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Evolutionary perspective

The evolution of multimodal warning displays

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Abstract. Multimodal warning displays combine visual signals with components produced in other sensory modalities, for instance, aposematically coloured insects often produce a pungent odour or harsh sound when they are attacked. Recent research has focussed upon a particular odour, pyrazine, which is commonly associated with warning coloration. Our experiments have shown that pyrazine elicits hidden unlearned biases against particular visual aspects of food in foraging domestic chicks. Here we asses the current state of our knowledge about these biases, reviewing our results using pyrazine and other odours, and also presenting new data showing that sound can produce similar effects. We will discuss potential psychological mechanisms by which these foraging biases are achieved in avian predators, and potential pathways for their evolution.

Key words: aposematism, hidden biases, multimodal warning signals, predation, pyrazine

The multimodal nature of warning signals

Conspicuous warning coloration is often accompanied by non-visual signals (e.g. Wallace, 1891; Carpenter and Ford, 1933; Cott, 1940; Alexander, 1964; Edmunds, 1974). These include pungent odours, harsh rattles, clicks or buzzes, frothing from spiracles, the shaking of brightly coloured wings, or a combination of these. 'Multimodal' displays communicate with predators along multiple sensory channels, and are common among many insect groups: Hemiptera (Wallace, 1891; Carpenter and Ford, 1933; Cott, 1940; Rothschild, 1961); Coleoptera (Wallace, 1891; Lane and Rothschild, 1959; Rothschild, 1961; Majerus, 1994); Lepidoptera (Carpenter, 1938; Ford, 1955; Haskell, 1956, 1961; Blest, 1957, 1964; Rothschild, 1961, 1965; Rothschild and Haskell, 1966; Dunning, 1968; Fullard and Fenton, 1979; Rothschild *et al.*, 1984); Orthoptera (Carpenter, 1921, 1938; Rothschild *et al.*, 1984); Hymenoptera (Carpenter and Ford, 1933; Gaul, 1952; Masters, 1979); and Phasmatisdae (Bedford and Chinnick, 1966). This widespread occurrence leads to the

question of why such complex, and presumably costly, multimodal displays have evolved? What are all these components for?

The hypotheses proposed to explain the evolution of multimodal warning displays are listed in Table 1, although it is not the purpose of this paper to review these in detail. One important question arising from these hypotheses is whether different components have evolved in response to selection form different predators searching for prey using different senses, or whether they could be aimed at a single predator. Although nocturnal predators can associate non-visual components with unpalatable prey, this does not conclusively demonstrate that these have evolved specifically for this purpose, and there is no supporting evidence that these odours and sounds are more effective than others in the learning process. In addition aposematically coloured insects do not restrict the production of odour to night time, but also use them in response to diurnal predators (e.g. Haskell, 1965; Rothschild and Haskell, 1966).

Table 1. Hypotheses concerning the evolution of multimodal warning displays

Hypothesis	Evidence for	Evidence against
1. Non-visual components are aimed at nocturnal predators (Rothschild, 1965; Pearson, 1989)	Predators can learn to associate sounds or odours with unpalatability (e.g. Dunning, 1968; Wheeler *et al.*, 1970; Masters, 1979; Guilford *et al.*, 1987; Roper and Marples, 1997)	Some insects produce non-visual components in response to diurnal predator activity (e.g. Haskell, 1965), and many insect displays involve two non-visual components (e.g. Blest, 1964)
2. Multiple components improve detection	Two components are detected faster than one and improve the chance of detection (see Rowe, 1999)	
3. Non-visual components startle predators	Sounds associated with sudden visual displays (e.g. Carpeneter, 1938; Haskell, 1956), and naïve predators can be startled by novel sounds (Dunning *et al.*, 1992)	Predators can overcome startle response and habituate to sounds (Bates and Fenton, 1990)
4. Non-visual components are secondary defences (Edmunds, 1974)	Some frothing may be distasteful (e.g. Wheeler *et al.*, 1970)	Unlikely for sound, odours do not appear to be aversive (e.g. Guilford *et al.*, 1987), odour mimicry by palatable insects (Rothschild *et al.*, 1984)
5. Nonvisual components enhance the psychological effects of visual components (Claridge, 1974; Rothschild *et al.*, 1984)	Odour can enhance learning of visual stimuli (Kaye *et al.*, 1989), non-visual components produce hidden biases against visual stimuli (Rowe and Guilford 1996)	

There are also many cases of insects producing two non-visual signal components integrated into their visual displays, suggesting that there could be benefits to multimodality in addition to independently being able to communicate with visually and non-visually searching predators. It is possible that each signal component could be aimed at separate predators, but our approach to understanding the evolution of multimodal warning signals has been to look at how these additional non-visual signal components might improve the efficacy of the visual signal for a single receiver (Guilford and Dawkins, 1991, 1993; Rowe, 1999).

