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ORIGINAL ARTICLE

A. D. Ham · E. Ihalainen · L. Lindström · J. Mappes

Does colour matter? The importance of colour in avoidance learning, memorability and generalisation

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Abstract Aposematic species exploit the ability of predators to associate, for example, conspicuous colouration with the unprofitability of prey. We tested the importance of colour for avoidance learning, memory and generalisation in wild-caught great tits (*Parus major*). First, we determined the birds' initial colour preferences for red, yellow, orange and grey artificial prey items. The birds showed some preferences, as they were more willing to eat grey prey as their first choice, but these were not strong preferences. We then trained birds to discriminate red, yellow or variable (red and yellow) signals from grey where colours signalled palatable and unpalatable food. In general, the birds learned the discrimination task equally well, irrespective of which colours signalled unpalatability, and subsequently remembered the distinction between previously palatable and previously unpalatable colours in the memorability test. We did not find strong evidence that variability in the signal affected learning or memory. Our results suggest that, in a task where birds must discriminate between palatable and unpalatable prey, it does not matter which specific colour signals unpalatability, although this might be context-dependent. To study whether training also affects responses to unconditioned stimuli, we included orange prey items in the memorability test. Although orange had been palatable in the initial preference test, the birds ate fewer orange prey items after they had been trained to avoid red, yellow or both colours (variable signal) as unpalatable prey, but did not change their preference when trained that these colourful signals were palatable. This indicates that generalisation occurred more readily after a negative experience than a positive experience, a situation that would potentially allow imperfect mimicry to occur.

Keywords Aposematism · Avoidance learning · Colour preference · *Parus major* · Predator psychology

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A. D. Ham
School of Life Sciences, John Maynard Smith Building,
University of Sussex, Falmer,
Brighton, Sussex, BN1 9QG, UK

E. Ihalainen · L. Lindström (✉) · J. Mappes
Department of Biological and Environmental Science,
University of Jyväskylä,
P.O. Box 35, 40014 University of Jyväskylä, Finland
e-mail: lilema@cc.jyu.fi
Tel.: +358-14-2604197
Fax: +358-14-2602321

Introduction

Aposematic animals use conspicuous signals, which may consist of sounds or odours but are often bright colour patterns, to advertise their unpalatability or toxicity to potential predators (Poulton 1890). As these signals are aimed at predators, predator psychology is likely to have had a large influence on the design of signals, and experiments studying predator psychology have found that there are several features of aposematic prey that make them more likely to be avoided by potential predators.

First, it has been shown that many birds have innate aversions to colours or combinations of colours that are commonly used in aposematic signals, i.e. black combined with red and yellow (see review by Schuler and Roper 1992; Mastrota and Mench 1995; Lindström et al. 1999a). This might be the result of novelty effects, such as neophobia (Schlenoff 1984) or dietary conservatism (Marples et al. 1998; Thomas et al. 2003). Second, there is evidence that predators learn to avoid unpalatable prey more quickly (e.g. Gittleman and Harvey 1980; Roper and Wistow 1986; Alatalo and Mappes 1996; Lindström et al. 1999b; Riipi et al. 2001) and recognise those prey more easily (Guilford 1986) if they are conspicuous (like typically warningly coloured animals), rather than cryptic. Finally, predators seem to remember the association between the signal and unpalatability for longer if prey items are more conspicuous (Roper and Redston 1987; Roper 1994).

It has been shown that typically aposematic colours are superior in promoting avoidance of unpalatable prey

(Mappes and Alatalo 1997). For instance, red unpalatable *Lygaeus* bugs had a higher survival rate against bird predators than grey unpalatable bugs, an effect that was irrespective of contrast (Sillén-Tullberg 1985a,b). Colour per se is therefore considered to be important for aposematic prey, although it should be noted that in these experiments, predators did not need to discriminate unpalatable prey from palatable prey. Usually, studies of warning colours include a discrimination task (e.g. Gagliardo and Guilford 1993; Rowe and Guilford 1996), where unpalatability is signalled by typically aposematic colours, without the inverse situation (e.g. yellow signals palatability and green signals unpalatability), or with colours (e.g. green and blue) that are not typically associated with unpalatability (e.g. Gittleman and Harvey 1980). Thus, it is unclear whether typically aposematic colours are better at signalling unprofitability than profitability, or if the mere difference in the appearance of different prey types is sufficient for successful discrimination (e.g. Alatalo and Mappes 1996; Lindström et al. 1999b). It has been suggested (Wallace 1867; Fisher 1930) that aposematic prey species benefit from bright colouration because it makes them most distinguishable from edible prey (Sherratt and Beatty 2003; see also Jansson and Enquist 2003).

Warningly coloured animals also benefit from the predators' capacity to generalise their learned avoidance of a signal to another similar signal (e.g. Duncan and Sheppard 1965; Goodale and Sneddon 1977; Alatalo and Mappes 1996; Mappes and Alatalo 1997; Gamberale-Stille and Tullberg 1999) and avoid new signals, despite lacking experience of these. For instance, generalisation is an important mechanism for Batesian mimicry, which is based on the fact that predators generalise their learned avoidance of warningly coloured prey, i.e. the "model species", to the similar but perfectly edible "mimic species" (Bates 1862). The generalisation curve is broader the more negative the experience of the "model" species (Duncan and Sheppard 1965; Lindström et al. 1997), and predators may be more likely to generalise if the environment is complex (Beatty et al. 2004). Generalisation can also affect food preferences. Domestic chicks (*Gallus gallus domesticus*) that are trained with food to prefer red and yellow, interpolate to show an even stronger preference for orange (i.e. the intermediate colour) in a subsequent unrewarded test (Jones et al. 2001; Ham 2003). This preference for the novel stimulus (although not entirely novel because it is intermediate between the two training colours) following the chicks' first encounter with it, is unlikely to be due to a non-specific "neophilia", but is more likely to be the result of training, and therefore based on sensory generalisation (Jones et al. 2001; Ham 2003).

