Better to be bimodal: the interaction of color and odor on learning and memory

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Defended prey frequently advertise to potential predators using multimodal warning displays. Signaling through more than one sensory pathway may enhance the rate of avoidance learning and the memorability of these learned avoidances. If this is so, then mimetic insects would gain more protection from mimicking a multimodal rather than a monomodal model. Day-old domestic chicks (Gallus gallus domesticus) were used to examine whether a common insect warning odor (pyrazine) enhanced learning and memorability of yellow prey, a common warning color. Pyrazine increased the rate at which the chicks learned to avoid unpalatable yellow prey, and how well this learned avoidance was remembered after a 96-h interval. After 96 h, mimics of the multimodal prey were avoided, whereas mimics of the monomodal prey were not. In the absence of pyrazine, chicks generalized their learned avoidance of the unpalatable yellow prey to palatable green prey; however, the presence of pyrazine reduced this color generalization. These results suggest that much is to be gained from signaling multimodally, for both models and mimetic prey species. The presence of multimodal prey in the habitat may also advantage the predators as it allows them to distinguish more easily between palatable and unpalatable prey. Key words: avian predators, learned avoidance, memorability, mimics, prey, pyrazine. [Behav Ecol 19:425–432 (2008)]

Animals need to signal for a multitude of reasons to a range of different receivers. Whatever the meaning of the signal, it is important that it is noticed, recognized, and correctly interpreted (Guilford and Dawkins 1991). It is a striking feature of signals that they often operate through several sensory modalities at once: visual displays are enhanced by odors, birdsongs by colorful plumage prominently displayed during the song, and so on. Such “multimodal signals” are abundant in the animal world (see Rowe 1999a for review), and it has been suggested that these combined stimuli may enhance the detectability, discriminability, and memorability of the message (Guilford and Dawkins 1991; Rowe 1999b). However, combining elements of signals may have consequences more complex than that of simply reinforcing the message. Individual component cues may affect how the other cues are perceived (Pearce 1997), either in a positive manner by potentiating learning about the signal (Mackintosh 1974; van Kampen and Bolhuis 1991, 1993) or negatively by blocking or overshadowing one another (Mackintosh 1976; Kehoe 1982), thus preventing the receiver from learning one cue in the presence of another. It is therefore necessary to study multicomponent signals holistically in order to understand how the additional signaling components affect the receiver’s perception of the signal (Rowe 1999a).

An alternative view is that multicomponent signals are not aimed at one receiver alone but are designed so that each component is in the favored sensory modality of different potential receivers (Hebets and Papaj 2005). For example, a prey species may use a visual signal to potential bird predators that are primarily visual foragers (Fernandez-Juricic et al. 2004), whereas an odor cue is for mammalian predators who rely more on olfaction when hunting (Ylönen et al. 2002). The sound component of the signal may communicate primarily to nocturnal attackers (Rucci and Wray 1999). If multicomponent signals are constructed as an amalgam of signals to different receivers, then we would not expect to find better learning and memory of such signals compared with monomodal signals when only the response of a single predator is considered.

In order to test the contrasting predictions of the hypotheses accounting for multimodal signals, in this study, we examine a display which is typical of one form of multimodal interspecific signaling, namely the warning displays of aposematic insects.

Aposematic insects advertise their defended state to potential predators using conspicuous warning displays (Poulton 1890; Cott 1940; Edmunds 1974), which Cott (1940) defined as signals that distinguish the signaler from the background. Conspicuous visual signals enhance the rate at which avian predators learn to avoid unprofitable prey (Gittleman and Harvey 1980; Lindström 1999; Riipi et al. 2001). They improve how well these lessons are remembered (Roper and Redston 1987; Roper 1994), reducing the number of prey sacrificed in order to educate a predator and prolonging protection once education is complete.

In addition to these conspicuous visual signals, many aposematic species use warning odors and/or sounds in their warning displays (Wallace 1891; Rothschild 1961; Rothschild and Haskell 1966; Rothschild et al. 1984; Moore et al. 1990). It has been suggested that these additional signal components may enhance the conspicuousness of a visual warning display and therefore improve learning and memorability (Rowe 1999b; Speed 2000). Studies using rats and rabbits as foragers support this view (Sutherland and Mackintosh 1971; Kehoe 1982, 1986) and show that compound stimuli are remembered for longer (Kehoe et al. 1994), at least in laboratory conditions. Rowe (2002) noted that a beeping sound sped up discrimination learning in chicks. Similarly, Skelhorn and Rowe (2005, 2006) observed that gustatory cues improved learning and memory.

