FOOD AVOIDANCE BY RED-WINGED BLACKBIRDS CONDITIONED WITH A PYRAZINE ODOR

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ABSTRACT.—Brown rice treated with 2-methoxy-3-methylpyrazine (one of a family of compounds reported to function as warning odors in nature) was not repellent to test groups of four Red-winged Blackbirds (*Agelaius phoeniceus*). But, after pairing with methiocarb, an illness-inducing agent, the odor of methylpyrazine was an effective conditioned stimulus that reduced the birds' rice consumption. Furthermore, rice consumption remained suppressed even after pyrazine was no longer present. A combination of red dye plus pyrazine odor proved no more effective a conditioned stimulus than red dye alone. Furthermore, the color seemed to interfere with the birds' ability to use the odor as a conditioned stimulus. Consequently, there was no residual suppression of rice consumption by groups exposed to the methiocarb-color-pyrazine treatment as was demonstrated by the groups exposed to just methiocarb plus pyrazine. *Received 15 September 1989, accepted 26 January 1990.*

CONDITIONED food-avoidance learning has been demonstrated in numerous animal species (Barker et al. 1977). The role of olfaction in the formation of conditioned aversions is less well-studied, although rats can be conditioned with odors, especially when the odor is potentiated by taste (Domjan 1980, Palmerino et al. 1980).

The olfactory capabilities of birds have received relatively little study especially in the context of feeding behavior. It is generally accepted that vision and, to a lesser extent, taste are the major sensory modalities in food selection behavior. However, some species can find food by olfaction (Stager 1964, Grubb 1972, Wenzel 1972, Harriman and Berger 1986), and the European Starling (*Sturnus vulgaris*) can discriminate among potential nesting materials on the basis of the plants' volatile properties (Clark and Mason 1987). Moreover, starlings can be conditioned to avoid food paired with various odors when consumption is followed by gastrointestinal malaise (Mason and Silver 1983).

Many plants and invertebrates possess strong, pungent odors (Rothschild 1961, Eisner and Grant 1980). One family of compounds, the pyrazines, is particularly widespread in nature, and it has been proposed that the odors of such compounds have a warning function. They may alert potential predators that prey are protected with noxious or distasteful chemicals (Rothschild and Moore 1987). Guilford et al. (1987) conditioned week-old domestic chicks (*Gallus gallus*) to avoid quinine-treated water paired with 2-methyl-3-isobutylpyrazine. Based on drinking-response latencies to water with and without pyrazine, the investigators concluded that the chicks detected pyrazine at a distance, probably by olfaction. These results support the idea that odors of pyrazine compounds can have a warning function.

We evaluated this concept more closely by examining the effects of another pyrazine compound, 2-methoxy-3-methylpyrazine, on the feeding behavior of an omnivorous, flock-feeding passerine—the Red-winged Blackbird (*Agelaius phoeniceus*). Specifically, we assessed the primary repellency of pyrazine when applied to a preferred food, the acquired repellency of pyrazine when used as a cue (conditioned stimulus) for postingestional illness, the relative importance of pyrazine taste and odor in the formation of an acquired aversion to the treated food, and the comparative effectiveness of color and odor as cues to unpalatability.

METHODS

We trapped male Red-winged Blackbirds in the vicinity of Gainesville, Florida, and held them in captivity 3–6 months. The captive birds were housed outdoors in groups of 20–25 birds in 1.8 × 1.2 × 1.2 m cages in a roofed aviary. For a given test, birds were chosen randomly from among those in captivity the longest, to minimize the length of time any individual was held. Birds were released after testing.

Because blackbirds are gregarious, we tested groups of 4 in two enclosures (3.2 × 9.7 × 1.8 m each). The

TABLE 1. Experimental treatments presented to Red-winged Blackbird groups during 4-day feeding trials.

