

# Pyrazine odour makes visually conspicuous prey aversive

Leena Lindström<sup>1\*</sup>, Candy Rowe<sup>2</sup> and Tim Guilford<sup>3</sup>

<sup>1</sup>*Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, FIN-40 351 Jyväskylä, Finland*

<sup>2</sup>*Department of Psychology, Ridley Building, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK*

<sup>3</sup>*Department 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 naive 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; Alatalo & Mappes 1996; Lindström *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; Lindström *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 (0.1 ml 2-isobutyl-3-methoxypyrazine dissolved in 10 ml pure ethanol and made up to 1 l with distilled water) was added to the Petri

\* Author for correspondence (lilema@cc.jyu.fi).

Table 1. *The mean number ( $\pm$  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.)*

experimental group	<i>n</i>	cryptic crumbs	conspicuous crumbs
experiment 1			
purple/odourless	7	4.00 $\pm$ 0.00	3.86 $\pm$ 0.14
green/odourless	8	3.75 $\pm$ 0.16	4.00 $\pm$ 0.00
purple/odour	8	3.25 $\pm$ 0.41	1.50 $\pm$ 0.57
green/odour	7	3.85 $\pm$ 0.14	3.14 $\pm$ 0.40
experiment 2			
purple/conspicuous familiar/odourless	14	4.00 $\pm$ 0.00	3.64 $\pm$ 0.20
green/conspicuous familiar/odourless	14	3.85 $\pm$ 0.14	3.71 $\pm$ 0.22
purple/conspicuous novel/odourless	16	3.75 $\pm$ 0.11	3.56 $\pm$ 0.20
green/conspicuous novel/odourless	14	3.64 $\pm$ 0.17	3.07 $\pm$ 0.35
purple/conspicuous familiar/odour	15	3.47 $\pm$ 0.23	1.93 $\pm$ 0.40
green/conspicuous familiar/odour	15	3.80 $\pm$ 0.11	3.33 $\pm$ 0.22
purple/conspicuous novel/odour	15	3.00 $\pm$ 0.29	1.93 $\pm$ 0.38
green/conspicuous novel/odour	15	3.60 $\pm$ 0.16	2.40 $\pm$ 0.36

dishes of approximately half of the chicks in each background colour condition. Therefore there were four experimental groups: purple background with pyrazine absent ( $n = 7$ ) or present ( $n = 8$ ); and green background with pyrazine absent ( $n = 8$ ) or present ( $n = 7$ ). We recorded which crumbs the subjects ate. Because pyrazine is a pervasive odour and it was impossible to use separate experimental rooms, chicks from the two control odourless groups were tested before the odour was added to the apparatus and the remaining two pyrazine groups were assessed. However, because the pyrazine subjects were likely to be marginally more hungry and more likely to eat all the crumbs presented to them, we consider any effect of order to be likely to dampen rather than exaggerate any effects of odour.

### (b) Experiment 2

In the first experiment, we did not control for the possible effects of conspicuous crumbs being novel, and so we designed a second experiment to explore these potential effects. Over three experimental weeks, we used a total of 118 chicks. Each batch was divided into groups upon arrival, and trained on days 1 to 3 (see §2(a)) either with brown crumbs on a brown background (i.e. cryptic crumbs,  $n = 60$ ) or with brown crumbs on a white background (i.e. conspicuous crumbs,  $n = 58$ ). Therefore, the first group repeated experiment 1, while the latter group familiarized birds with 'conspicuous' food prior to testing. On day 4, chicks in each training condition were divided into four further groups according to background colour and odour treatment as for experiment 1. Birds trained with cryptic crumbs were tested either on a green background with pyrazine absent ( $n = 14$ ) or present ( $n = 15$ ), or on a purple background with pyrazine absent ( $n = 16$ ) or present ( $n = 15$ ). Birds trained with conspicuous crumbs were tested either on a green background with pyrazine absent ( $n = 14$ ) or present ( $n = 15$ ), or on a purple background with pyrazine absent ( $n = 14$ ) or present ( $n = 15$ ). All other experimental details are as for experiment 1.