In this study, we first investigated whether and to what extent wild-caught adult great tits (*Parus major*) prefer certain colours. We used artificial prey with typically aposematic colours (red, yellow and orange) together with grey, and expected to see a bias against the aposematic colours. We designed the colours of artificial prey by using a trichromatic receptor space (excluding UV wavelengths) (see Osorio et al. 1999a,b), which was important because birds perceive colours differently from the way we do. We then trained the birds in a task where they had to discriminate between palatable and unpalatable prey items, to test whether typical warning colours are more easily associated with unpalatability than with palatability. We used both single-coloured and double-coloured training stimuli to test whether variability in a signal slows down the learning process (but see Rowe et al. 2004).

For some birds in the experiment, palatability was signalled with red and/or yellow and unpalatability was signalled with grey, while others were presented with unpalatable red and/or yellow prey and palatable grey prey. In a situation where colour per se is important for aposematic prey, we would expect the discrimination task to be easier when the typically aposematic colours (red and/or yellow) are unpalatable, compared with the situation where grey signals unpalatability. However, if the difference between the colours of palatable and unpalatable prey is sufficient to produce avoidance learning, we would expect the degree of discrimination to be the same, regardless of the colour of the unpalatable prey. We also tested whether there were differences in memorability depending on which colour(s) the birds had been trained to avoid, and whether variability in the training signal affected learning and memorability. Finally, we tested how the birds reacted to orange, which they had previously encountered as palatable (in the preference test), but was not used to signal unpalatability in the discrimination task.

Materials and Methods

Experiments were conducted at Konnevesi Research Station (central Finland) during autumn and winter 2002–2003.

Predators

Great tits are visual foragers, common in central Finland during winter. They are able to learn comparatively complex tasks, such as the handling of the artificial prey used in these experiments. We were able to keep wild great tits in captivity and use them in experiments by permission of the Central Finland Regional Environment Centre (permission numbers LS-55, KSU-2002-L382/254, LS-6, KSU-2003-L52/254) and the Experimental Animal Committee of the University of Jyväskylä (permission numbers 29/4.6.02, 7/28.1.2003). Birds were captured using a simple trap (13×17×40 cm) containing peanuts as bait, from which they had previously learnt to feed. The trap was closed by pulling a string from a distance, which allowed the selective capture of only great tits, and also only those that had not been used before in other experiments. The birds were kept for experiments for an average of 3 weeks, before being ringed and released back into the wild at the same location where they had been captured.

Trapped birds were housed individually in plywood cages (65×50×80 cm) with a photoperiod of 11.5 h light and 12.5 h dark. The cages were illuminated by two energy-saving light bulbs (Osram dulux el longlife, 7 W). The floors of the cages were covered with brown paper, so that they were easy to keep clean. There were three perches in the cages for handling food. Birds were unable to see out of these plywood cages and were therefore calm; they also retained better plumage conditions in plywood cages than in common metal birdcages. Birds were provided with sunflower seeds, peanuts and a vitamin-mix for food and had fresh water ad libitum. From 2 h prior to testing, the birds were deprived of food to ensure they were motivated to sample the artificial prey.

Prey

We used pieces of peanut glued under squares of paper (1×1 cm) as experimental prey. The paper stimuli were doubled-sided; both surfaces were printed with a specifically designed colour (see below for details) and were waterproofed with a transparent sticky-tape. Non-toxic glue (UHU Stic), which has a mild odour to humans, was used to glue a small piece of chopped peanut to the underside of each square. During experimental sessions (see below), these prey items were presented on a small plywood tray (9×9 cm), which was painted white and had 16 small, equally spaced wells (diameter 6 mm) drilled into it to accommodate the peanuts attached to paper prey. The wells were arranged in four rows and four columns. Birds were pre-trained in a step-wise manner to handle artificial prey items, which were not coloured (unlike the testing stimuli), so that they learnt to flip the paper and find the peanut. As the peanuts were attached under the coloured paper, we could ascertain whether the birds were just pecking the coloured paper or if they were actually gathering experience (pecking and eating) of the peanut.

In the training phase of the experiment (see below) half of the peanuts were treated to make them unpalatable by soaking them in a solution of 30 ml of water and 2 g chloroquinine phosphate (malaria drug Heliopar). After soaking, the peanut pieces were dried overnight at room temperature. Soaking the peanuts in chloroquinine makes them unpalatable, but otherwise, this chemical is not harmful to the birds. Because malaria drugs contain titanium dioxide (TiO_2), peanuts soaked in chloroquinine look slightly different to birds, compared with untreated ones (Lyytinen et al. 2001). To prevent birds from choosing peanuts based on their colour difference in UV and without looking at the coloured paper, palatable peanuts were treated with TiO_2. TiO_2 is a tasteless, non-toxic chemical, widely used in pharmaceutical products, which does not have any adverse effect on these birds.