This review brings together our findings and explores their significance for the evolution of complex warning signals. Specifically, we will review a series of experiments that show interactions between visual and non-visual components of warning displays in eliciting avoidance behaviour in naïve birds. Most of these experiments have investigated the role of odour in aposematic displays, but new data from an experiment using sound suggests that components from other non-visual modalities could elicit similar behaviour. Whilst much of the review summarises previously published data, more detail is given where previously unpublished experiments are presented. Our aim in the final part of the paper is to interpret these recent results in the light of the possible psychological mechanisms being employed, and to attempt to explain how these interactions might have evolved.

Experiments with pyrazine: unlearned interactions between colour and odour

Why pyrazine?

Pyrazines are odours found in a wide range of aposematically coloured insects, and often associated with deterrent displays (Rothschild, 1961; Moore *et al*., 1990; Woolfson and Rothschild, 1990). Rothschild (1961, 1964; Rothschild and Moore, 1987) has long maintained that pyrazines may hold a special significance in the warning displays of insects, perhaps as alerting signals or learning enhancers, and we established early on that birds, the probable primary receivers of aposematic colour patterns with which pyrazines are associated, are sensitive to the odour. Guilford *et al*. (1987) demonstrated that domestic chicks (*Gallus gallus domesticus*) can use the odour of pyrazine alone to discriminate distally between quinine tainted and untainted water once they have learnt the association. Furthermore, in a separate experiment without quinine, after initial hesitancy to drink pyrazine tainted water there appeared to be no aversion to pyrazine. Further experiments have shown that pyrazine, and other odours, can enhance the learning of a visual task, confirming that they could potentially perform this function in

multimodal signals (Kaye *et al*., 1989; Roper and Marples, 1997). Our own pilot experiments demonstrated that in a discrimination learning task between green palatable and yellow unpalatable crumbs chicks avoided yellow faster in the presence of pyrazine than in its absence. Although initially we considered this support for a learning enhancer role, subsequent experiments with better controls now suggest otherwise. It is to these subsequent experiments that we now turn in more detail.

The experiments that we have been conducting over the past few years use domestic chicks preying upon coloured starter chick crumbs. Although individual experiments differ in detail, as a general protocol we trained single naïve domestic chicks to eat untreated chick starter crumbs from a series of sunken wells in a circular walled arena. We then gave them equal numbers of two types of palatable coloured crumb, placed singly in the bottom of each well. Chicks ate (or rejected) crumbs presented in a random sequence, and the relative preference or avoidance of a particular colour was measured as the difference in the number of crumbs of each type that a chick ate. Crumbs were coloured by food-dyeing standard brown chick starter crumbs which were then dried and sieved to get crumbs of a uniform size. In each experiment, half the chicks had 2-isobutyl-3-methoxypyrazine solution added to all petri dishes under all crumbs (both colours), and the odour was allowed to pervade the arena through holes pierced in their lids (see Rowe and Guilford, 1996, 1999 for further experimental detail). Therefore, pyrazine was not associated with any particular crumbs and was not a discriminatory signal, but instead acted as a local background stimulus to a visual food choice task.

Pyrazine elicits a hidden bias against colours typically associated with aposematism

First, we gave chicks the choice between equal numbers of palatable green and palatable yellow crumbs. Chicks showed no aversion to either green or yellow crumbs, but the presence of pyrazine elicited an attack bias against yellow crumbs, with more green crumbs being eaten and more yellow being rejected (Rowe and Guilford, 1996, 1999; Jetz *et al*., in press). These results indicate that unlearned colour biases in birds may only be seen in the presence of a second warning signal component, pyrazine. This may explain why the results of previous experiments investigating innate colour biases in birds have been so variable (Roper and Cook, 1989; Sílen Tullberg, 1985; Mastrota and Mench, 1985; Roper and Marples, 1996; Marples *et al*., 1998). These results also suggest an alternative explanation for our pilot data. It was the unlearned aversion to yellow triggered by the presence of pyrazine that accelerated the discrimination against yellow unpalatable crumbs, *not* a general learning enhancement. To see if this effect of pyrazine could be generalised to other

colours commonly associated with warning signals, we repeated this experiment with a choice between green and red crumbs: chicks avoided red crumbs more when pyrazine was present than when it was absent (Rowe and Guilford, 1996).