Pyrazines are common warning odors found in a diverse range of aposematic insects across many taxa and geographical locations (Rothschild 1961; Moore et al. 1990; Woolson and Rothschild 1990). Rothschild and Moore (1987) suggested that they may act as alerting signals, drawing the
predator’s attention to the food and therefore aiding differ-
etiation of the signal from its background, making it more
conspicuous. However, to date, there is little direct empirical
evidence for this effect, although several experimental results
are consistent with the idea (Barnea et al. 2004). Pyrazine
odor has also been shown to increase innate avoidance of
warningly colored and novel prey (Marples and Roper 1996;
Rowe and Guilford 1996, 1999a, 1999b).

Once the predator has overcome its initial neophobia and
started to sample the novel prey, pyrazines may also act as
discriminant cue for learned avoidances in the absence of
visual cues, as has been observed for both chicks (Guilford
further demonstrated that pyrazine increased the speed with
which chicks learned to avoid distasteful red water. Two other
nonwarning odors, almond and vanilla, have also been ob-
served to enhance avoidance learning and memorability
(Roper and Marples 1997a).

If a signal is to work as an effective defense, once a predator
has formed an association between the warning display and the
distastefulness of the prey, it must remember this associa-
tion each time it meets the prey (Guilford and Dawkins 1991;
Speed 2000). Guilford et al. (1987) trained chicks to avoid
tainted water accompanied by pyrazine odor. After a 24-h re-
tention interval, the chicks showed no avoidance of the water;
however, they did show more signs of distress during the sub-
sequent test than untrained chicks, suggesting some recollec-
tion of the meaning of the pyrazine odor. However, this study
was conducted without a color cue, so represents the memo-
rability of pyrazine as a lone signal. Roper and Marples
(1997a) noted that after a 24-h retention interval, almond
odor prolonged memorability of a learned avoidance of un-
palatable water. Barnea et al. (2004) noted that pyrazine odor
prolonged memorability of unpalatable water in red, yellow,
and green tubes.

In their study of the memory-enhancing effects of a conspic-
uous visual signal, Roper and Redston (1987) made their prey
unpalatable using methyl anthranilate. Methyl anthranilate
has a noticeable odor (Marples and Roper 1997); therefore,
the distasteful prey of Roper and Redston (1987) possessed
a multimodal warning signal, although the importance of this
was not recognized at the time or mentioned in their paper.
Their results show that unpalatable odorous-conspicuous prey
were avoided more than unpalatable odorless-cryptic prey;
therefore, it is possible that the odor cue may have interacted
more with the conspicuous than the cryptic visual signal.

All previous studies investigating the effect of pyrazine on
learned avoidances have used unpalatable water rather than
unpalatable solid food (Guilford et al. 1987; Kaye et al. 1989;
Barnea et al. 2004). Roper and Marples (1997b) noted that
birds respond differently to liquids and solids. It would seem
that solid prey more accurately represents insect prey, and
therefore, this current study seeks to investigate whether py-
razine odor enhances learned avoidance of solid yellow prey.

Many undefended insect species gain protection by mimick-
ing the warning signals of aseptic species, thereby spoofing
the predator into generalizing the learned avoidances of the
model to the mimic species (Wallace 1870; Duncan and Shep-
pard 1965). Mimics and models are not always sympatric and
may be separated spatially (Waldbauer 1988a) or temporally
(Waldbauer 1988b), or both (Joron 2003). As a result, long-
term memorability of learned avoidance of the model is im-
portant for the protection of the mimic. If defended insects
that signal multimodally are better protected and remem-
bered for longer, then it is to be expected that mimics of
multimodal model species will be better protected than mimics
of monomodal species. There are many examples of
multimodal mimicry by insect species (Rothschild 1984;
Moore et al. 1990), but until this present study, no research
has investigated the difference in protection gained by mim-
icking a monomodal or a multimodal signal. There is evi-
dence to suggest that even poor visual mimics of the model
can often gain some level of protection (Cuthill and Bennett
1993; Dittrich et al. 1993), though they are not as well pro-
tected as perfect mimics (Azmeh et al. 1998). It is unknown
whether mimics of multimodal models need to mimic all com-
ponents of the multimodal signal or whether mimics of the
visual component alone is sufficient to give full protection.