Designing colours for stimuli

A trichromatic receptor space was used to design experimental stimulus colours (see Osorio et al. 1999a,b). A colour was represented by its location in the receptor space, which was designed from calculated excitations of S (blue), M (green) and L (red) avian single cones. Double cones are not thought to contribute to colour vision and were therefore not incorporated into the receptor space (Osorio et al. 1999b). Additionally, UV cones were excluded from the receptor space for practical reasons. The need to consider UV cones was also excluded because bulbs emitting minimal UV light were used to illuminate boxes. The centre of the space, where receptor excitations were equal, was an achromatic grey.

Differences between loci in the receptor space corresponded to differences in hue and saturation. The distance from the central, achromatic point determined saturation, and hue was determined by the coordinate within the space somewhere about this point. Colours were then chosen according to their position within the receptor space and relative to one another. We used red, yellow and orange stimuli in this experiment because these produce the strongest responses in similar experiments using domestic chicks (Jones et al. 2001; Ham 2003). They are also typically aposematic colours and, therefore, are often targets of learning in natural situations.

Experimental boxes

The trays of prey items were offered to the birds in controlled conditions. The experimental boxes were made of plywood (50×50×70 cm). There was one handling perch on the wall and a water bowl in the box. The floor was covered with brown paper. There was a door (12.5×6.5 cm) in the wall of the experimental box, through which the trays could be pushed. Observations were made through a small mesh-covered window in one side of the box and experiments took place in a dark room, so that birds were less aware of the presence of the observer. Boxes were illuminated with non-UV-emitting bulbs (Oy Airam Electric, 3 W/14 V E10, 230 V) to minimise light effects.

Experiments

Birds were trained and tested individually. Prior to the test, the birds were familiarised with an experimental box for at least 1 h. During tests, the birds were presented with trays, one after the other. Each tray held four prey items (the colours of these varied according to the phase of the experiment; see below), which were arranged randomly in the grid of wells. Prey handling was divided into four categories: (1) leaving untouched; (2) pecking or flipping the coloured paper, but without tasting the peanut; (3) tasting (i.e. the bird clearly pecks the peanut and shows a behavioural response) and (4) eating. Tasted and eaten prey items were considered as “killed”. The order of the pecking

(2) and killing (3–4) of prey items was recorded for every tray. Each prey item was given a score; the first item to be killed was given a score of 1, the second a score of 2, and so on. Similar scores were given for the pecking of prey, which was analysed separately to killing in the initial preference test. In situations where birds were not required to eat all prey items, preys gained a score of 5 if they had not been killed by the end of the trial. The least-chosen colours therefore gained the highest scores. We used killing scores to test learning because birds only gained information about the palatability of prey items by tasting or eating them.

This experiment was divided into three phases. First, the colour preferences of the birds were determined in an initial preference test. Three to 5 days after the preference test, the birds were trained to discriminate between palatable and unpalatable prey items in a training phase. Finally, 1 week after the end of training, the birds' memory for the prey was tested. These three phases are described in more detail below and shown in Fig. 1.

Initial preference test

It was important to first examine whether the birds had an initial preference for one or more of the colours used in the experiment. This test consisted of five trays containing prey items, consecutively presented to each bird (*N*=67). Each tray held one of each of the following prey items: red, yellow, orange and grey. All of the prey items were palatable (Fig. 1). Birds had to "kill" all of the prey items on each tray before the next one was placed into the experimental box. This ensured that birds had equal experience of all colours during the course of the initial test; for example, if they had been allowed to eat only one colour, they might have developed a preference for this. Emptied trays were replaced with new ones as soon as they were finished, unless the bird did not seem hungry; in which case, it was given a short break.

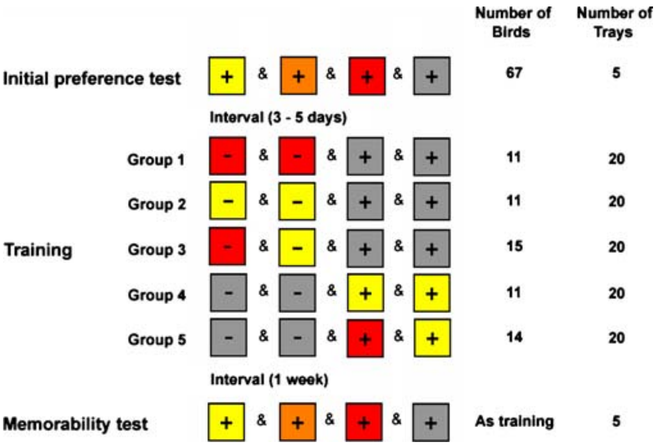


Fig. 1 Experimental design. Each row represents the contents of a single tray of prey items, presented to birds in the three different tests. Prey were either palatable (+) or unpalatable (−) and yellow, orange, red or grey, depending on the test. Sample sizes and the number of trays in each test are also given

Training phase

The training phase not only taught birds the discrimination task, but also allowed learning to be measured. Birds were separated into five treatment groups, where unpalatability was signalled by red (group 1); yellow (group 2); red and yellow, i.e. variable signal (group 3) or grey (groups 4 & 5); and palatability was signalled by grey (groups 1, 2 & 3); yellow (group 4) or both red and yellow, i.e. variable signal, (group 5). The trays presented to birds thus contained four prey items each, with colours according to the treatment group, as shown in Fig. 1.