We conducted a further experiment with a choice between two colours that are not typically associated with insect warning colours, namely green and purple. Figure 1 shows the results for two groups of 8 chicks that were trained to eat single brown chick crumbs from a series of 8 wells as in previous experiments (Rowe and Guilford, 1996, 1999), and then given the choice between 4 palatable green crumbs and 4 palatable purple crumbs, either in the absence or presence of pyrazine. The mean attack bias is measured as the number of green crumbs eaten minus the number of purple crumbs eaten, and therefore scores of 0 indicate no colour bias. There is no obvious bias in colour choice, and pyrazine does not elicit any hidden bias (Mann–Whitney U-test: $Z = 0.056$; $n = 16$ NS). So in this case, where both colours have no obvious association with aposematism, pyrazine does not have any effect. Of course, because we have not measured the spectral qualities of our coloured crumbs it could be that chicks are avoiding another aspect of the coloration, perhaps

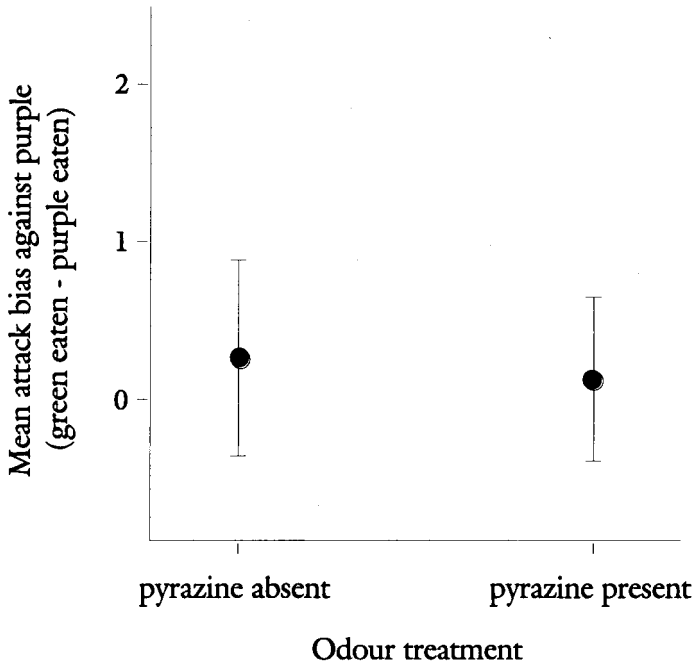

Figure 1. Mean attack bias against purple crumbs made by chicks given the choice between palatable green and palatable purple crumbs, either in the presence or absence of pyrazine. Scores of 0 indicate no relative colour preference. n = 16.

contrast or brightness, rather than these specific hues *per se*. However, the results of these experiments are at least consistent with the idea that pyrazine elicits biases against colours typical of warningly coloured insects (red and yellow) rather than any other attribute of the colours.

Pyrazine elicits a bias against novel coloured crumbs

Avian predators are often deterred from eating food that is novel in appearance upon first encounter (e.g. Coppinger, 1970; Jones, 1986; Marples *et al*., 1998). Marples and Roper (1996) showed that naïve domestic chicks had longer latencies to approach novel coloured (green or blue) food or water when it was associated with pyrazine than when in an odourless condition. Subsequently, Jetz *et al*. (in press) gave chicks the choice between brown crumbs (with which they were familiar) and either yellow crumbs or green crumbs. Chicks had no tendency to avoid the novel crumbs, but showed a significant bias against novel crumbs in the presence of pyrazine. From the results of the previous experiments, we might have expected chicks to avoid yellow crumbs more than the green, but this was not the case: the degree to which each novel colour was avoided relative to familiar brown crumbs was similar. This result is supported by another experiment where, instead of using untreated brown crumbs, chicks were fed and trained with either green crumbs or yellow crumbs, before being given the choice between green and yellow crumbs in a single choice trial. Those chicks that were given pyrazine alongside this trial showed a much stronger aversion to the novel colour than chicks that were in an odourless trail, but the size of the effect was identical whether that colour was green or yellow (Rowe, 1998). Therefore, there appears to be a significant effect of pyrazine that increases neophobic reactions in naïve chicks, but if colour biases are also involved, why is this not greater for the colour yellow than for the colour green? One possibility is that neophobic aversions seem to be stronger than those seen against specific colours (Jetz *et al*., in press). Therefore, any bias against yellow might have been swamped by the degree of enhanced neophobia (the interaction between novelty and coloration will be discussed further below).

