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Colour biases are more than a question of taste

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Unpalatable insects often advertise their defences to predators using conspicuous colours, such as red and yellow. Perhaps not surprisingly, birds show unlearned biases against warningly coloured food. These biases are particularly evident when other components of insect warning displays, such as sounds and odours, are also present. Quinine, a bitter-tasting toxic chemical, can also enhance unlearned biases against red and yellow prey in naïve birds. However, whether this behaviour is performed specifically in response to quinine (which is chemically similar to many insect toxins) or can be elicited by other bitter-tasting chemicals is not known. The aim of our experiments was twofold. First, we investigated whether Bitrex, a bitter-tasting, nontoxic, man-made chemical, elicits colour biases similar to those elicited by quinine. Second, since avoidance learning can be affected by the number of bitter chemicals present in a prey population, we investigated whether the presence of both quinine and Bitrex enhanced unlearned biases against red crumbs compared to either chemical alone. We found that only quinine elicited attack biases against red prey, and there was no evidence that quinine and Bitrex in combination produced a stronger bias against red crumbs than quinine alone. These results indicate that colour biases incited by defence chemicals are chemical specific and may occur only in response to natural or toxic chemicals.

Keywords: aposematism; domestic chicks; evolution; *Gallus gallus domesticus*; innate bias; learning; receiver psychology; warning coloration

Aposematic insects use conspicuous colour patterns to advertise their unprofitability to potential avian predators (Poulton 1890; Cott 1940), and birds can learn to avoid aposematic insects on the basis of their visual appearance. Although avoidance learning plays a key role in the success of insect warning signals against avian predators (Gittleman & Harvey 1980; Guilford 1990), birds can also express unlearned aversions to particular colours and patterns associated with warning signals (e.g. Schuler & Hesse 1985; Sillén-Tullberg 1985; Roper & Cook 1989; Mastrota & Mench 1995; see Schuler & Roper 1992 for review), but the results of these studies are not always consistent (e.g. Fischer et al. 1975; Roper 1990; Roper & Marples 1997; Jones & Carmichael 1998).

However, adaptive unlearned aversions have been consistently found in the context of multimodal warning signals. Warning displays of many aposematic insects are multimodal, in that they often combine conspicuous coloration with sounds and odours (Cott 1940; Haskell 1966; Edmunds 1974). When sounds and odours are presented alongside food colour choice tasks, naïve foraging domestic chicks (*Gallus gallus domesticus*) bias their behaviour against food with visual traits associated with aposematism, such as food that is conspicuous, red or yellow, or novel (Rowe & Guilford 1996, 1999a,b; Marples & Roper 1996; Jetz et al. 2001; Lindström et al. 2001; Rowe & Skelhorn 2005). These additional components are thought to provide additional cues which birds can use to reduce their attack rates on coloured prey that are more likely to be defended (Rowe & Guilford 1999b; Gambarale-Stille & Tullberg 2001).

Another component that birds could use to bias their attacks is the taste of the prey because many insects secrete bitter-tasting defence chemicals in their warning displays (see Brower 1984 and Nishida 2002 for reviews). Recently, we found that naïve domestic chicks that were given bitter-tasting quinine before a colour choice task were less likely to attack red and yellow crumbs than when they were given only water (Rowe & Skelhorn 2005). Although the bitter taste of quinine appeared to

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bias birds against attacking yellow and red prey, quinine can also have postingestion effects, because at very high doses it appears to be emetic to birds (Alcock 1970). Therefore, chicks may simply find quinine distasteful or they may be able to detect the potentially toxic nature of the chemical, making it impossible to predict whether all bitter-tasting chemicals cause birds to bias their foraging decisions against warningly coloured prey.