### (c) Statistical analysis

Since the structure of the data did not meet the assumptions of parametric statistics, non-parametric statistics were applied. When the assumptions of parametric ANOVA were not met, we performed a non-parametric multiway ANOVA for ranked

variables of the data. The sum of squares and mean sums of squares were estimated as in parametric multiway ANOVA, the test values  $H (= SS_{\text{source}}/MS_{\text{total}})$  follows asymptotically the  $\chi^2$ -distribution with d.f.<sub>source</sub> (Zar 1996). All  $p$ -values are two-tailed.

## 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 (Marples & 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),

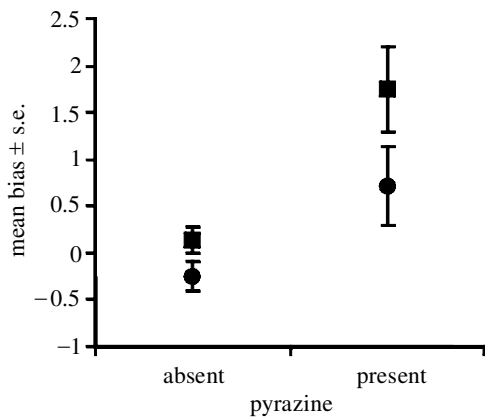


Figure 1. The mean bias against conspicuous crumbs eaten for all groups in experiment 1. Bias against conspicuous crumbs was calculated as the number of cryptic crumbs eaten minus the number of conspicuous crumbs eaten. Therefore, zero indicates no bias with both types of crumb being eaten equally, while positive scores indicate that birds ate more cryptic than conspicuous crumbs. Bars represent one standard error above and below the mean. Squares indicate that birds were tested on a purple background and circles that they were tested on a green background.

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 ( $H = 12.892$ , 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 ( $H = 0.025$ , 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 warningly 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

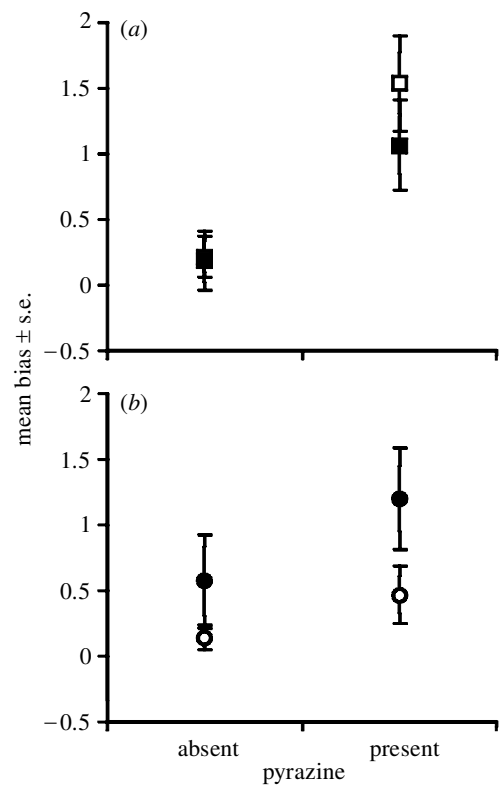


Figure 2. The mean bias against conspicuous crumbs eaten for all groups in experiment 2 (calculated as for figure 1), (a) where crumbs were presented on a purple background, and (b) where crumbs were presented on a green background. Bars represent one standard error above and below the mean. Open symbols indicate that conspicuousness was familiar to the birds and filled symbols indicate where it was novel.