Pyrazine elicits a hidden bias against conspicuous coloured crumbs

Having established that pyrazine can enhance unlearned aversions in birds against specific colours and against novel coloured prey, we conducted a further experiment that tested whether pyrazine elicited a similar effect against conspicuous food, since conspicuousness seems to be an almost ubiquitous feature of aposematic displays (Guilford and Dawkins, 1993). For this experiment, chicks were trained with brown crumbs on a brown background,

i.e. crumbs were cryptic during training. We then gave chicks a choice between purple and green crumbs (for which there is no colour bias, even in the presence of pyrazine, see Figure 1) which were presented either on a purple background (green crumbs were conspicuous), or on a green background (purple crumbs were conspicuous). In an odourless condition, chicks did not show any colour bias. However, with pyrazine present, chicks showed a significant bias against conspicuous coloured crumbs, regardless of which colour was conspicuous (Lindström *et al*., in press). This bias could either be against contrast itself, or simply an enhanced neophobic reaction to the novelty of contrasting food.

The role of novelty

Obviously visual novelty is important in its own right with pyrazine eliciting biases against visually novel crumbs, but how does it interact with conspicuousness and hue? In our experiments investigating biases against specific colours, all the crumbs presented in the test trial were novel, in that chicks had not experienced any coloured crumbs during training. To investigate directly the role of novelty in the elicitation of biases against specific colours in the presence of pyrazine, we next gave chicks different levels of experience during training with green and yellow crumbs (Rowe and Guilford, 1999). So, chicks either had 0, 24, 48 or 72 trials of green and yellow crumbs (half of each colour) before being given a test trial in the presence of pyrazine. The foraging bias elicited in the presence of pyrazine was not immediately extinguished by prior exposure to yellow and green crumbs in the absence of pyrazine. Induced aversion was even seen in birds that had experienced 48 crumbs (24 yellow and 24 green), and only disappeared in those birds having encountered 72 crumbs (36 yellow and 36 green) prior to exposure to pyrazine. Even when chicks are given plenty of encounters in which to learn that yellow and green are equally palatable, they still show an innate aversion to yellow when pyrazine is encountered. In our view this demonstrates surprising resilience in the hidden bias, whose triggering in the context of a bimodal 'display' is resistant to erosion by experience with the same visual stimuli outside this context. Indeed, this contextual isolation may be important if appropriate reactions are to be elicited at all by multimodal warning signals, as we shall see below. More significantly perhaps, we have now found that the aversive reaction that pyrazine causes to conspicuous food, regardless of actual colour, is also resilient to prior experience with conspicuousness (Lindström *et al*., in press). So, two aspects of aposematic colour patterns, that they contain specific colours such as yellow and that they are conspicuous, become the target of aversions from birds when a warning odour, pyrazine, is encountered simultaneously, but not in the odour's absence. Furthermore, even if birds are previously rewarded for eating yellow or

conspicuous crumbs outside the multimodal signalling context, these aversions are still triggered within the multimodal context. Since predators may encounter both yellow and conspicuous prey that are palatable, this contextual isolation is likely to be necessary (though perhaps not sufficient) if both multimodal warning displays and their receivers are genuinely receiving selective advantage from these reactions.