In the following experiment, we used chicks (Gallus gallus
domesticus) foraging on colored crumbs to examine whether
pyrazine odor enhances learned avoidances of unpalatable
yellow prey and how well these learned avoidances are remem-
bered after a retention interval. We then compared the avoid-
ance of perfect and imperfect mimics of the model after the
same retention interval in order to examine how interactions
between the component cues of the multimodal display affect
memory. Finally, we investigated whether the presence of pyr-
azine odor affects generalization of the response toward the
defended prey species to palatable species in close proximity.
Such generalization based on a secondary cue has not been
considered in previous studies.

MATERIALS AND METHODS

Subjects and housing

One hundred one day-old male domestic chicks (Ross strain)
were delivered to the laboratory from a commercial hatchery
(Carlton Hatchery, Monaghan, Ireland) and held under li-
cence number B100/2756 held by Nicola Marples. The chicks
were housed in batches of 50, in 150 × 60 × 60cm wooden boxes,
the floors of which were covered in wood shavings.
They were subject to a 12:12 h light:dark cycle using
uncovered fluorescent lights, and there was also natural light
in the laboratory. The temperature was maintained between
24 and 25°C using radiators and ceramic heat lamps. Water was
provided ad libitum throughout the experiment, and brown
chick starter crumbs were provided ad libitum except for 1 h
prior to learning and extinction trials, when the chicks were
food deprived. All food deprivation was carried out in accord-
ance with European Union guidelines (86/609/EC).

On the day of arrival, all the chicks were individually
marked on their heads using permanent marker pens. This
procedure appeared to have no adverse effects on the study
subjects and they did not appear to respond to the marks on
their own or other chicks’ heads.

The laboratory consisted of 2 rooms separated by a door. All
chicks were housed in the first room, and the odorless treat-
ments were trained and tested in this room. The pyrazine
treatments were tested in the other room (termed the “odor
room”). Extractor fans ran in both rooms throughout the
experiment, and the door between the 2 rooms was kept shut
at all times when odor was present in the odor room. The
pyrazine solution was kept in a sealed container in the odor
room in order to prevent cross-contamination of the odor to
the nonodor room.

Artificial prey

The prey were chick starter crumbs, colored using food dye.
Prior to coloring, the yellow crumbs were made unpalatable
using Bitrex, which is a bitter, odorless substance that has
been used in previous studies to induce taste aversions in
chicks (Skelhorn and Rowe 2005). Five drops of 2.5% Bitrex were added to 90 ml of tap water which was then mixed with 150 g of sieved chick starter crumbs. The crumbs were sieved to remove dust and standardize the crumb size.

The crumbs were then allowed to dry for 24 h prior to coloring (Sugarflair Colors Ltd, Benfleet, Essex, UK) Spruce Green and Egg Yellow were used to color the crumbs. For each color, 0.5 ml of the dye was diluted to make 90 ml of solution using tap water and then mixed with 150 g of chick starter crumbs. The colored crumbs were then allowed to dry for 24 h prior to use in the experiment.

During the learning and extinction trials, the odor treatments had 1 drop of pyrazine solution placed beneath the yellow crumbs in the experimental arena (see below). This pyrazine solution consisted of 100 µl of 2-isobutyl-3-methoxy-pyrazine diluted to 1000 ml using tap water.

Experimental arena

The experimental arena was a 30 × 21 × 22-cm cardboard box, with a 10 × 21-cm section divided off using chicken wire. Two “buddy chicks,” previously fed to satiation, were placed in this smaller section and the test chick in the larger section. The buddy chicks reduced potential stress from placing the test chick alone in the arena (Marple and Roper 1996; Skelhorn and Rowe 2005) and were never used as experimental chicks.

A Perspex feeding tray, 20 cm in diameter, was used to present the artificial prey in the test arena. The tray consisted of 2 layers of Perspex, each of which was punctured by 24 wells, 12 mm in diameter. The wells in the top layer had a mesh floor, and the wells in the bottom layer had a solid floor. Twelve green crumbs and 12 yellow crumbs were presented, 1 in each of the 24 wells, on top of the mesh. The spatial arrangement in which the crumbs were presented was determined using a randomly generated map constrained to have not more than 4 of the same crumb color in adjacent wells.