Training consisted of 20 trays, 15 of which were presented to the birds on the first day of training, while the last five were presented on the following day. In a few cases (*N*=5), where birds were stressed or slow to learn, the number of trays presented was spread out differently over the 2 days.

To ensure the birds had some idea that there were both palatable and unpalatable items on the tray, they had to taste each colour of prey at least once from the first three trays. Each prey item therefore gained a score of 1–4 from each of these trays. Subsequently, it was possible for birds to leave some prey items completely untouched if they so chose, so that prey could now score 1–5 from each tray. Killing (rather than pecking) was used as a measure of choices in this test, because only by tasting or eating the prey items could the birds gain information about their taste.

Emptied trays were replaced with new ones when there were no more selections made for 2 min following the killing of a prey item. This interval allowed the birds the chance to make all the selections they wanted and meant we could be more confident of their learning if unpalatable items were still untouched after that time.

Memorability test

We tested whether the birds remembered their learned avoidance of the previously unpalatable colours and preference of the previously palatable colours, as well as whether the different colours differed in their memorability. Memorability was defined (after Speed 2000) as the "maintenance and retrieval of long-term memories" (i.e. the amount of time taken to forget a stimulus, rather than the time taken to learn and commit it to memory), whereas learning was regarded as the process of acquiring a memory. We were also interested to study whether birds generalised their learned responses (either preference or avoidance) towards red and/or yellow to the orange stimulus, which was intermediate between these two training colours.

This test, which took place 1 week after the end of training, was similar to the initial preference test, but birds did not have to eat, or even touch, all of the prey items. The test consisted of five trays, which held one of each of the following palatable prey items: red, yellow, orange and grey (Fig. 1). Trays were replaced with new ones when no more selections were made for 1 min following the killing of a prey item to be sure that the birds had finished making their selections.

Statistical analysis

Following the preference test, we used Chi-square tests to analyse whether the birds pecked or killed the four different colours equally often as their first choice. We subsequently calculated whether there was an association between the colour and the order of pecking or the order of killing in the first trial, and used likelihood tests to study whether there was a connection between the colours and the order of pecking or the order of killing as related to the results of the preference test. Two birds refused to eat all four of the prey items in the first trial of the preference test and their scores were therefore obtained from only four trials. Finally, we calculated the mean preference scores (which ranged from 1 to 4) for each colour from the five trays.

We measured learning rates by using a repeated measures ANOVA. We divided the 20 trays into four sets of five trays and then calculated a mean killing score for each of these sets of trays, using these as a dependent variable in the repeated measures ANOVA. Factors were colour (typical warning colour or not) and the variability in the signal (uniform or variable). Because the structure of the data from mean killing scores for each trial did not meet the assumptions of parametric statistics, we transformed the data to rank them over the whole training phase. The mean killing scores of the conditioned unpalatable prey from the last five trays of the training phase were used to measure asymptotic learning. In the memorability test, we used mean killing scores as the dependent variable to test for differences between each colour.

Finally, we tested how birds reacted to orange prey in the memorability test. Because all colours were palatable in the memorability test, birds could have re-learned that these prey items were not unpalatable. To remove any effect of re-learning, we counted in which trial birds ate orange prey and compared it to situations where the aversive signal was either a typical warning colour or grey, and used this as a measure of generalisation. We also counted the total number of orange prey eaten in the memory test to assess whether generalisation led to meaningful avoidance from the perspective of the prey. We compared each bird's score of orange in the memorability test to their initial preference scores, to study whether the birds had changed their reaction to orange. If birds' reactions to orange were changed without associative training of unpalatability and orange, then this would indicate that learned avoidance had been generalised between colours. Because orange is similar to red and yellow in terms of hue, we subtracted the mean score of the trained colourful prey (the unpalatable colour for groups 1, 2 & 3 and the palatable colour for groups 4 & 5; i.e. not grey) from the mean score of orange. When both red and yellow were present in training (groups 3 & 5), we averaged the scores of red and yellow and subtracted this from the mean score of orange. If this difference between the colourful prey and orange, i.e. the generalisation score, did not significantly differ from 0, it indicated that the birds had responded to orange prey in the same way that they had learned to respond to the conditioned colourful stimuli.

As scores were discontinuous, we used non-parametric statistics in our tests, except for learning rates which were tested by using parametric statistics. Statistical tests were performed using the Statistical Package for the Social Sciences 11.5.1 statistical package. All *P* values are two-tailed.

Results

Initial preference test

There was no significant initial preference for the colours when we analysed the very first pecks of the birds (χ^2=6.25, df=3, *P*=0.10). Prey items eaten first were random (χ^2=7.09, df=3, *P*=0.069). Similarly, when we analysed whether the order of pecks (1–4) was evenly divided between the four colours over the whole experiment (five trials), there was no significant preference (G^2=13.29, df=9, *P*=0.150). However, when we analysed the order of prey items eaten during the course of the preference test, we found that birds did not eat the colours in a random order (G^2=18.65, df=9, *P*=0.028). When analysing the first-eaten prey items for the five trays (Fig. 2), it seemed that the birds were more willing to eat the grey prey items as their first choice, rather than the coloured prey items (χ^2=11.40, df=3, *P*=0.01). There seemed to be a slight hesitation to eat the red prey as a first choice (Fig. 2).