Up to this point, we have not discussed whether pyrazine needs to be novel in order to elicit these visual biases. Surprisingly, then, we have now shown that a single training trial with palatable brown crumbs being eaten in the presence of pyrazine is sufficient to extinguish any foraging bias against yellow or novel coloured foods (Rowe and Guilford, 1999; Jetz *et al.*, in press). That pyrazine needs to be novel in order to exert its effects raises the possibility that any novel odour could also produce the same foraging biases. Indeed, this appears to be true: methyl salicylate, or oil of wintergreen (a natural plant compound associated with pathogen resistance) and ethyl acetate (a man-made odour with no known signalling function) both produce foraging biases against yellow food and novel food, but again, only if these odours are novel. These results contrast with those of Marples and Roper (1996) who found that it was only pyrazines (2-methoxy-3-isobutyl pyrazine and 2-methoxy-3-sec-butyl pyrazine) and almond odour which enhanced neophobia towards novel coloured food and water, whilst thiazole (a man made odour) and vanilla odour had no significant effect. This difference could be due to the different odours used in each set of experiments, or that odour concentrations differed and that novel odours simply do not work if they are not strong enough. However, the fact that other novel odours can work equally well as pyrazine suggests that there is nothing special about pyrazine itself, but if this is the case, why do so many insects produce pyrazine (Rothschild *et al.*, 1984; Moore *et al.*, 1990)? Rothschild (Rothschild 1961; Rothschild *et al.*, 1984; Moore *et al.*, 1990) suggests that the prevalence of pyrazine in models (and some mimics) indicates that some predators can use pyrazine as a warning signal, allowing both Müllerian and some Batesian mimicry to evolve. However, because research in this area has been dominated by an interest in pyrazine, it could be instead that there are many different odours that insects use, to which we have not paid much attention. Alternatively, pyrazine may be favoured because it has some advantages over other odours, for example, perhaps it is relatively cheap to manufacture or more novel than other odours in the environment. Whilst these answers may seem unsatisfactory, what it is important to remember is that odour novelty seems to be an important factor in the production of visual foraging biases, more important than visual novelty. A possible explanation for this is that particular odours may be more reliably associated with particular prey if many more prey have similar coloration in nature than they do similar odours.

Summary of odour experiments

Our conclusions from this series of experiments using odours are:

1. Novel pyrazine can elicit and enhance biases against three aspects of visual warning signals, namely novelty, conspicuousness and some property of the colours yellow and red (but not green or purple).
2. Pyrazine only has this effect if it is novel, i.e. when birds have not had any previous experience with the odour.
3. All three odours that we have tested (pyrazine, ethyl acetate and methyl salicylate) elicit visual biases against novel colours and specifically against the colour yellow. However, this only occurs if the odours are novel.
4. Experience of yellow and green food can eliminate the bias against yellow crumbs, whilst experience of conspicuous food does not seem to dampen any bias against conspicuous food that novel pyrazine elicits.

Our results so far suggest that odour novelty is vital for eliciting visual biases. This points towards a general mechanism for inducing visual foraging biases, where any novel tests might induce similar effects. The following experiment tests this idea, by looking at whether a novel sound can produce the same foraging biases in a naïve avian predator as a novel odour.

Can sound produce unlearned visual foraging biases?

Studies of aposematic multimodal displays have concentrated upon the interaction between colour and odour, but many displays also involve the use of sound: authors describe hearing clicking (Blest, 1957), swishing or hissing (Carpenter, 1921, 1938; Bedford and Chinnock, 1966), rattling (Carpenter, 1938; Rothschild, 1965; Haskell and Rothschild, 1966; Masters, 1979), or buzzing (Gaul, 1952; Haskell, 1961). As with odours, these sounds are often produced upon attack and so may function in a similar manner. In this experiment, we investigated whether buzzing could elicit unlearned visual foraging biases against novel or warningly coloured food.

Methods

In the each of two consecutive weeks, 36-day-old chicks were trained in groups and then singly to eat brown crumbs from a circular training arena. On day 3 post-hatch, chicks were trained to eat single brown crumbs from a series of 8 sunken wells in a circular arena (as described elsewhere, see Rowe and Guilford, 1996, 1999). On day 4, chicks were given a random sequence of eight