In the bottom layer, below the mesh floor, each well contained a small piece of filter paper, 1 cm2. In the odor treatment immediately prior to the start of each trial, a drop of the pyrazine solution was placed on the filter paper beneath the wells containing yellow crumbs, whereas a drop of water was placed beneath the wells containing green crumbs. In the odor treatments, pyrazine therefore acted as an additional discriminatory signal for the avoidance of the yellow prey as it was present beneath only the yellow and not the green crumbs. In the nonodor treatments, a drop of water was placed in all the wells. During the learning and memory trials, each experimental chick, in turn, was placed in the middle of the tray and allowed to forage freely from the wells.

Pretraining

On the day of their arrival (day 1), the chicks were pretrained in pairs to accustom them to the apparatus. They were allowed to eat brown crumbs from the feeding tray (in the absence of any odor) for two 10-min sessions. Each chick then received 6 more pretraining sessions of 5 min each, accompanied by 2 buddy chicks in the buddy chamber. By the end of day 1, all the chicks that continued into the experiment ate readily from the feeding trays.

Learning trials

On day 2, the chicks were deprived of food for approximately 1 h before their first learning trial, during which they were offered 12 unpalatable yellow crumbs and 12 palatable green crumbs in the feeding tray. The chicks were randomly assigned to 1 of 2 treatments, an odorless treatment and a pyrazine treatment. The chicks in the pyrazine treatment had a drop of pyrazine solution placed beneath each yellow crumb and a drop of water beneath each green crumb, whereas those in the odorless treatment had water beneath every crumb.

The number of yellow and green crumbs attacked during the learning trial was noted. The trial continued either until the chick had attacked 12 of the 24 crumbs or for 3 min, whichever occurred first. A total of 7 learning trials were conducted, 3 on each of days 2 and 3 and 1 on day 4.

The chicks from the odorless treatment were then randomly assigned to 1 of 2 treatment groups (T1 and T2) and the chicks from the pyrazine treatment to either T3 or T4 (see Table 1). Each of these 4 treatments was then subdivided into 2 retention intervals, 3 or 96 h, giving a sample size of 13 chicks per treatment for the 3-h retention interval and 12 chicks per treatment for the 96-h retention interval.

Extinction trials

Three hours after completion of the last learning trial, chicks in the 3-h retention interval group from each of the 4 treatments took part in an extinction trial in which all crumbs were palatable. This trial was considered a further test of learning because memory formation processes in the chick continue for several hours after learning (Tsunova et al. 1998; Hale and Crowe 2002). During this period, a process called "consolidation" occurs during which recent memories are committed to long-term memory, and learned performance continues to improve. This first extinction trial was therefore considered a better measure of the chicks’ final learned avoidance level than the final learning trial and will consequently be referred to as the "consolidation trial."

On day 7, chicks in the 96-h retention interval group from each of the 4 treatments received an extinction trial designed to test the chicks’ memory of the learned avoidance. The numbers of green and yellow crumbs attacked during the consolidation and extinction trials were noted.

Data analysis

The data did not conform to the assumptions of parametric statistics and could not be transformed by any standard method; therefore, the data were analyzed using nonparametric tests, Mann–Whitney U, and Kruskal Wallis tests. Dunn’s post hoc test was used to make pairwise comparisons in cases where more than 2 treatments were tested together (Zar 2005). In the cases where Dunn’s post hoc results were quoted, the Kruskal–Wallis results were always less than P = 0.05.

Table 1

Treatments received during the learning and extinction trials, indicated as crumb color and odor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Learning trials</th>
<th>Extinction trials</th>
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<tbody>
<tr>
<td>1</td>
<td>Green; yellow</td>
<td>Green; yellow</td>
</tr>
<tr>
<td>2</td>
<td>Green; yellow</td>
<td>Green; yellow with pyrazine</td>
</tr>
<tr>
<td>3</td>
<td>Green; yellow with pyrazine</td>
<td>Green; yellow</td>
</tr>
<tr>
<td>4</td>
<td>Green; yellow with pyrazine</td>
<td>Green; yellow with pyrazine</td>
</tr>
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Yellow crumbs were unpalatable in the learning trials. All crumbs were palatable in the extinction trials.
RESULTS

Learning
All chicks learned to avoid the unpalatable yellow crumbs over the course of the 7 learning trials, attacking significantly fewer crumbs during trial 7 than during trial 1 (Mann–Whitney U test, odorless treatments: \( U = 846.00; n = 50, 50; P < 0.01 \); and pyrazine treatments: \( U = 424.00; n = 50, 50; P < 0.001 \), respectively; Figures 1 and 2).