Treatment groups[a]	Condition of brown rice			
	Day 1	Day 2	Day 3	Day 4
Control	Plain	Plain	Plain	Plain
M	Plain	Methiocarb	Plain	Plain
C	Plain	Red dye	Red dye	Plain
P-T	Plain	Pyrazine taste	Pyrazine taste	Plain
MC	Plain	Methiocarb + red dye	Red dye	Plain
MP-T	Plain	Methiocarb + pyrazine taste	Pyrazine taste	Plain
MP-O	Plain	Methiocarb + pyrazine odor	Pyrazine odor	Plain
CP-O	Plain	Red dye + pyrazine odor	Red dye + pyrazine odor	Plain
MCP-O	Plain	Methiocarb + red dye + pyrazine odor	Red dye + pyrazine odor	Plain

[a] Abbreviations: M (methiocarb), C (color), P-T (pyrazine-taste), MC (methiocarb + color), MP-T (methiocarb + pyrazine taste), MP-O (methiocarb + pyrazine odor), CP-O (red dye + pyrazine odor), and MCP-O (methiocarb + red dye + pyrazine odor).

test enclosures had shaded perches at each end, and water was provided *ad libitum*. Except as described below, a mixture of brown rice, wheat, cracked yellow corn, and layer crumbles was always available.

For each test group, we conducted feeding trials on consecutive days after 2 days of acclimation to the enclosures. On each test day, the birds' food was removed at 0730. One hour later, we put one bowl (14-cm diameter, 8.5-cm deep) of test food and one bowl of alternative food in each enclosure. Positions of the test and alternative food were determined randomly on the first day and did not change during the test. Food bowls were at ground level and were separated by a 1.3-m-high canvas partition.

At the start of the 3-h test period, 100 g of food were available in each bowl. The contents were reweighed at the end of the test period, and group consumption was determined by subtraction. Two bowls of food, identical to those presented to the birds, were exposed daily to measure weight gain due to moisture absorption. Consumption data were adjusted accordingly.

Throughout the trials, the test food was medium-grain brown rice and the alternative food was F-R-M® Layer Crumbles (Flint River Mills, Bainbridge, Georgia). On days 1 and 4, only untreated food was offered. On days 2 and 3, the condition of the rice offered to the birds depended upon the test treatment (Table 1).

Color was applied by mixing rice with enough water-soluble commercial red food dye to impart a bright red color (Spinel Red, Smithe 1975) to the rice. Methiocarb (Mesurol® 75% wettable powder), an illness-inducing agent, was applied at a rate (0.125% active ingredient by weight) previously shown to produce reliable repellency (Holler et al. 1985).

The olfactory stimulus was 2-methoxy-3-methylpyrazine. We prepared a solution of 100 mg of the chemical in 1 l of distilled water, and applied 5 ml to 100 g of brown rice just before presentation to the birds. Birds that received the pyrazine-taste (P-T) and methiocarb plus pyrazine-taste (MP-T) treatments had direct access to pyrazine-treated rice. For the color plus pyrazine-odor (CP-O), methiocarb plus pyrazine-odor (MP-O), and methiocarb plus color plus pyrazine-odor (MCP-O) groups, direct access was prevented (i.e. birds could not taste the pyrazine). Brown rice (100 g) treated with methylpyrazine was placed in the food bowl as before. Then a wire screen supported by an aluminum ring inside the food bowl was inserted. On top of the screen, ca. 4 cm above the pyrazine-treated rice, 100 g of brown rice treated with red dye, methiocarb, or red dye plus methiocarb was added.

To examine the effectiveness of the various treatments, we calculated suppression ratios (Mason and Reidinger 1983) for brown rice consumption and for total food consumption. Each group's daily consumption was divided by the sum of that day's consumption plus that on day 1. Lower ratios correspond to greater avoidance, with indifference being indicated by a value of 0.5. We analyzed the resulting ratios by a two-way repeated measures analysis of variance to evaluate the initial and residual effects of the treatments. Tukey HSD tests (Sokal and Rohlf 1969) were applied *a posteriori* to isolate significant differences among means ($P < 0.05$).

RESULTS

Overall, there were no significant differences among groups in brown rice consumption on day 1. Analysis of suppression ratios showed significant ($P < 0.0001$) main effects for groups and days, and a significant ($P < 0.0001$) group × day interaction. Birds that received methiocarb plus a sensory cue had significantly ($P < 0.05$) lower suppression ratios over all days than did those exposed to methiocarb or sensory cues alone (Tukey tests). Across groups, suppression of rice consumption was greatest on day 2 and least on day 4.