crumbs of two different colours (four of each colour), according to their experimental group. There were three visual treatments: (1) brown (familiar) crumbs versus green (novel) crumbs, (2) brown (familiar) crumbs versus yellow (novel) crumbs, and (3) green (not a typical warning colour) versus yellow (a classic warning colour) crumbs. Therefore, the first two groups looked for a bias against novel coloured crumbs, whilst the third group investigated the relative avoidance of green and yellow crumbs. In each colour treatment, half the chicks had sound played continuously during the trial, whilst the other half had no buzzing and was a silent control. There were 6 chicks in each group in each week. The sound played to the chicks was that of a bumble bee (*Bombus terrestris*) which produced high-pitched buzzing when caught in a net. The buzzing was recorded using a Sony DAT walkman (TCD-D7) and microphone (Sony ECM-909A), and then played through a Macintosh Powerbook running Canary software. The buzzing began as the chick was put into the arena, and was played from a single speaker positioned in the centre of the arena, equidistant from all 8 wells. We played the buzzing at a level that we had previously established was not aversive to chicks (20 chicks were trained to eat 10 brown crumbs from a well and then half were given a trial with buzzing played underneath the food, whilst the others had a silent treatment: buzzing did not increase attack latency (Mann–Witney: $Z = 0.378$, NS) or time to eat all 10 crumbs ($Z = 1.17$; $n = 20$ NS)).

Results

The number of crumbs of each colour that each chick ate (see Table 2) was used to calculate the 'attack bias'. In groups 1 and 2, the attack bias was calculated as the number of familiar brown crumbs eaten minus the number of

Table 2. The mean (±SE) number of yellow and green crumbs eaten by each colour treatment when buzzing was present or absent

Experimental group	Brown crumbs eaten	Green crumbs eaten
Brown versus green, silent	4 ± 0	3.92 ± 0.08
Brown versus green, buzzing present	3.75 ± 0.25	3.25 ± 0.33
	Brown crumbs eaten	Yellow crumbs eaten
Brown versus yellow, silent	3.75 ± 0.13	3.92 ± 0.08
Brown versus yellow, buzzing present	3.83 ± 0.11	3.0 ± 0.41
	Green crumbs eaten	Yellow crumbs eaten
Green versus yellow, silent	3.58 ± 0.23	3.33 ± 0.40
Green versus yellow, buzzing present	3.58 ± 0.23	3.0 ± 0.44

novel coloured (green or yellow) crumbs eaten during the session. Therefore, positive scores show a bias against novel crumbs, whilst scores of 0 indicate no bias. The third group replicated our earlier experiment where chicks were given the choice between yellow and green crumbs, and here the attack bias was calculated as being the number of green crumbs minus the number of yellow crumbs eaten, and this time a positive score shows a bias against yellow crumbs. Figure 2 clearly shows that buzzing elicits hidden visual biases in naïve chicks. Across all colour treatments, there was a significant effect of sound (general non-parametric ANOVA: $H_1 = 10.6$, $p < 0.01$), but no effect of colour group ($H_2 = 0.68$, NS), and no interaction between colour and sound treatments ($H_1 = 1.45$, NS).

