Pyrazine odour makes visually conspicuous prey aversive

Leena Lindström1*, Candy Rowe2 and Tim Guilford3

1Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, FIN-40351 Jyväskylä, Finland
2Department of Psychology, Ridley Building, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK
3Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

Unpalatable insects frequently adopt multimodal signals to ward off predators, incorporating sounds and odours into their colourful displays. Pyrazine is an odour commonly used in insect warning displays, and has previously been shown to elicit unlearned biases against common warning colours, e.g. yellow and red in naïve predators. We designed two experiments to test for similar effects of pyrazine on the conspicuousness of prey, perhaps the most ubiquitous aspect of aposematic coloration. In the first experiment, we offered predators (Gallus gallus domesticus) a choice between conspicuous crumbs and cryptic crumbs in the presence or absence of pyrazine. In the second experiment, we manipulated the birds’ experience of conspicuous prey during an initial training phase. Only in the presence of pyrazine did birds show a bias against conspicuously coloured food, and this occurred whether or not they had previously experienced food that contrasted with the background. This emergent behaviour relied upon the visual and odorous signal components being presented together. These unlearned, yet hidden, responses against conspicuousness demonstrate that there are initial benefits to prey being conspicuous when the multimodal nature of warning signals is accounted for.

Keywords: aposematism; conspicuousness; hidden bias; pyrazine; receiver psychology

1. INTRODUCTION

It has long been known that toxic prey species protect themselves from predation using complex multimodal signals combining vivid coloration with odours and sounds (Poulton 1890; Cott 1940; Rothschild 1964; Edmunds 1974). The function of the striking warning or ‘aposematic’ coloration has generally been attributed to enhancing avoidance learning in predators, particularly through its conspicuous nature (Gittleman & Harvey 1980; Roper & Redston 1987; Ataloto & Mappes 1996; Lindstrom et al. 1999a). Unlearned responses against particular colours and patterns associated with aposematism have also been shown in avian predators (Schuler & Hesse 1985; Sillén-Tullberg 1985; Roper & Cook 1989; Roper 1990; Lindstrom et al. 1999b), although these have never been shown to be against visual conspicuousness per se. Although the evolutionary significance of warning coloration has been relatively well explored, recent experiments have shown that it is misleading to assume that different components of a signal function in isolation (e.g. Hughes 1996; Rowe & Guilford 1996, 1999; Kilner et al. 1999). We recently showed (Rowe & Guilford 1996) that unlearned responses to particular colours that are commonly used in warning displays (yellow and red) may remain hidden in avian predators until triggered by the presence of a second warning signal component (pyrazine odour). Since conspicuousness is the most prominent and generic feature of visual warning signals (Guilford & Dawkins 1993), we conducted two experiments to investigate whether conspicuousness itself could evoke hidden innate reactions in young birds when presented in conjunction with pyrazine odour.

2. MATERIAL AND METHODS

(a) Experiment 1

We used a model laboratory system (Rowe & Guilford 1996, 1999), with domestic chicks (Gallus gallus domesticus) as predators of dyed chick starter crumbs. Thirty-five day-old chicks were obtained on day 1 from a local hatchery, and were trained to eat brown chick starter crumbs on a background that matched the natural crumb colour in a high-walled training arena. All birds became accustomed to eating on their own in the arena by the end of the first day.

On days 2 and 3, subjects were trained to forage in an experimental runway, where palatable crumbs were presented in a series of eight sunken wells. At the bottom of each well was a Petri dish with a hole pierced in its lid, filled with chick crumbs that provided a matching background upon which to present crumbs. Subjects learned to walk through the runway and eat single crumbs from the lids of each dish, and by the end of day 2 would readily feed on their own in the apparatus. On the third training day, 30 subjects attained the training criterion of eating all eight brown crumbs in two consecutive sessions. These chicks were food deprived overnight before testing the following day.

On day 4, all chicks were given a random sequence of four palatable green and four palatable purple crumbs in one out of four experimental conditions. We used purple and green crumbs as in a previous experiment, chicks did not show a colour preference either with or without odour present (Rowe 1998). Crumbs and backgrounds (paper placed on the lid of each Petri dish) were dyed using standard food dyes. Half the chicks were given the crumbs on a purple background (i.e. purple crumbs were cryptic and green crumbs were conspicuous), and the other half had the crumbs presented on a green background (i.e. green crumbs were cryptic and purple crumbs were conspicuous). Cotton wool containing four drops of pyrazine solution (0.1ml 2-isobutyl-3-methoxypyrazine dissolved in 10ml pure ethanol and made up to 11 with distilled water) was added to the Petri

*Author for correspondence (ilema@cc.jyu.fi).
Table 1. The mean number (± s.e.m.) of conspicuous and cryptic crumbs eaten by each group in both experiments. (Purple and green refer to the background colours, and therefore, also indicate the colours of the cryptic crumbs.)