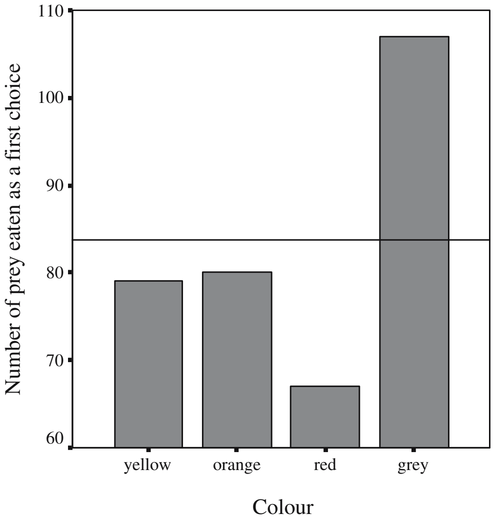


Fig. 2 The distribution of prey colours preferentially eaten by birds (all five preference trials included per bird). The *solid line* refers to random predation, calculated as the mean of summed scores possible as a result of ordering the attacks on prey (1–4)

Training phase

The results from the final five trials of the training phase show that, in general, birds had learned to effectively associate palatability/unpalatability with certain stimuli (Fig. 3). Observations of killing rather than pecking were used to measure learning. All birds learned to avoid the unpalatable prey, measured by the change in the mean rank of unpalatable prey eaten from the four sets of five trays (repeated measures ANOVA, $F_{3,171}$=138.92, P<0.001). There were no differences in the learning rates depending on whether the unpalatable prey was typically warning-coloured (learning*colour $F_{3,171}$=0.553, P=0.647) or whether there was variability in the signal (learning*variability $F_{3,171}$=0.619, P=0.604). Nor was there any three-way interaction (learning*colour*variability $F_{3,171}$=0.295, P=0.829). Thus, all birds learned in a similar manner, irrespective of the treatment. For the last five trays of the training phase, scores of unpalatable prey were typically greater than 4, which meant that birds chose the colours that were unpalatable in training last, or left them untouched (for all groups, P≤0.008 in Wilcoxon signed ranks tests comparing choices of palatable and unpalatable prey from the last five trays).

To judge whether the discrimination task was more difficult when grey signalled unpalatability than when aposematic colours signalled unpalatability, we compared avoidance scores for unpalatable grey and unpalatable yellow (which was the most effectively avoided of the aposematic colours tested; see Fig. 3). The aversion to unpalatable grey was similar in magnitude to the avoidance of unpalatable yellow (Mann–Whitney U test, U=126.5, $N1$=11, $N2$=25, P=0.694). Thus, in our discrimination task it did not matter whether grey, yellow or yellow and red together signalled palatability or unpalatability.

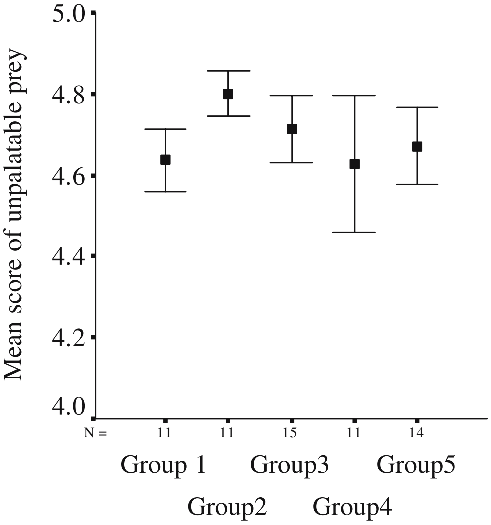


Fig. 3 Mean score of unpalatable prey from the last five trays of the training phase, according to the experimental group. *Bars* represent the standard error of the mean

Memorability test

Following discrimination training, birds were presented with all four colours in a memorability test, which was similar to the initial preference test. In the first trial, we analysed the birds' behaviour towards colours, and killing scores were used to measure memorability. In all five groups, birds showed clear preferences (χ^2≥22.54 and P≤0.001 in Friedman tests for all groups; Fig. 4b–f). The training was sufficient, because all groups of birds avoided the previously unpalatable signals and preferred the previously palatable signals (for all groups, Z≥−2.852 and P≤0.004 in Wilcoxon signed ranks tests of the differences between average scores).

