward. Indeed, avoidance of the yellow flowers disappeared as soon as the moths had obtained one or two rewards from the blue flowers (Fig. 1). After, on average, four such rewards, almost all of the animals (95%) began visiting yellow flowers as well as blue flowers (Fig. 1), and they finally learned to prefer yellow over blue in about 80% of choices (statistically different from chance after 40 visits,  $P < 0.001$ ,  $\chi^2$ -test on 10 consecutive choices for all animals).

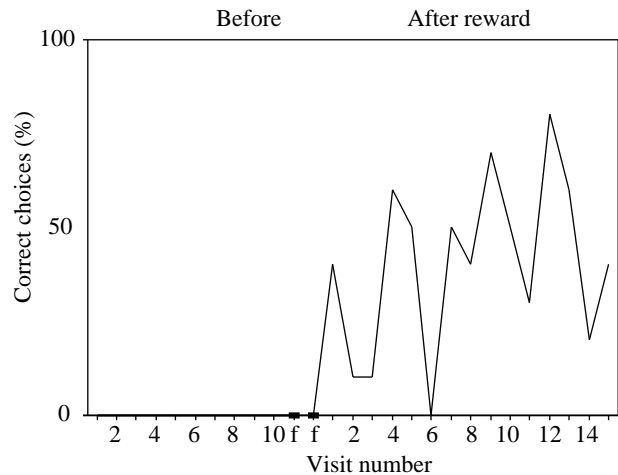


Fig. 1. Change in motivation caused by feeding. Animals were trained to artificial flowers; yellow was the rewarded and blue was the unrewarded colour. Results are percentage choices of the yellow flowers, data for 10 animals. The diagram shows 10 choices preceding and 15 choices following two rewards given in blue artificial flowers (indicated by f on the abscissa). The moths did not visit the yellow flowers before they had been rewarded twice at the blue ones (see text).

Thus, one or a few rewards from blue flowers motivated the moths to search for food sources at other colours, rather than enforcing their innate preference for blue by learning (Fig. 1). A similar observation was made by Giurfa *et al.* (1995), who tested the colour preferences of naive honeybees.

### Colour learning and 'forgetting'

In all later experiments (Figs 2–4) the moths' innate preference for the colour blue was exploited to increase the

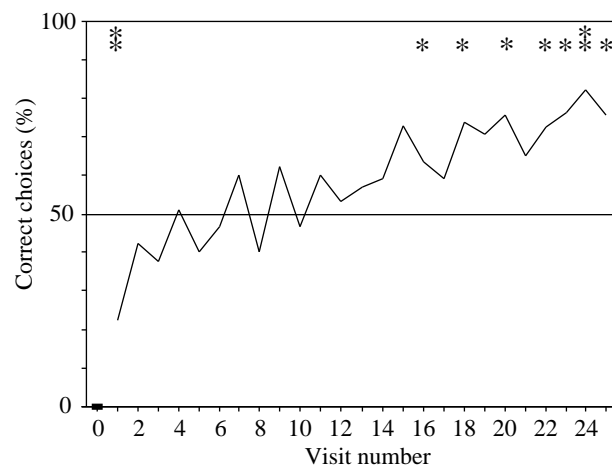


Fig. 2. Colour learning. Percentage correct choices made by 45 moths following the first rewarded visit to a red flower (indicated by a filled square). Almost all animals chose the wrong colour (blue, unrewarded) immediately following the first reward. Their preference for the rewarded colour (red) increased as training continued and was significantly different from the 50% level after 15–20 visits ( $\chi^2$ -test, \*\* $P < 0.001$ , \* $P < 0.005$ ).

attractiveness of the rewarded artificial flowers by providing them with a blue 'nectar guide' in the centre of the flower (see Materials and methods). Forty-five animals were trained using light red for the rewarded flowers and light blue for the unrewarded ones, both with a medium blue 'nectar guide'.

After one reward on a red flower, almost all of the animals still preferred blue (Fig. 2, note that the choice following the first reward is different from the 50% level,  $P < 0.001$ ,  $\chi^2$ -test). In the following 20–25 visits, however, they alternated between the two colours (Fig. 2), and after 30–60 visits achieved a stable level of about 80% correct choices (see Fig. 4). In an unrewarded test after 3 days of training to rewarded red flowers, moths preferred the correct colour in 87% of the visits (189 choices by 13 animals).