In the first trial, there were no significant differences in the number of yellow crumbs attacked in the presence or absence of the pyrazine odor (Mann–Whitney U test, \( U = 1290.50; n = 50, 50 \); nonsignificant [NS]; Figures 1 and 2). Therefore, contrary to previous findings (Rowe and Guilford 1996, 1999b), pyrazine did not increase the innate avoidance of novel yellow crumbs in this instance. By trial 3, chicks in the pyrazine treatment attacked significantly fewer yellow crumbs than the chicks in the odorless treatment (Mann–Whitney U test, \( U = 987.00; n = 50, 50; P < 0.05 \); Figure 1). This difference was maintained during all subsequent learning trials, indicating faster acquisition of the learned avoidance in the presence of pyrazine. In the last learning trial, chicks in the pyrazine treatment attacked significantly fewer yellow crumbs than chicks in the odorless treatment (Mann–Whitney U test, \( U = 826.50; n = 50, 50; P < 0.01 \); Figure 1). This indicates that consolidation had indeed occurred. There was no such difference shown by chicks in treatments 3 and 4 during these 2 trials (Dunn’s post hoc test for treatment 3: trial 7 vs. 8, NS; treatment 4: trial 7 vs. 8, NS; Figure 2).

Consolidation of learning
The difference in avoidance levels between the 4 treatments was no longer detectable after the 3-h consolidation period (Kruskal–Wallis test, \( \chi^2 = 1.439 \); degrees of freedom [df] = 3; NS; Figure 2). Chicks in treatments 1 and 2 (the odorless treatments) attacked significantly fewer yellow crumbs during the consolidation trial than they did during their final learning trial (Dunn’s post hoc test for treatment 1: trial 7 vs. 8, \( P < 0.001 \); treatment 2: trial 7 vs. 8, \( P < 0.001 \); Figure 2) showing that consolidation had indeed occurred. There was no such difference shown by chicks in treatments 3 and 4 after the 3-h extinction trial (Dunn’s post hoc test treatment 1: trial 7 vs. 8, \( P < 0.001 \); treatment 2: trial 7 vs. 8, \( P < 0.001 \); treatment 3: trial 7 vs. 8, \( P < 0.001 \), suggesting that they forgot their learned avoidance to some extent. However, they attacked significantly fewer crumbs than they did during the memory trials remember the learned avoidance to an intermediate level. Avoidance in this treatment was intermediate between chicks that were offered yellow, pyrazine crumbs in both the learning and memory trials, and chicks that learned to avoid the odorless yellow crumbs.

Mimicry
Chicks in treatments 1, 2, and 3 attacked significantly more crumbs during the 96-h extinction trial than they did during the 3-h extinction trial (Dunn’s post hoc test treatment 1: trial 8 vs. 9, \( P < 0.001 \); treatment 2: trial 8 vs. 9, \( P < 0.001 \); treatment 3: trial 8 vs. 9, \( P < 0.001 \), suggesting that they forgot their learned avoidance to some extent. However, they attacked significantly fewer crumbs than they did during the 3-h extinction trial.
first learning trial (Dunn’s post hoc test treatment 1: trial 1 vs. 9, \( P < 0.001 \); treatment 2: trial 1 vs. 9, \( P < 0.001 \); treatment 3: trial 7 vs. 9, \( P < 0.001 \)), suggesting that they had not forgotten their avoidance completely.

Chicks in treatment 4 showed no evidence of forgetting after the 96-h retention interval. There were no significant differences in the number of yellow crumbs attacked during the last learning trial, the 3- and 96-h extinction trials (Kruskal–Wallis test, \( \chi^2 = 1.893; \text{df} = 2; \ P = \text{NS} \); Figure 2). Thus, the yellow, pyrazine crumbs were as well protected after the 96-h interval as during the 3-h consolidation period.