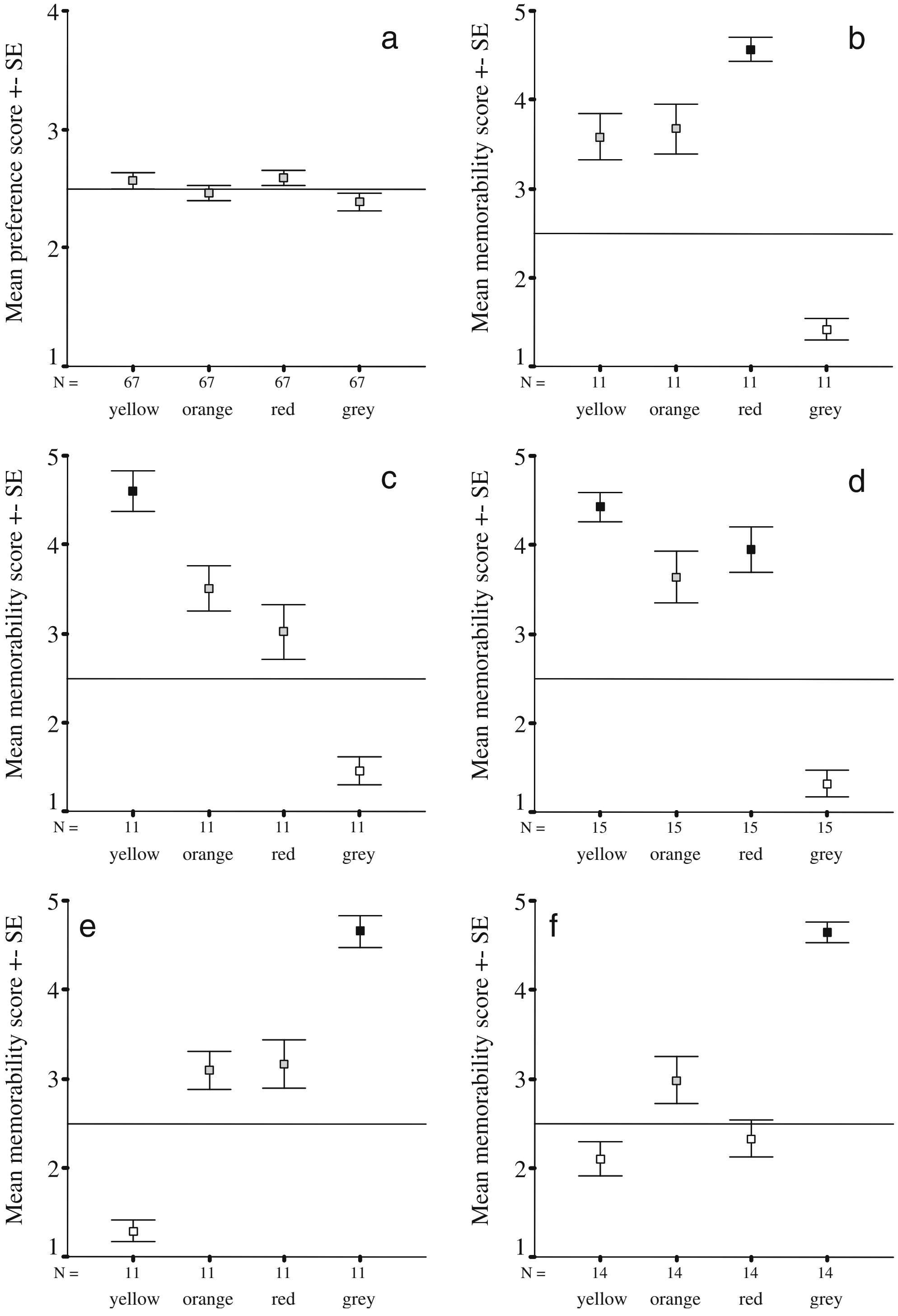
After the birds had been trained to avoid a single colour (either red, yellow or grey, groups 1, 2 & 4, respectively), they remembered to avoid these colours equally well (Kruskal–Wallis test, χ^2_2=1.23, P=0.541). According to our results, typically aposematic colours did not benefit from increased memorability compared with grey.

We then tested whether two colours were more difficult to remember than one colour (i.e. whether variability in the signal had an effect on memorability). When red and yellow had been previously unpalatable, birds remembered equally well to avoid red, irrespective of whether it had been presented alone or as part of a variable signal in training [Mann–Whitney U test, U=56.0, $N1$=11 (group 1), $N2$=15 (group 3), P=0.164], and the same was true for yellow [Mann–Whitney U test, U=68.0, $N1$=11 (group 2), $N2$=15 (group 3), P=0.436]. However, within the group that had simultaneously experienced red and yellow unpalatable prey items (group 3), birds surprisingly subsequently avoided yellow more than red (Wilcoxon signed ranks test, Z=−2.057, N=15, P=0.040). When the unpalatable signal had been grey in training (groups 4 & 5), this colour was equally well avoided, irrespective of whether the opposing palatable signals had been variable or not (Mann–Whitney U test, U=69, $N1$=11 (group 4), $N2$=14 (group 5), P=0.641). Furthermore, within group 5 there was no difference between preferences for red and yellow (Wilcoxon signed-ranks test, Z=−0.63, P=0.529). Thus, our results provide limited evidence that variability within signals could affect their memorability.

Generalisation to orange

We were interested to test how birds would react to the original orange stimuli following training with red and yellow signals. As a test of generalisation, but excluding the possibility for re-learning, we tested whether orange was eaten from the first tray or from subsequent trays, depending on the colour of the unpalatable prey. There were no differences depending on whether the unpalatable colours were (typically unpalatable) red, yellow or red/yellow (groups 1, 2 & 3) or (typically palatable) grey (groups 4 & 5) in training (Fisher's exact test, P=0.573). However, there was a difference in the total number of orange prey eaten during the whole memory test, which