On the second day of training to rewarded red flowers, moths appeared to have partially 'forgotten' the colour they learned on the previous day (Fig. 3A). The proportion of correct choices (that had reached almost 100% by the end of the first training session) dropped again to random at the beginning of the second day (the first five choices were not significantly different from the 50% level,  $P > 0.5$ ,  $\chi^2$ -test). Although, on the second day, blue was not preferred over red (as opposed to the first several choices at the beginning of the first session, see Fig. 2), the innate preference for blue still influenced the animals' choice behaviour. Whether the partial return to the preference for blue depends on the number of rewarded visits made on the first day or on the time elapsed between the first and the second session remains to be investigated. However, this pattern might also depend on the colour of the rewarded flowers. For example, moths rewarded on yellow flowers with blue nectar guides (light-blue flowers being unrewarded) did not show this pattern: they chose the yellow flowers at the beginning of the second session with the same frequency as they did at the end of the first session on the previous day (about 80% for seven animals,  $P > 0.4$ ,  $t$ -test for paired samples), whereas moths rewarded on green flowers without blue 'nectar guides' returned to their initial preference for the blue flowers when tested on the next day (results not shown).

Moths rewarded on blue flowers on the first day, and on red flowers on the second and third days, initially chose red less often than did the animals trained to red for all three days ( $P < 0.001$ ,  $t$ -test). After a time, however, they learned red as reliably as did the naive animals, and there was no reduction in the percentage of correct choices between the second and third day (Fig. 3B). It seems that naive, newly eclosed animals (Fig. 3A) rely much more strongly on their innate preferences than do more experienced animals and tend to 'forget' the learned colour if it does not agree with their innate search image (see also Discussion).

#### Reciprocal colour learning

Moths rewarded on the light-red flowers for 3 days and then on the light-blue flowers for a further 2 days (Fig. 4A) reversed their preferences, the shape of the two learning curves being very similar. These animals clearly abandoned their initial spontaneous preference for blue, and then learned this colour

in the same way as they had previously learned red. Even when the rewarded flower was switched between red and blue more than once (Fig. 4B), the percentage of correct choices reached a high level every time. The moths are able to reverse their colour preferences depending on whether blue or red is rewarded.

#### Avoidance of the unrewarded colour

In the final set of experiments, moths learned not only to prefer the rewarded colour but, in addition, to avoid the unrewarded one. Two groups of moths were trained to feed on green artificial flowers, the unrewarded flower was light blue for one group, and yellow for the second group. After 3 days