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>N</th>
<th>Cryptic Crumbs</th>
<th>Conspicuous Crumbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple/odourless</td>
<td>7</td>
<td>4.00 ± 0.00</td>
<td>3.86 ± 0.14</td>
</tr>
<tr>
<td>Green/odourless</td>
<td>8</td>
<td>3.75 ± 0.16</td>
<td>4.00 ± 0.00</td>
</tr>
<tr>
<td>Purple/odour</td>
<td>8</td>
<td>3.25 ± 0.41</td>
<td>1.50 ± 0.57</td>
</tr>
<tr>
<td>Green/odour</td>
<td>7</td>
<td>3.85 ± 0.14</td>
<td>3.14 ± 0.40</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purple/conspicuous familiar/odourless</td>
<td>14</td>
<td>4.00 ± 0.00</td>
<td>3.64 ± 0.20</td>
</tr>
<tr>
<td>Green/conspicuous familiar/odourless</td>
<td>14</td>
<td>3.85 ± 0.14</td>
<td>3.71 ± 0.22</td>
</tr>
<tr>
<td>Purple/conspicuous novel/odourless</td>
<td>16</td>
<td>3.75 ± 0.11</td>
<td>3.56 ± 0.20</td>
</tr>
<tr>
<td>Green/conspicuous novel/odourless</td>
<td>14</td>
<td>3.64 ± 0.17</td>
<td>3.07 ± 0.35</td>
</tr>
<tr>
<td>Purple/conspicuous familiar/odour</td>
<td>15</td>
<td>3.47 ± 0.23</td>
<td>1.93 ± 0.40</td>
</tr>
<tr>
<td>Green/conspicuous familiar/odour</td>
<td>15</td>
<td>3.80 ± 0.11</td>
<td>3.33 ± 0.22</td>
</tr>
<tr>
<td>Purple/conspicuous novel/odour</td>
<td>15</td>
<td>3.00 ± 0.29</td>
<td>1.93 ± 0.38</td>
</tr>
<tr>
<td>Green/conspicuous novel/odour</td>
<td>15</td>
<td>3.60 ± 0.16</td>
<td>2.40 ± 0.36</td>
</tr>
</tbody>
</table>

3. RESULTS

(a) Experiment 1

In the first experiment, chicks were given initial training using cryptic brown crumbs, and then presented with four cryptic and four conspicuous prey, either in the presence or the absence of pyrazine. Pyrazine did not reduce the ingestion of cryptic crumbs (Mann–Whitney, \(Z = -1.01, n = 30\), not significant), but did significantly depress the ingestion of conspicuous crumbs (Mann–Whitney, \(Z = -3.675, n = 30, p = 0.001\)) (Table 1). This is most clearly shown by their relative preferences for cryptic crumbs (Figure 1). While chicks ate conspicuous and cryptic crumbs roughly equally in the absence of odour (Wilcoxon, \(Z = -0.577, n = 15\), not significant), they significantly avoided conspicuous crumbs in its presence (Wilcoxon, \(Z = -2.844, n = 15, p = 0.004\)). When the difference between conspicuous and cryptic crumbs was a dependent variable, the bias was more present in the pyrazine group (non-parametric multiway factorial ANOVA for ranked data, \(H = 9.131, d.f. = 1, p < 0.005\)). There may have been a marginal effect of background colour (\(H = 2.819, d.f. = 1, p = 0.093\)), but there was no significant interaction between this and odour (\(H = 0.570, d.f. = 1, not significant\)).

(b) Experiment 2

Pyrazine odour induced an aversion to conspicuous prey in experiment 1, but because chicks had no experience with conspicuous food, it is possible that the odour-induced effect was confounded by a response against novel presentation (Marple & Roper 1996). Our second experiment, therefore, repeated experiment 1 but this time only half of the chicks were trained using brown crumbs on brown backgrounds (conspicuousness novel),
with the other half being trained with brown crumbs on white backgrounds (conspicuousness familiar). The results of this experiment confirmed that of the first, and showed that while pyrazine had a small but significant effect at depressing overall ingestion (Mann-Whitney, $Z = -2.654$, $n = 118$, $p = 0.01$), its main effect was to cause aversions against conspicuous crumbs (Mann-Whitney, $Z = -4.524$, $n = 118$, $p < 0.001$) (see table 1). In relative terms (figure 2), there was a significant avoidance of conspicuous prey in the presence of pyrazine ($H = 12.392$, d.f. = 1, $p < 0.001$). Novelty of conspicuousness, however, showed no main effect ($H = 0.580$, d.f. = 1, not significant), no interaction with pyrazine ($H = 0.023$, d.f. = 1, not significant), and no three-way interaction with background type and odour ($H = 0.720$, d.f. = 1, not significant). There was a small non-significant interaction between background colour and novelty of conspicuousness ($H = 3.559$, d.f. = 1, $p = 0.059$). In addition to replicating the effect of pyrazine in inducing a foraging bias against conspicuous prey, these results show that conspicuousness itself does not have to be novel for birds to avoid it.