Fig. 4 Mean preference scores for each colour in the initial preference test (**a**) and memorability tests (**b**–**f**, groups 1–5, respectively). *Black squares* denote the colour that was unpalatable and *open squares* denote the colour that was palatable during training. *Grey squares* indicate coloured stimuli that were not used to condition birds in training. *Bars* represent the standard error of the mean. The *reference line* indicates random predation if all four colours are eaten



depended on the unpalatable colour [Mann–Whitney U test, *U*=−2.71, *N*1=37 (groups 1, 2 & 3), *N*2=26 (groups 4 & 5), *P*=0.007], indicating that birds trained to avoid red, yellow or red/yellow also avoided orange prey more during the memory test. The same result was obtained when we compared, for each individual bird, the mean scores of orange in the memorability test (Fig. 4b–f) with those in the preference test (Fig. 4a). Although the birds had experienced orange as palatable in the preference test, they avoided orange after they had been trained to avoid red, yellow and red/yellow as unpalatable (groups 1, 2 & 3: Wilcoxon signed ranks tests, *Z*=−2.938, *P*=0.003; *Z*=−2.35, *P*=0.019 and *Z*=−2.79, *P*=0.005, respectively). These birds changed their preference for orange, indicating that they associated orange with unpalatable prey following training (compare Fig. 4a–c). For the two remaining groups (4 & 5), where grey was unpalatable, the preference for orange did not change significantly from the original preference test (Wilcoxon signed ranks tests, $Z=-1.62$, $P=0.106$; $Z=-1.60$, $P=0.109$ and compare Fig. 4a–f). Although there was no significant difference, the power of the tests was low (0.218 and 0.37, respectively) so we cannot conclusively accept the null hypothesis. However, the difference between the groups corresponded to the size of a medium effect (0.53), which suggests that the possible difference between killing scores of orange in preference and memorability tests is not very large. Therefore, the results indicate that birds do not associate palatable prey with colourful stimuli as easily as they do with unpalatable prey.

As the preference for orange changed only in the groups where unpalatability had been signalled with red and/or yellow (groups 1, 2 & 3), we tested the intensity of avoidance by comparing whether birds treated orange similarly to red and/or yellow signals in these groups. We obtained a generalisation score by calculating the mean difference between the killing scores of orange and either red or yellow, or their average (where the birds had experienced both colours in training). Only after birds had experienced both red and yellow as an unpalatable signal was the generalisation score not different from 0 (i.e. they chose orange as often as red and yellow in the memorability test) (Wilcoxon signed ranks test, $Z=-1.54$, $P=0.123$) (Fig. 5).

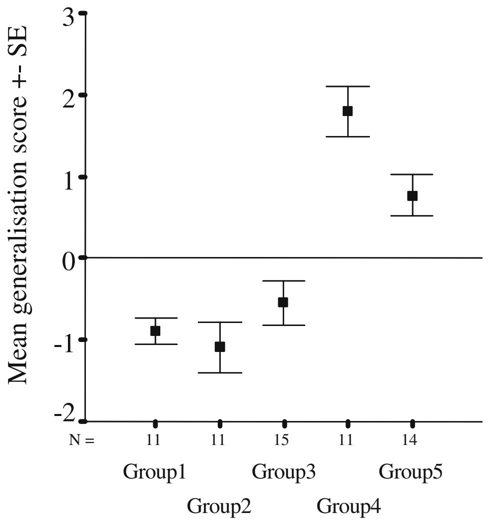


Fig. 5 Mean generalisation score, i.e. the difference between orange and conditioned colourful stimuli (red, yellow or red/ yellow) for all training groups. The *zero line* indicates where orange was generalised to these colours. This was the case for group 3, where birds had been trained to avoid red and yellow simultaneously. *Negative values* indicate that the orange prey was eaten before the conditioned stimuli (unpalatable red or yellow alone), while *positive values* indicate that orange prey items were eaten after the conditioned stimuli (palatable yellow and palatable red and yellow simultaneously). *Bars* represent the standard error of the mean

Discussion

The colour preferences of wild-caught great tits were observed and related to learning and memory of different-coloured prey items in situations where these colours signalled palatability or unpalatability. Birds seemed more willing to eat the grey prey items first than would be expected if choices were random, although no preferences were shown in the initial pecking order. Previous studies have similarly shown that some birds have biases against the colours used in aposematic displays (see review by Schuler and Roper 1992; Exnerová et al. 2003). This avoidance can be innate (Smith 1975, 1977; Schuler and Hesse 1985; Mastrota and Mench 1995; Lindström et al. 1999a) or based on experience (Evans and Waldbauer 1982; Roper 1990; Thomas et al. 2003).

The avoidance of warningly coloured prey items was not particularly strong because only 39% of the birds attacked the grey prey as their first choice (the expected attack rate was 25%). Previously, Lindström et al. (1999a) found that adult great tits showed a weaker bias against black-and-yellow-striped prey than juveniles, which shows that the age and experience of subjects may affect results (see also Schmidt and Schaefer 2004). The lack of strong colour preferences may therefore be explained by the fact that our adult birds, being more experienced, could have been aware of the artificial context of signal presentation (see Gamberale-Stille and Tullberg 2001). The results of an experiment by Rowe and Guilford (1996) may reflect the same phenomenon; a significant avoidance of red and yellow by naïve chicks that were offered coloured artificial prey items only showed up when a novel odour was also present, i.e. when the colour was presented in a different context. Furthermore, we have to consider that great tits are generalist foragers and have a varied diet in nature; they will eat a variety of flying insects and larvae, as well as some seeds and berries (von Haartman et al. 1967; Royama 1970). This may make them less wary of unfamiliar situations and food items.