**Generalization**

The chicks were offered palatable green crumbs as an alternative food to the yellow crumbs throughout the experiment. When the relative proportion of each crumb odor attacked was examined, an additional effect of the pyrazine odor was detected. As they learned avoidance, chicks in all treatments reduced the number of yellow crumbs they attacked, but their response to the green crumbs differed. In the odorless treatment, the chicks also reduced the number of green crumbs attacked, so the relative number of yellow crumbs attacked did not change across the learning trials (Kruskal–Wallis test, \( \chi^2 = 4.203; \text{df} = 6; \ P = \text{NS} \); Figure 3). In contrast, the chicks trained in the presence of pyrazine differentiated between the colors, continuing to attack the green crumbs, so the relative proportion of yellow crumbs attacked reduced across the learning trials (Kruskal–Wallis test, \( \chi^2 = 39.530; \text{df} = 6; \ P < 0.001 \); Figure 3). After 4 learning trials, the chicks in the pyrazine treatment had learned to avoid the unpalatable yellow crumbs and to distinguish between the colors (Figure 3; for post hoc tests of these comparisons, see Table 2). This result suggests that pyrazine odor reduced the chicks’ tendency to generalize between the colors and made them better able to discriminate against the unpalatable yellow crumbs, eating proportionately more palatable green crumbs.

**DISCUSSION**

This experiment clearly demonstrates that the addition of pyrazine odor to a yellow visual signal increased the rate, degree, and memorability of the learned avoidance in chicks. Barnea et al. (2004) observed a similar effect of pyrazine with red but failed to see an increased learning rate with yellow. They offered chicks quinine-tainted water in colored tubes rather than solid prey as used in this experiment. As Roper and Marples (1997b) noted chicks differ in their responses to solids and liquids, it is unsurprising that there were differences in the responses observed in this and in the experiment of Barnea et al. (2004).

There has been much debate about the mechanism through which visual conspicuousness enhances learned avoidances. Gittleman and Harvey (1980) argued that conspicuous prey are more easily detectable and therefore endure a higher initial rate of attack by naive predators, which may account for the increased rate of learned avoidance observed with conspicuous prey. In our experiment, the relatively more conspicuous, yellow, pyrazine prey were attacked less frequently than the yellow, odorless prey, suggesting that any differences in the rate of learning found in our experiment were due to the characteristics of the warning display and not the encounter rate.

During the final learning trial, the chicks in the odorless treatment attacked significantly more yellow crumbs than the chicks in the pyrazine treatment. However, after the 3-h consolidation period, this difference disappeared. These results support the view of Hale and Crowe (2002) that memory consolidation proceeds for several hours after learning. It also suggests that the presence of pyrazine made learning so effective that the consolidation had already happened by the seventh learning trial, so birds in treatments 3 and 4 did not improve their avoidance during the consolidation period. Consolidation of the learned avoidance by treatments 1 and 2 may therefore have caused the difference in attack level between all treatments to disappear.

After the 96-h retention interval, the chicks remembered a learned avoidance of the yellow crumbs that smelt of pyrazine but forgot that of the odorless yellow crumbs. This confirms the result of Barnea et al. (2004) that pyrazine odor prolongs memorability of learned avoidances in a similar manner to other odors such as almond (Roper and Marples 1997a). These results are in keeping with the suggestion of Roper and Redston (1987) that conspicuousness prolongs memorability. That the chicks in all treatments consolidated their learning to the same level suggests that any difference in the memory recollection of these learned avoidances is therefore due to some aspect of the signal that makes it memorable rather than to an enhanced
encounter rate or a higher level of learning, as debated by Gittleman and Harvey (1980) and Roper and Redston (1987). Speed (2000) pointed out that to date no studies have examined how 2 signals of a multimodal display interact with regards to memorability. The design of this current experiment sought to address this issue by allowing the chicks to learn to avoid the yellow, pyrazine crumbs and later compared their memorability of this learned avoidance in the presence of only the color cue or both the color and odor cue used in our multimodal signal.

Our results showed that the chicks which had learned to avoid the yellow, pyrazine crumbs but were offered only the color cue during the extinction trials increased the number of yellow crumbs they attacked between the 3- and 96-h extinction trials, suggesting progressive forgetting during the retention interval. No such effect was observed in chicks that were offered crumbs that smelt of pyrazine in both the learning and the extinction trials. This suggests that the pyrazine odor did not merely serve to potentiate learning about the unpalatable yellow prey but also serves either as an alerting signal as suggested by Rothschild and Moore (1987) or as an “aide-memoire” (Rothschild 1984) or both.