In this study we compared biases produced by quinine with those produced by Bitrex, a bitter-tasting but nontoxic chemical. Chicks can detect the differences in the tastes of these two chemicals, and both quinine and Bitrex taste sufficiently unpleasant to produce learned aversions in chicks (Skelhorn & Rowe 2005a,b). If birds rely on taste to bias their food choices against warningly coloured food, Bitrex should produce the same food colour biases as quinine. We also considered how birds change their behaviour when given both quinine and Bitrex together. Insect defences can be highly variable, both within and between species (e.g. Blum 1981; Pasteels et al. 1995), and the presence of multiple chemicals can affect the avoidance learning process. We found that birds learned to avoid red prey more quickly when they could be defended with either quinine or Bitrex than when they were defended with either quinine or Bitrex alone or with a cocktail of the two (Skelhorn & Rowe 2005a). If birds avoid these prey more quickly because of the uncertainty of any potential toxic effects of eating prey that taste of two defence chemicals (Skelhorn & Rowe 2005a) then we might equally expect that they will also bias their unlearned choices against warningly coloured prey more strongly in the presence of multiple chemicals. These experiments therefore investigated the relative roles of taste and toxicity in unlearned colour biases expressed by naïve avian predators.

EXPERIMENT 1: DO CHEMICALS INTERACT TO ENHANCE BIASES?

Methods

Subjects and housing

Fifty-four domestic chicks (*G. g. domesticus*) of both sexes were hatched in the laboratory and housed in two cages measuring 100 × 50 × 50 cm. One cage housed 40 experimental chicks, and the other cage housed 14 buddy chicks (see below). They were all subject to a 14:10 h light:dark cycle using uncovered florescent lights with no UV component, and temperatures were maintained at 24–25°C using room heaters and heat lamps. All subjects were marked with nontoxic 'child-friendly' coloured marker pens which did not appear to have any adverse effects on chick behaviour. Water was provided ad libitum, as were brown chick starter crumbs except during training and experimenting when food deprivation was necessary. When being food deprived, chicks had access to water but not food. Weights were monitored for welfare purposes throughout the experiment, and all chicks gained weight as the experiment progressed. All deprivation periods were in accordance with Home Office regulations and guidelines, and at the end of the experiment all chicks were donated to free-range small holdings.

Artificial prey

To produce palatable, quinine-flavoured and Bitrex-flavoured brown crumbs, 150 g of brown chick starter crumbs was sprayed with 100 ml of either water, 2% quinine sulphate solution, or one drop of 2% Bitrex solution made up to 100 ml with water. These concentrations of quinine and Bitrex were chosen because birds learn to avoid red crumbs sprayed with these solutions at similar rates (Skelhorn & Rowe 2005a).

Both red and green palatable crumbs were produced by spraying 150 g of chick starter crumbs with either 2 ml of Supercook red food dye (Supercook, Leeds, U.K.) diluted to 90 ml with tap water or 0.5 ml of Sugarflair spruce-green food dye (Sugarflair, Benfleet, Essex, U.K.) diluted to 90 ml with tap water. These concentrations were chosen because they produced a similar degree of colour saturation in the crumbs. All crumbs were allowed to dry for 24 h before being sieved to ensure that they were of similar size.

Experimental arena

The arena consisted of a cage similar to the housing cages, with a section measuring 25 × 50 × 50 cm partitioned off using a wire mesh screen to create a separate 'buddy arena.' In all training and experimental trials, two chicks were placed in the buddy arena to reduce any potential distress from placing experimental chicks alone in the arena. These buddy chicks were selected from a stock of individuals not used in the experiments and were changed every three trials. They had free access to food and water throughout the experiment. The floor of the experimental arena was covered in white paper that was changed every trial. The purpose of the white paper was to ensure that crumbs of different colours appeared equally conspicuous.

Training

During the first 2 days posthatch, the 40 experimental chicks were trained to eat brown crumbs from the white floor of the experimental arena. On the first day posthatch, chicks were placed in the experimental arena for three training sessions in groups of three, followed by one session in pairs. These trials allowed chicks to habituate to the arena and no food deprivation was necessary. However, on day 2, chicks were food deprived for approximately 1.5 h before each training session. This deprivation period does not seem to distress the chicks nor have any adverse effects on their daily weight increases. In the first of these trials chicks were placed in the arena in pairs, whereas in the following three trials chicks were placed in the arena individually. All training sessions lasted for approximately 3 min, and all chicks ate readily in the arena at the end of this training. Unfortunately, one chick died of causes not related to the experiment on the night of day 2, leaving 39 experimental chicks.