The theory of aposematism suggests that predators more easily learn to avoid signals that contrast strongly with their viewing background (e.g. Cott 1940; Gittleman and Harvey 1980; Roper and Redston 1987; Lindström et al. 1999b; Gamberale-Stille 2001), although colour per se has also been shown to be important in avoidance learning (Sillén-Tullberg 1985b; Gamberale-Stille and Guilford 2003). We trained some groups of birds to discriminate palatable grey from unpalatable aposematic colours (red and/ or yellow), while other birds were presented with the inverse situation. In general, all birds learnt the discrimination task equally well, irrespective of whether the colour of the unpalatable prey was typically aposematic or not. Thus, our study did not provide strong evidence that typically aposematic colours enhanced avoidance learning. Grey, which is not typically associated with unpalatability in nature, was equally well avoided compared with the aposematic colours, although yellow was avoided slightly more than red at the end of training (see Fig. 3) (see also Rowe and Guilford 1996). Additionally, we found no evidence that a variable signal was more difficult to learn than a signal consisting of a single colour (see also Rowe et al. 2004). This is interesting, as the theory of Müllerian mimicry assumes that dissimilarity slows down the learning process, so that individuals that look alike are better protected from predation. Beatty et al. (2004) recently suggested that the benefits of similarity in Müllerian mimicry might only appear when a predator has to learn several prey patterns instead of only two. It is also important to note that during training in our experiment, the discrimination task essentially remained the same for all groups in that grey prey items always opposed the same number of colourful prey items (red and/or yellow), irrespective of their palatability or unpalatability.

In the memorability test, birds generally remembered to prefer the colours they were trained to associate with palatability and avoid the unpalatable colours (Fig. 4b–f). Furthermore, the specific colours, whether typically aposematic or not, did not differ in their memorability. Interestingly, however, when birds had been trained with an unpalatable variable signal (red and yellow, group 3), they subsequently avoided yellow more than red in the memorability test (Fig. 4d). It is possible that the variability itself confused these birds by affecting their memorability of red, although this seems unlikely because the memorability of red and yellow was the same (see also Ham 2003), regardless of whether they were previously presented alone or as part of the variable treatment in training (yellow in group 1 vs group 3; red in group 2 vs group 3). Alternatively, yellow could be a slightly more memorable signal than red.

At the beginning of the memory test, birds attacked orange prey irrespective of whether they were trained to avoid or prefer red and/or yellow. However, during the course of the whole test, and although the birds had eaten orange prey that were palatable in the preference test, they killed fewer orange prey items overall, following training to avoid red or yellow or red/yellow. Only birds that had learnt to avoid both red and yellow in training (group 3) subsequently avoided orange as much as these two colours in the memorability test. For the remaining groups, although orange was not as clearly generalised to colourful signals, it was still treated differently from grey. When grey had been palatable and either red or yellow unpalatable in training (groups 1 & 2), birds changed their reaction to orange and avoided it more than they had done in the preference test. Thus, orange subsequently benefited from its similarity to the previously unpalatable colourful stimuli, because it survived better than grey and was left untouched in 49.7% of cases. This could imply that experienced predators might be likely to leave novel but typically aposematic coloured prey untouched, and this would indicate that even a crude resemblance to traditional aposematic signals (e.g. bright colouration) may be beneficial for novel prey (Alatalo and Mappes 1996; Mappes and Alatalo 1997; Thomas et al. 2003). If this is true, it may in turn maintain polymorphism in warning signal designs.

When grey had been unpalatable in training (groups 4 & 5), orange was not treated similarly to grey, yellow or even the combination of red and yellow stimuli in the memorability test. Furthermore, the killing score in the memorability test remained the same in comparison to the preference test. These results suggest that relatively unfamiliar signals may be more easily associated with coloured stimuli that signal unpalatability than those that signal palatability. However, contrary to this experiment, domestic chicks trained to prefer red and yellow stimuli (similar to those used here) subsequently showed an even stronger preference for a novel orange in a memorability test (Jones et al. 2001; Ham 2003), suggesting that predators may also generalise a positive association.

It should be noted that the preferences of birds can change if additional objects are presented (Bateson et al. 2003). Our test of generalisation to orange may therefore be affected by the fact that some birds were conditioned with three colours (groups 3 & 5) while others were conditioned with only two (groups 1, 2 & 4), although all were tested with four colours in the memory test. If the mere presence or absence of an additional unconditioned prey item had had an effect, it would have shown in the generalisation score. The presence of an extra unconditioned stimulus (groups 1, 2 & 4) could have pushed the score of orange closer to 0 because the risk of attack was shared between two unconditioned colours (orange and red/yellow), while its absence (groups 3 & 5) could have had the opposite effect. However, unlike this “shared risk expectation”, it was group 3 which showed a generalisation score that did not differ from 0 (Fig. 5). The possible psychological effects of an extra, unconditioned colour therefore cannot be extracted from these data.

According to our results, colour does matter in the sense that it facilitates learning and memory of an unconditioned stimulus (in this case, palatability or unpalatability of prey), although it does not matter which specific colour signals the quality of the prey. This is interesting because theories of aposematism assume that prey unpalatability is best signalled by certain colours (Poulton 1890; Cott 1940; Mappes and Alatalo 1997). However, it might be more crucial that signals with opposing messages are distinguishable (Sherratt and Beatty 2003; Jansson and Enquist 2003). Perhaps our findings can also be explained by the fact that aposematic prey also tend to contrast with their natural background, and this may be a more important feature than the colours of signals per se (but see Sillén-Tullberg 1985b; Gamberale-Stille and Guilford 2003). Additionally, birds may react to the context in which signals are presented (e.g. see Bateson et al. 2003) and it would therefore be interesting to study whether this result holds if the predators are presented with real insects that are coloured.

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