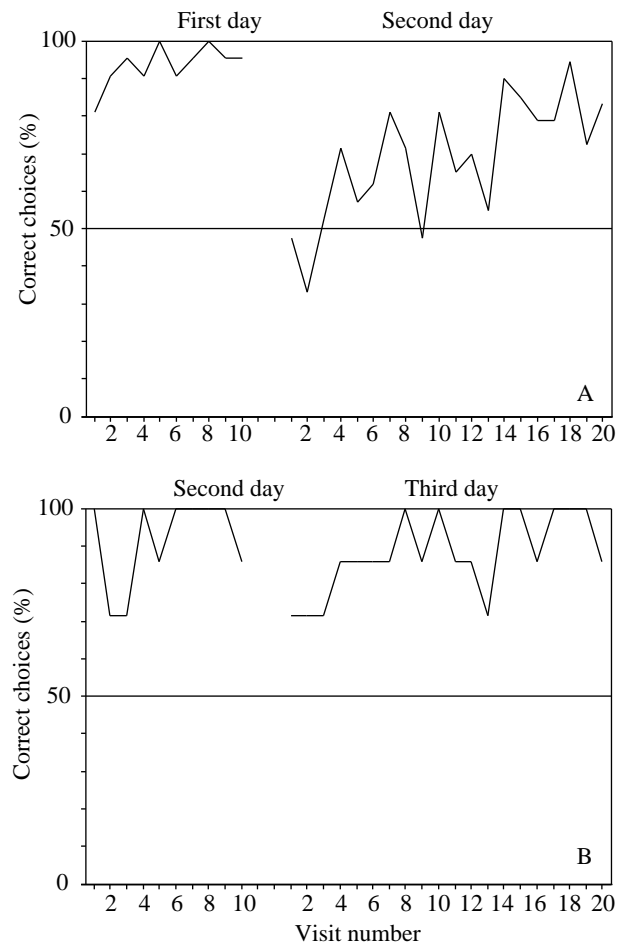


Fig. 3. Results for two successive days of training. (A) The percentage of correct choices of moths rewarded at red flowers in the last 10 visits on the first day of training and the initial 20 choices on the second day are shown. Only those animals ( $N=22$ ) that had performed between 30 and 100 flower visits and performed at least eight correct choices out of the last 10 on the first day of training are included. The difference between the last 10 choices on the first day and the initial 10 choices on the second day is highly significant ( $P < 0.0001$ ,  $t$ -test for paired samples). (B) Seven animals trained to blue flowers on the first day, then presented with rewarded red flowers on the second and third days. There is no significant difference between the last 10 choices of day 2 and the initial 10 choices of day 3 in this case ( $P > 0.5$ ,  $t$ -test for paired samples).

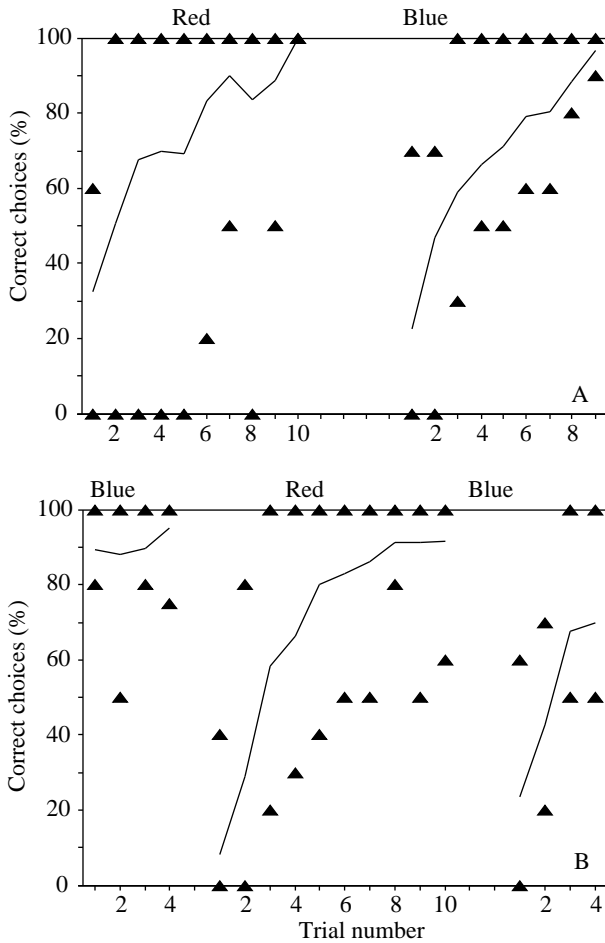


Fig. 4. Learning and reversal of learning. The percentage of correct choices is shown. 10 consecutive visits by one animal are taken as a trial. The lines show the mean values, the upper and lower triangles represent the minimum and maximum values. (A) 3 days of training to red flowers followed by 2 days of training to blue flowers (12 animals). (B) 1 day of training to blue flowers followed by 3 days of training to red flowers and finally 1 day of training to blue flowers (11 animals). Moths trained to one colour against another learn to reverse their preferences when the reward is switched to the previously unrewarded colour. The last two trials of each training situation were tested for difference from the random level using the  $\chi^2$ -test; the result was significant in all cases ( $P < 0.001$ ).

of training, unrewarded tests were performed in which all animals were given the choice between three flowers, green, light blue and yellow. The colour that had been unrewarded during training was visited less frequently than the colour that the animals had never seen before (Table 1). A further experiment using red (rather than green) as the rewarded colour gave similar results (not shown). Avoidance of the unrewarded stimulus has also been found in pattern-learning experiments in honeybees (Srinivasan *et al.* 1993).

### Discussion

The results demonstrate that *Macroglossum stellatarum* is

Table 1. Avoidance of unrewarded flowers

Test colour	Percentage of choices	
	Training colour: green (rewarded), blue (unrewarded)	Training colour: green (rewarded), yellow (unrewarded)
Green	53.3	49.9
Yellow	25.9	15.0
Blue	20.8	35.1

Percentage of choices in an unrewarded test in which the moths had to choose among the previously rewarded colour (green), the unrewarded colour (blue or yellow as indicated) and a novel colour (yellow or blue as indicated). The novel colour is chosen more frequently than the unrewarded colour; the innate preference for blue has an obvious influence.