On day 2, suppression ratios of all groups

TABLE 2. Suppression ratios (±SD) of rice consumption by Red-winged Blackbird groups ($n = 4$ per treatment) on days 2, 3, and 4 of feeding trials with color, taste, and olfactory stimuli. Means sharing the same letter are not significantly different ($P > 0.05$, Tukey HSD test).

Treatment[a]	Day of trial 2	Day of trial 3	Day of trial 4
MC	0.05 ± 0.03 A	0.02 ± 0.01 A	0.53 ± 0.04 C
MCP-O	0.07 ± 0.05 AB	0.06 ± 0.05 A	0.48 ± 0.02 BC
MP-T	0.11 ± 0.11 AB	0.06 ± 0.06 A	0.25 ± 0.17 A
M	0.16 ± 0.04 ABC	0.44 ± 0.06 B	0.54 ± 0.04 C
MP-O	0.18 ± 0.06 ABC	0.16 ± 0.17 A	0.31 ± 0.23 AB
CP-O	0.25 ± 0.13 BC	0.40 ± 0.20 B	0.54 ± 0.07 C
C	0.34 ± 0.18 C	0.48 ± 0.18 BC	0.57 ± 0.07 C
P-T	0.59 ± 0.08 D	0.59 ± 0.08 BC	0.59 ± 0.09 C
Control	0.60 ± 0.09 D	0.61 ± 0.09 C	0.57 ± 0.10 C

[a] Treatments as described in Table 1.

exposed to methiocarb did not differ statistically among themselves (Table 2). The methiocarb plus color (MC) group showed the greatest suppression (Fig. 1), and their consumption was significantly lower than that of the color (C) and color plus pyrazine-odor (CP-O) groups. The methiocarb plus pyrazine-odor (MCP-O) and methiocarb plus pyrazine taste (MP-T) groups also exhibited significantly greater suppression of rice consumption than did C only. Only the control and the pyrazine-taste (P-T) groups showed no evidence of reduced rice consumption on day 2.

On day 3, the 4 groups exposed to methiocarb plus a sensory cue (i.e. MC, MP-O, MP-T, and MCP-O) were statistically distinct from the other treatments (Table 2) as the conditioned stimuli continued to inhibit rice intake. Conversely, consumption by the C and CP-O groups returned to pretreatment levels. The response of the methiocarb (M) groups did not differ from those exposed only to sensory cues.

Removal of pyrazine and color cues on day 4 returned rice consumption to pretreatment levels in the MC and MCP-O groups (Fig. 1). The response was not observed in the MP-T and MP-O groups where rice consumption on day 4 reached just 37% and 57%, respectively, of that on day 1. The MP-T groups differed significantly from all except MP-O, and the latter was statistically distinct from all others except MCP-O (Table 2).

Analysis of total consumption suppression ratios revealed significant differences among treatments ($P < 0.0005$) and days ($P < 0.00001$). There was also a significant ($P < 0.0001$) treatment × day interaction.

On day 2, total food consumption was suppressed most dramatically in groups exposed to methiocarb without the color cue: M, MP-O, and MP-T (Fig. 2). Somewhat less suppression was exhibited by groups exposed to methiocarb-color-pyrazine (MCP-O), and their response was not significantly different ($P > 0.05$) from that of groups given the methiocarb-color treatment (MC). On days 3 and 4, there were no significant differences among treatments in suppression of total food consumption.

DISCUSSION

All groups that received methiocarb-treated rice on day 2 reduced rice consumption (Fig. 1), but only those groups that received methiocarb in the absence of the color cue (M, MP-O, MP-T) also substantially reduced total food consumption (Fig. 2). Evidently the latter groups were not immediately repelled by the methiocarb treatments and curtailed consumption only after suffering postingestional consequences associated with methiocarb intoxication (Rogers 1974, 1978). By contrast, the MC and MCP-O groups, immediately alerted by a visual signal (red color) that itself may have been somewhat repellent, ate only a small amount of the treated rice, were probably not incapacitated, and maintained their total consumption by increasing intake of the alternative food.