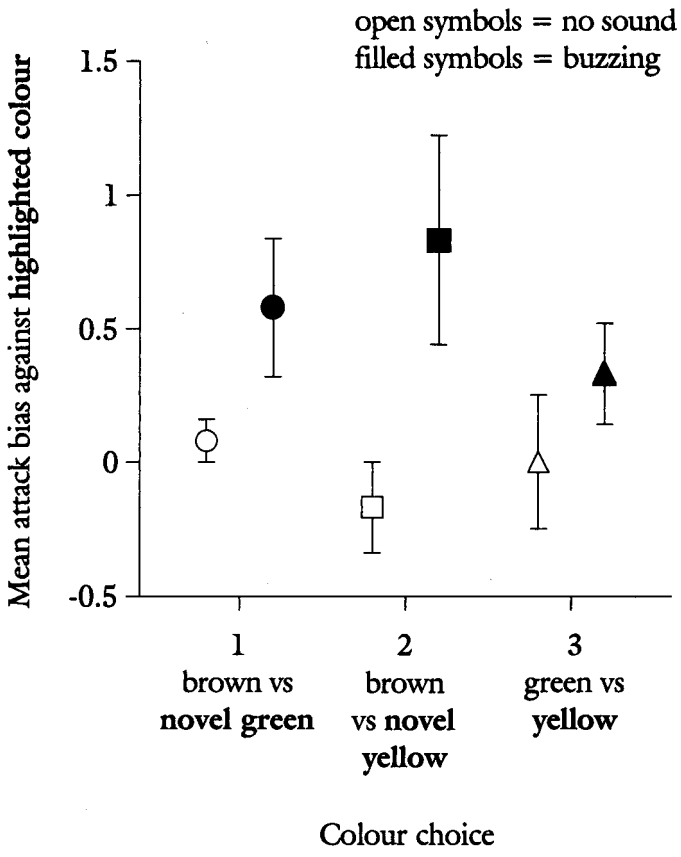

Figure 2. Mean attack bias shown by groups of chicks against (1) novel green crumbs (circles), (2) novel yellow crumbs (squares), and (3) yellow crumbs (triangles) where sound was absent (empty symbols) or present (solid symbols). Scores of 0 indicate no colour preference. n = 72.

Discussion

These results confirm that signal components in sensory modalities other than olfaction elicit hidden unlearned biases in naïve avian predators. The production of sound at a level that is not aversive to chicks elicits hidden biases against novel-coloured foods, and also against yellow food. The size of the biases do not seem to be as large as those produced by odours, but this could be indicative of differences in stimulus intensity rather than any qualitative difference between olfactory and auditory stimuli.

We do not know whether this effect is specific to buzzing, but as for odour, it may be that any sound will elicit a similar effect, as long as it is novel. This leads to an interesting idea that perhaps visual biases can be elicited by all novel stimuli in any non-visual sensory modality. This could include taste: insects can often be rejected on taste alone without the food being eaten, and in such cases it could be that taste is advertising underlying toxicity to avian predators (e.g. Coppinger, 1970; Marples *et al*., 1994). Obviously, prey palatability could span from individuals being mildly distasteful to being highly toxic, with predators' reluctance to eat being mapped on to such a scale. However, it would seem that taste qualitatively differs from sound and odour in that in some cases it could be a deterrent, whilst in others a reliable indicator of toxic chemicals.

At present, we can only hypothesise that taste might also evoke hidden aversions in birds, but perhaps one source of evidence for this is where birds have difficulty in learning colour avoidance tasks. For example, several studies show that naïve domestic chicks learn to avoid unpalatable crumbs faster if the crumbs contrast with their background and are conspicuous than if they are cryptic and match the background (Gittleman and Harvey, 1980; Roper and Wistow, 1986; Roper and Redston, 1987). However, it could be argued that none of these studies actually present any evidence that chicks learn to associate cryptic crumbs with unpalatability. The same effect is seen in our own colour discrimination tasks: when chicks are given yellow and green crumbs where one colour has been sprayed with quinine solution, chicks only learn the discrimination if the unpalatable crumb colour is yellow, and not when it is green (Rowe 1998). One further piece of evidence which suggests that some biases may exist in the presence of unpalatability comes from Marples *et al*. (1994) who manipulated the components of ladybird (*Coccinella septempunctata*) warning displays. Japanese quail (*Coturnix coturnix japonica*) readily ate palatable beetles that tasted and smelled of ladybird reflex blood, and yet started to reject them at a high rate once the coloured elytra were added. This was not entirely due to the colour pattern alone, nor to an interaction between the smell and colour as groups of quail given these combinations did not show such a high and sustained avoidance rate. Marples *et al*. (1994) concluded that

there was an interaction between taste and colour, although no mechanism was proposed. It is therefore conceivable that this interaction is the same as those witnessed with odour and sound, and the same general psychological process is producing all of these effects. In the following section we consider the possible mechanisms by which non-visual components elicit visual foraging biases, and address the question of how these displays have evolved.

Evolutionary considerations

So far we have said little either about the evolutionary stability of the reactions we have uncovered and the multimodal signals that cause them, or about the underlying mechanisms involved. As a starting position we can assume that innate aversions to stimuli, or combinations of stimuli, will not be evolutionarily stable unless these stimuli are either inherently dangerous (fitness reducing in some way) or genuinely correlated with harmful agents. It is one of the interesting aspects of the effects we report that neither pyrazine odours, buzzing nor the visual stimuli with which they interact are of themselves considered aversive by chicks, ruling out the first possibility that they have detrimental effects. But what about the second: are pyrazines, sounds, and aposematic or conspicuous colours genuinely correlated with dangerous attributes? There is no *a priori* reason why they should be, and so we must consider the question from a co-evolutionary perspective.