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 & Hesse 1985; 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 been attributed to enhancing associative learning (Gittleman & Harvey 1980; Roper & Redston 1987; Schuler & Roper 1992; Alatalo & Mappes 1996; Lindström *et al.* 1999a; but see

Guilford 1986). This result is the first to clearly point to specific unlearned biases against conspicuousness that can not 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 predatory 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

- Alatalo, R. V. & Mappes, J. 1996 Tracking the evolution of warning signals. *Nature* **382**, 708–710.
- Cott, H. B. 1940 *Adaptive coloration in animals*. London: Methuen.
- Edmunds, M. 1974 *Defence in animals. A survey of anti-predator defences*. New York: Longman.
- Gittleman, J. L. & Harvey, P. H. 1980 Why are distasteful prey not cryptic? *Nature* **286**, 149–150.
- Guilford, T. 1986 How do warning colours work? Conspicuousness may reduce recognition errors in experienced predators. *Anim. Behav.* **34**, 286–288.
- Guilford, T. & Dawkins, M. S. 1993 Are warning colors handicaps? *Evolution* **47**, 400–416.
- Hughes, M. 1996 The function of concurrent signals: visual and chemical communication in snapping shrimps. *Anim. Behav.* **52**, 247–257.
- 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.)
- Kilner, R. M., Noble, D. G. & Davies, N. B. 1999 Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature* **397**, 667–672.
- Lindström, L., Alatalo, R. V., Mappes, J., Riipi, M. & Vertainen, L. 1999a Can aposematic signals evolve by gradual change? *Nature* **397**, 249–251.
- Lindström, L., Alatalo, R. V. & Mappes, J. 1999b Reactions of hand reared and wild-caught predators towards warningly colored, gregarious and conspicuous prey. *Behav. Ecol.* **10**, 317–322.
- Marples, N. M. & Roper, T. J. 1996 Effects of novel colour and smell on the response of naive chicks towards food and water. *Anim. Behav.* **51**, 1417–1424.
- Partan, S. & Marler, P. 1999 Communication goes multimodal. *Science* **283**, 1271–1273.
- Poulton, E. B. 1890 *The colours of animals. Their meaning and use. Especially considered in the case of insects*. London: Kegan Paul, Trench, Trübner & Co.
- Roper, T. J. 1990 Responses of domestic chicks to artificially coloured insect prey: effects of previous experience and background colour. *Anim. Behav.* **39**, 466–473.
- Roper, T. J. & Cook, S. E. 1989 Responses of chicks to brightly coloured insect prey. *Behaviour* **110**, 276–293.
- Roper, T. J. & Redston, S. 1987 Conspicuousness of distasteful prey affects the strength and durability of one-trial avoidance learning. *Anim. Behav.* **35**, 739–747.
- Rothschild, M. 1964 A note on the evolution of defensive and repellent odours of insects. *Entomologist* **97**, 276–279.
- Rowe, C. 1998 Multicomponent signals. DPhil thesis, University of Oxford, UK.
- Rowe, C. 1999 Receiver psychology and the evolution of multi-component signals. *Anim. Behav.* **58**, 921–931.
- Rowe, C. & Guilford, T. 1996 Hidden colour aversion in domestic chicks triggered by pyrazine odours of insect warning displays. *Nature* **383**, 520–522.
- Rowe, C. & Guilford, T. 1999 Novelty effects in a multimodal warning signal. *Anim. Behav.* **57**, 341–346.
- Schuler, W. & Hesse, E. 1985 On the function of warning coloration: a black and yellow pattern inhibits prey-attack by naive domestic chicks. *Behav. Ecol. Sociobiol.* **16**, 249–255.
- Schuler, W. & Roper, T. J. 1992 Responses to warning coloration in avian predators. *Adv. Stud. Behav.* **21**, 111–146.
- Sillén-Tullberg, B. 1985 Higher survival of an aposematic than of a cryptic form of distasteful bug. *Oecologia* **67**, 411–415.
- Sillén-Tullberg, B. & Leimar, O. 1988 The evolution of gregariousness in distasteful insects as a defence against predators. *Am. Nat.* **132**, 723–734.
- Tullberg, B.-S., Leimar, O. & Gamberale-Stille, G. 2000 Did aggregation favour the initial evolution of warning coloration? A novel world revisited. *Anim. Behav.* **59**, 281–287.
- Yachi, S. & Higashi, M. 1998 The evolution of warning signals. *Nature* **394**, 882–884.
- Zar, J.-H. 1996 *Biostatistical analysis*, 3rd edn. Upper Saddle River, NJ: Prentice-Hall.