When presented separately, the warning color and the warning odor affected the birds differently. The red color itself caused some reduction in rice consumption, and when paired with methiocarb, the visual stimulus suppressed rice consumption more (though not sta-

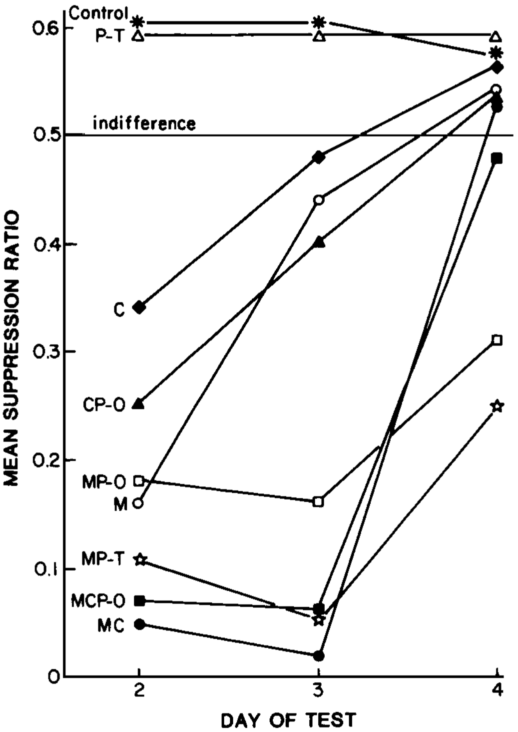

Fig. 1. Daily mean rice consumption suppression ratios for Red-winged Blackbird groups on days 2, 3, and 4 of feeding trials involving visual, taste, and olfactory stimuli. Treatment group designations are as described in Table 1.

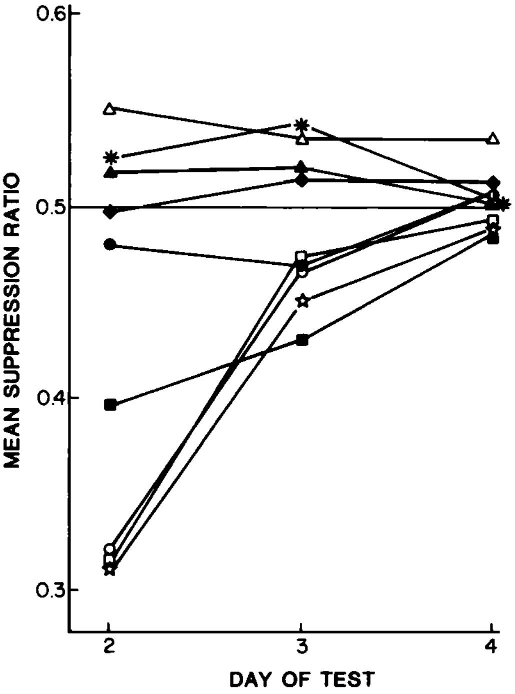

Fig. 2. Daily mean suppression ratios for total food consumption (brown rice plus Layer Crumbles) of Red-winged Blackbird groups exposed to rice treated with various repellent-stimulus combinations. Group symbols are the same as in Fig. 1.

tistically more) than did either methiocarb-pyrazine treatment. This difference disappeared on day 3 (Table 2), when the separate conditioned stimuli suppressed mean rice consumption equally (Fig. 1). On day 4 the birds responded to color removal with an immediate increase in consumption to pretreatment levels. In contrast, even in the absence of the volatile stimulus, the pyrazine groups exhibited substantial residual reduction in rice consumption (Table 2, Fig. 1). The presence and absence of the warning color had immediate and dramatic effects; the warning-odor effect was more gradual and persistent. We believe that the pyrazine was a more ambiguous signal than the color, and the birds reduced their consumption of brown rice rather than risk further illness.

When the color and the odor were presented together (treatment groups CP-O), the pyrazine may have enhanced the neophobic effect of the red color. The unusual appearance of the dyed rice may have made the birds more aware of the pyrazine odor so that the compound stimulus suppressed rice consumption slightly more (Fig. 1) than did the color alone (treatment groups C). The birds did not respond to the pyrazine (treatment groups P-T) on plain brown rice because, at the concentration used, the pyrazine itself caused no apparent discomfort, and the rice looked normal. Of course, at higher concentrations, pyrazine compounds (like certain other volatiles) may be aversive and irritating in themselves (Mason et al. 1989).