If triggering innate aversions in predators against warningly coloured prey is the sole fitness benefit of having multimodal warning signals, this will favour cheats who mimic the signals but are not genuinely unpalatable or toxic, and the system will destabilise. This, of course, exactly parallels the situation for unimodal warning coloration itself, where only genuinely noxious prey can afford to signal conspicuously, since edible prey who do so put themselves at greater risk from the attacks of naïve predators. What stabilises warning coloration, therefore, is the presence of predators that can distinguish cheats from honest signallers – and these cannot be predators with innate aversions (Guilford, 1992). There are specific exceptions to this, which lead to Batesian mimicry, but in general this must be true. In some cases, predators with innate aversions to warning colours may be tolerated by the system (e.g. Smith, 1975; Caldwell and Rubinoff, 1983), but they cannot be the general explanation. However, since we used domestic chicks in our experiments for the precise reason that they have been domesticated from generalist ancestors (Wood-Gush, 1989), it is unlikely that the effects that we have uncovered are an example of a specific co-evolved system.

So, in our view, these biases will not be evolutionarily stable as innate avoidance cues. However, as we pointed out above, it is the presence of

discerning predators that maintains aposematic signalling systems, and so a further associated possibility remains. Namely, that the function of the reactions we find is not actually to induce aversion to signal components, but to induce greater hesitancy in attack (see also Schuler and Hesse, 1985 for a similar effect produced by yellow and black stripes). Whilst we have recorded and presented the reactions of domestic chicks to various food stimuli as relative aversions, it is possible that these responses measure an increased hesitancy to attack or eat particularly coloured prey in the presence of the second stimulus (odour or sound). Given the choices presented to the birds, hesitancy towards a particular colour, such as yellow, could easily induce the bird to move on to the next crumb prematurely and therefore appear as an aversion. The reason this matters is that if predators are induced, by specific reactions to combinations of stimuli, to treat particular prey types more carefully (yellow, conspicuous, or novel, for example), then they may make a more accurate assessment of the prey's actual edibility. A prey that contains a chemical defence may be tested in such a way as to reveal the defence without completely ingesting the entire prey and its associated toxin dose. This kind of reaction will be favoured in predators, because it will help to expose genuine profitability differences between prey types at the same time as reducing risk of injury or toxicosis. Inducing this reaction will also be favoured in prey, but only as long as they are genuinely noxious. In addition, it may facilitate the individual selection of chemical or other delayed-acting defences (Guilford, 1990). This idea is a multimodal variant of the 'go-slow' signalling hypothesis proposed for the evolution of warning coloration itself (Guilford, 1994).

However, innate hesitancy cannot fully account for our results, in that chicks will avoid yellow prey in the presence of pyrazine even when they have experienced it sufficiently to associate the colour with edible food (Rowe, 1998; Rowe and Guilford, 1999). In this case, there is no reason for chicks to show hesitation in eating yellow coloured food with which they are already familiar. This finding suggests that the presence of the odour can alter the perceived value associated with the colour. It is almost as if the presentation of even a familiar colour in the presence of pyrazine creates a different visual stimulus, one with which potentially new outcomes may become associated. Therefore, a multisensory combination of stimuli could create a perceptually distinct unit that is relatively isolated from any prior experience that a predator may have had with the stimuli outside the multimodal context. In the same sense that conspicuous warning colours and patterns may be designed to be highly discriminable and distinctive to aid avoidance learning and recall where there is genuine toxicity, so the addition of non-visual components may enhance this distinctiveness. The perceptual isolation created by stimulus combinations would allow greater discriminability of warning stimuli, and therefore enhanced efficacy of the warning signal in a more conventional role.

Although some of our data are consistent with this hypothesis, others suggest that effects may be asymmetric between components. That is, prior experience of particular colours may be relatively well isolated from the same colours within the multimodal display, whilst prior experience of the odour may not be (Rowe and Guilford, 1999). The significance of this is currently unclear to us, but it does not in principle negate the hypothesis that perceptual isolation is part of the function of multimodal warning signalling. However, the perceptual isolation hypothesis alone cannot account for foraging biases, but may work in conjunction with the hesitancy hypothesis presented above. And hesitancy may benefit from being biased since for traditional warning colour reasons, novel, conspicuous, and particularly coloured prey (yellow or red for example) *are* in fact more likely to be worth being careful with, at least initially.

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