### 4. DISCUSSION

These experiments demonstrate that a common warning odour, pyrazine, can elicit unlearned aversions to conspicuous food in birds which are not apparent in the absence of the odour. The effect can not be attributable to differences in the detectability of the two prey types since (i) conspicuous food is likely to be easier to find than cryptic, and (ii) there were no biases exhibited by the control groups. This odour-induced ingestion bias is similar in form to those that we have already shown against prey that is typically waringly coloured (red and yellow) (Rowe & Guilford 1996), or that which is novel in appearance (Marples & Roper 1996; Jetz et al. 2000). However, this aversion to conspicuous prey is not reduced through prior experience with conspicuous food unlike the colour biases we have previously shown (Rowe & Guilford 1999), and hence is not dependent on an inevitably transient neophobic reaction against conspicuous food. The more robust nature of this particular bias may be a consequence of how prey are encountered in nature: predators will encounter the same prey species on a variety of backgrounds, and even cryptic species will find it difficult to be camouflaged all the time. Therefore, conspicuousness can not be a wholly reliable cue upon which predators should base their foraging decisions, but those insects that are unprofitable to birds are likely to be conspicuous. The olfactory signal component appears to make avian predators more hesitant to attack conspicuous prey, and in nature may help a predator to discriminate between conspicuous prey that is aposematic, and that which is profitable to eat.

Although it has been possible to show unlearned foraging biases against particular colours and patterns associated with aposematism without pyrazine present (Schuler & Hese 1983; Sillén-Tullberg 1985; Roper & Cook 1989; Roper 1990; Lindström et al. 1999b), this is the first evidence for a bias against conspicuous prey per se. Indeed, the function of conspicuousness in warning coloration has previously mainly been attributed to enhancing associative learning (Gittleman & Harvey 1980; Roper & Redston 1987; Schuler & Roper 1992; Alatalo & Mappes 1996; Lindström et al. 1999a; but see...
This result is the first to clearly point to specific unlearned biases against conspicuousness that cannot be attributed to other visual aspects of the food, such as novelty or colour per se. This has important implications for our understanding of the initial costs to aposematism. Conspicuousness of visual warning signals has previously been assumed to be costly to prey in encounters with naive predators, since it increases both detection and attack rate (Gittleman & Harvey 1980; Roper & Redston 1987; Guilford & Dawkins 1993; Yachi & Higashi 1998; Alatalo & Mappes 1996; Lindström et al. 1999a). However, considering the multimodal nature of warning signals, conspicuousness may in fact be beneficial even initially. This mechanism could help explain the initial evolution of conspicuous warning coloration without the prerequisite of gregarious behaviour (Sillén-Tullberg & Leimar 1988; Tullberg et al. 2000). However, it remains also possible that the bias against conspicuous prey in the presence of pyrazine is a result from predator–prey evolution and is therefore a predator adaptation to a world that consists of many toxic species.

Many signalling systems are multimodal in nature, and yet we have only recently begun to explore the potential for intersensory interactions between components (Rowe & Guilford 1996; Kilner et al. 1999; Partan & Marler 1999; Rowe 1999). These results emphasize how by investigating multiple components in displays, we can improve our understanding of signal function significantly. Since some receiver responses to a signal can remain hidden in the presence of a single component and only emerge when presented with multiple components, it seems important to study interactions between components to understand fully how the signal works as a whole.

We thank J. Howe for immense help in the laboratory, and T. Mappes and S. Puustinen for statistical advice, The Academy of Finland (L.L.) helped finance the study. C.R. was supported by the Mary Lunt Senior Scholarship from St Hugh’s College, University of Oxford during these experiments, and the Sir James Knott Research Fellowship while this work was being prepared for publication.

REFERENCES

Cott, H. B. 1940 Adaptive coloration in animals. London: Methuen.
Jetz, W., Rowe, C. & Guilford T. 2000 Non-warning odors trigger innate color aversions— as long as they are novel. Behav. Ecol. (In the press.)