Some birds are hesitant to attack novel prey (e.g. see Coppinger 1970), and so possession of a warning odor in addition to conspicuous color may have survival value. However, unless novel stimuli are associated with adverse consequences, birds habituate rapidly (e.g. Mason and Reidinger 1981). Thus, the C and CP-O treatments did not effectively suppress rice consumption after day 2 (Fig. 1).

On the other hand, formerly innocuous stimuli can acquire salience in certain contexts. Even in the absence of the color stimulus, the pyrazine odor proved an effective deterrent to rice consumption after pairing with methiocarb (treatment groups MP-O). On day 3, the rice was safe to eat, but was "protected" by the pyrazine odor much as a visually perfect Batesian mimic would be. This "protection" did not differ statistically on day 3 from that afforded by the color stimulus alone (treatment groups MC), but it persisted even in the absence of the olfactory cue on day 4. Thus, predators that experience adverse consequences from contacting an insect with aposematic odor may thereafter not only be averse to the odor but to the feeding situation (type of prey, location of prey, etc.) itself (Kaye et al. 1989).

The MP-T, MP-O, and MCP-O groups exhibited similar levels of suppressed rice consumption on day 3, but on day 4 consumption by the MCP-O group returned to pretreatment levels, while the MP-T and MP-O groups increased only modestly. We suggest that the presence of the color interfered with the birds' ability to associate pyrazine taste and odor with induced intoxication. A similar asymmetrical response to sensory cues was described in domestic chicks by Gillette et al. (1983: 55), who found that "visual cues . . . are so predominant in feeding that they 'block' flavour cues almost completely." The implication is that for some diurnal bird species, a decision to eat or not depends almost exclusively on the appearance of potential prey items with olfactory or taste cues of secondary importance.

If under natural field conditions olfactory aposematism is important (Rothschild 1961, Eisner and Grant 1980), then certain attributes of the models and mimics can be inferred. For example, many species display gregarious behavior as an antipredator defense (Sillén-Tullberg and Leimar 1988). Because odor is volatile and not necessarily identifiable with an individual organism, close proximity to an individual emanating pyrazine odors may afford some protection to a similarly looking but neutral smelling individual. Palatable visual mimics that aggregate with their models may not have warning odors even if their models do. Such mimics would have to be accurate copies of the models because in a side-by-side comparison, birds could readily distinguish imperfect mimics (e.g. Shideler 1973, Terhune 1977). As model and mimic become more solitary in their behavior, it is likely that the constraint of exact visual resemblance will be relaxed, and natural selection should act to favor mimics that possess a warning odor like that of the model. These conjectures are amenable to laboratory testing, and field data bearing on this subject would be welcome.

A warning odor may help protect nonmimetic prey that are brightly colored but nontoxic by stimulating a greater neophobic response in avian predators. Similarly, a warning odor might benefit prey that are inconspicuously colored but emetic by enhancing a conditioned avoidance response with residual repellency. Pyrazine-like odors are not likely to be effective against avian predators if the prey is aposematic and emetic (color overshadows the odor and interferes with residual repellency effect), or if the prey is nonaposematic and nontoxic (pyrazine itself is not repellent), unless the prey is a mimic of a toxic model. Effective mimicry without aposematic coloration has been demonstrated experimentally (Avery 1985, 1989), but evidence for the existence of such a system in nature is lacking.

Generally, most invertebrates are not preyed upon exclusively by birds. Possession of both strong odor and conspicuous color will probably deter a suite of potential predators with various sensory capabilities (Mason 1989, Pearson 1989). For example, the aposematic beetle (*Metriorrhynchus rhipidius*) possesses a pyrazine warning odor, is reportedly distasteful, but is preyed upon by bats, dragonflies, and birds (Moore and Brown 1981).

Pyrazine compounds occur in numerous ecological contexts (Rothschild et al. 1984), and information on their role in prey avoidance of avian predators is inconclusive. For example, the monarch butterfly (*Danaus plexippus*) is subject to bird predation, and possesses warning coloration, toxins, and pyrazine compounds. But the odor of the butterfly seems to depend upon its larval food plant, and it is possible that the adult butterfly uses pyrazine odor in selecting oviposition sites (Rothschild et al. 1984). Thus, the presence of the pyrazine in the insect may be incidental and not directly related to predator avoidance.

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