When the number of yellow crumbs attacked by the 4 treatments during the 96-h extinction trial was examined, an NS trend was observed which suggested that learning to avoid a conspicuous signal in the presence of an odor cue may prolong memorability, even if the odor component of the signal is no longer present at the time of memory recollection. Similarly, when Roper and Redston (1987) offered their chicks red odorous crumbs in the learning trials and then odorless red crumbs in the extinction trials, the chicks showed progressive levels of forgetting over time but still appeared to recall some level of avoidance up to 72 h after learning. Further work is needed to determine whether additional protection is to be gained by signaling using both components of a multimodal display and how this benefit changes as forgetting progresses.

When the proportion of each color of crumb attacked during the learning trials was examined, it became clear that the presence of pyrazine improved discrimination between the palatable green crumbs and the unpalatable yellow crumbs. In the odorless treatment, the chicks generalized their learned avoidance of yellow to include the green crumbs, so the palatable crumbs gained protection through their association with the defended crumbs. Mappes et al. (1999) noted that palatable species living in proximity to unpalatable species may gain protection due to avoidance of all prey in the presence of pyrazine improved discrimination between the palatable green crumbs and the unpalatable yellow crumbs. The generalization result suggests that palatable prey which are visibly different from the defended prey were better protected than mimics of the monomodal model. This is in keeping with the Barnea et al. (2004) results in which they observed that the presence of pyrazine helped chicks to remember their learned avoidance. The addition of pyrazine to a yellow mimic of an odorless model did not enhance avoidance. Crumbs that mimicked only the visual component of the multimodal signal appeared to be protected to an intermediate level between crumbs that mimicked both components of the multimodal signal and mimics of the monomodal crumbs. Rothschild (1984) suggests that mimetic insects living sympatrically with a multimodal model would gain no extra protection from mimicking both the visual and the olfactory components of a multimodal model’s display as the visual component provides sufficient protection.

After a 96-h retention interval, the mimics in all treatments were better protected than they would have been through innate avoidance alone. However, mimics of the multimodal signal were better protected than mimics of the monomodal model. This is in keeping with the Barnea et al. (2004) results in which they observed that the presence of pyrazine helped chicks to remember their learned avoidance. The addition of pyrazine to a yellow mimic of an odorless model did not enhance avoidance. Crumbs that mimicked only the visual component of the multimodal signal appeared to be protected to an intermediate level between crumbs that mimicked both components of the multimodal signal and mimics of the monomodal crumbs. Rothschild (1984) suggests that mimics do not need to perfectly replicate their model’s defenses and that a “reminder of the danger involved is sufficient.” Further work is required to determine whether a mimic needs to replicate both components of a multimodal display in order to gain full protection.

The generalization result suggests that palatable prey which are visibly different from the defended prey may not gain protection from close proximity to aposematic prey if the model prey utilize pyrazine odor as part of their warning signal because under these conditions visual discrimination is enhanced.

If these results reflect the responses of wild birds to their insect prey and if we make the further assumption that other warning color and odor combinations will have a similar effect, then some interesting conclusions can be drawn about the effects of olfactory cues on predator behavior. It would appear that multimodal insect warning displays are of benefit to both the predator and prey. Multimodal signals may speed up avoidance learning, so fewer individuals are sacrificed in order to educate the predator, who subsequently ingests fewer toxic individuals thus reducing the risk of being poisoned. Once educated, the predator remembers the avoidance of the multimodal signal for longer, and therefore, the prey species is better protected over time. The presence of the multimodal signal helps the bird to discriminate between defended and palatable prey types, thereby avoiding the toxic insects but continuing to exploit palatable species. And finally, by
signaling multimodally an insect may make itself more difficult to mimic, thus protecting itself against the erosion of its defenses by potential mimics. Previous work suggests that auditory (Rowe 2002) and gustatory signals (Skellhorn and Rowe 2005, 2006) may have similar effects on learning and memory as those observed with pyrazine. However, no previous experiments examine whether sound and taste component cues affect generalization and mimicry in the same way as pyrazine. This is an area that warrants further research.

**CONCLUSIONS**

Pyrazine enhanced the rate at which learned avoidances of unpalatable yellow crumbs were acquired and enhanced the ability of the chicks to remember this aversion over time. Pyrazine also decreased the extent of generalization between the palatable green and unpalatable yellow prey. This study represents the first demonstration that pyrazine odor enhances learned avoidance of yellow prey and improves discrimination between 2 prey types. The results also show that mimics of 1 multimodal model are better protected than mimics of the corresponding visual cue–only model and highlights the interaction between the color and odor components of the multimodal display in reference to memorability.

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