691 choices by 15 moths that were trained to blue as the unrewarded colour, 646 choices by 13 moths that were trained to yellow as the unrewarded colour. The two distributions differ significantly from each other ( $P < 0.001$ ,  $\chi^2$ -test).

able to learn different corolla colours within about 20 rewarded visits. In comparison with honeybees, the moths reach a similar proportion of correct choices, but they learn much more slowly. The learning rate is comparable to that of bumblebees, but higher than that of the solitary carpenter bee (Dukas and Real, 1991). Compared with other lepidopteran species, *M. stellatarum* learns well. Only a few species of Lepidoptera could be colour-trained successfully (*Vanessa* sp., Ilse, 1928; *Heliconius charitonius*, Swihart and Swihart, 1970; Swihart, 1971; *Battus philenor* and *Agraulis vanillae*, M. R. Weiss, unpublished results). Experiments with three *Pieris* species have revealed a strong tendency to visit the innately preferred colour and thus poor learning ability (Lewis, 1986; Scherer and Kolb, 1987; Goulson and Cory, 1993). Training experiments with several other butterfly species (Ilse, 1928) also did not reveal a significant learning capacity.

It cannot be determined, from the present experiments, whether the cue by which the moths learned to distinguish between the artificial flowers is the spectral distribution or the intensity difference between them. Indeed, the red colour chosen is likely to be seen as achromatic by these moths because they lack a red-sensitive photoreceptor (K. Bartsch, personal communication; own unpublished results). To solve this problem, learning experiments using spectral colours will be performed in future studies. The aim of the present study was, however, to confirm the suggestion of Knoll (1922) that *Macroglossum stellatarum* has a capacity for learning flower characteristics, and red was chosen for this purpose because of its low attractiveness.

Rapid learning and reversal of learning might be understood as adaptations by *Macroglossum stellatarum* to its habitat. The moths not only have a relatively long life span (up to 4 months in the laboratory) compared with butterflies such as *Pieris napi* (12.4 days on average in the laboratory; Goulson and Cory, 1993), but they also migrate over long distances from the

Mediterranean across the Alps to central and northern Europe (see Pittaway, 1993). Accordingly, they must encounter changing resources as well as different vegetations and seasons. They might have to change the flower species on which they feed quite often, either on their way north or seasonally as plant species cease to bloom. Thus, it makes sense for them to try different flowers even if their current staple food plant is still in bloom. Another aspect for consideration might be their requirement for large amounts of nectar (up to 1 ml of 20% sugar solution daily in the laboratory; M. Pfaff, personal communication). From this point of view, it would seem worthwhile to pay attention to any new flower species which might provide larger amounts of nectar. This interpretation is also in accordance with the findings that a few initial rewards on a spontaneously preferred colour are followed by an increasing readiness to visit the alternative colour (see Fig. 1) and that totally novel colours are preferred over a previously unrewarded colour (see Table 1).

Results for the moths in the present study resemble the behaviour which Heinrich (1979) termed 'majoring' and 'minoring' in bumblebees. Bumblebees only specialize on a single species of flower if it is very readily available; when this species declines in availability, they start visiting up to four other species at a time.

Recent results by Giurfa *et al.* (1995) suggest another explanation for the moths' behaviour after the first reward. Flower-naïve honeybees were trained to either an achromatic or a chromatic stimulus. After a single reward, they did not choose colours similar to the rewarded one, as experienced bees would do. In naïve bees, learning might still be influenced to some degree by innate preferences. A similar explanation might apply to *Macroglossum stellatarum*. However, in contrast to the naïve honeybees, the moths not only visited colours that they innately preferred, after one or several rewards on blue, but were motivated to visit unattractive colours as well. The differences between Fig. 3A and Fig. 3B suggest that the initial influence of spontaneous colour preference on the insect's choice behaviour disappears with increasing experience (see also Fig. 4). An innate search image, containing features such as colour or odour (see Giurfa *et al.* 1995), is necessary for newly eclosed animals to find their first nectar source. In animals capable of learning, it can subsequently be replaced by learned associations between food and flower features.

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