

Chemical Repellency in Birds: Relationship Between Chemical Structure and Avoidance Response

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ABSTRACT We examined how molecular structure of 24 anthranilate and benzoic acid derivatives correlated with drinking behavior in European starlings *Sturnus vulgaris*. The effectiveness of bird repellents was associated with basicity, the presence of an electron-donating group in resonance with an electron-withdrawing carboxylic group on a phenyl ring, and a heterocyclic ring in the same pi cloud plane as the phenyl ring. Of the benzoic acid derivatives tested in this study, methyl, ethyl, dimethyl, and linalyl anthranilate as well as anthranilic acid and 4-ketobenzotriazine were repellent to birds. Water consumption was significantly reduced relative to control levels at concentrations as low as 0.05% (weight/volume) for the best repellents. Further statistical tests showed that reduction in consumption for the best repellents was absolute, not significantly different from zero consumption. Anthranilic acid isomers were moderately good repellents. The ability to generate a model predicting repellency allows for the efficient identification and development of ecologically sound, nonlethal, taxa-specific repellents to be used for the protection of wildlife in agricultural and industrial applications.

Avoidance of a compound can be based on post-ingestional factors, e.g., toxicity, where a conditioned aversion to a sensory cue is learned. Avoidance can also be mediated via purely sensory cues. In this case, no post-ingestional conditioning occurs, nor is there chemical or physical damage to the organism. For purely sensory repellents, the emerging picture is that there are clear perceptual differences between birds and mammals. For example, mammals find capsaicin (the agent responsible for the hotness in *Capsicum* chili peppers) irritating, whereas birds are indifferent to concentrations as high as 20,000 ppm (Szolcsanyi et al., '86). Alternatively, methyl or dimethyl anthranilate (grape flavoring) is highly repellent to birds, yet mammals are either indifferent to or prefer the compound (Kare, '61; Mason et al., '85; Glahn et al., '89).

Stimulation of trigeminal fibers is an important component of repellency in vertebrates (Alarie, '90). In birds, olfaction and trigeminal chemoreception underlie the aversiveness of methyl and dimethyl anthranilate, suggesting that avoidance is based on odor quality and irritation (Mason et al., '89). This is in sharp contrast to earlier studies, which supported the popular belief that the limited taste capacities of birds mediated repellency (e.g., Kare and Pick, '60; Rogers, '78). Recent studies support the thesis that birds are fully capable of making quantitative and qualitative odor discriminations

(Walker et al., '79; Mason and Silver, '83; Clark and Mason, '87, '69; Mason et al., '89; Clark and Smeraski, '90; Clark and Shah, '91; Clark, '91). Presumably, stimulation of the trigeminal nerves provides information on irritancy only, while stimulation of the olfactory receptors provides information on irritancy only, while stimulation of the olfactory receptors provides qualitative sensory cues useful in stimulus identification (Mason and Silver, '83; Silver et al., '88).

In contrast, compounds that repel both birds and mammals always appear to operate in a post-ingestive mode via conditioned avoidance, e.g., thiram and methiocarb (Johnson et al., '82). This raises fundamental questions about the nature of receptors and coding for purely sensory irritants between these two taxonomic classes. From an anatomical and histological perspective, the two taxa appear to be similar, though comparative taxonomic information on the structure of the trigeminal system as it pertains to chemical signals is limited. Explanation of this taxonomic difference is of both fundamental and practical interest. For example, irritation may reflect phylogenetic constraints present at the time of divergence for each group or an

evolutionary response to selective pressures relating to chemical ecology prevailing at the time of divergence. Practically, the ability to identify ecologically sound avian repellents has numerous advantages, the most important of which is that birds can be kept away from crops, formulated pesticides or rodenticides, or areas where they pose a risk to themselves and/or humans, e.g., airports, waste water impoundments.

At present, the discovery of general or taxon-specific repellents is empirical. A more systematic and theoretical approach would be of great utility for practical as well as comparative evolutionary purposes. A series of studies and observations led us to concentrate our research on derivatives of a basic phenyl ring structure and, specifically for this study, benzoic acid derivatives. Methyl and dimethyl anthranilate (MA and DMA, respectively) are ester derivatives of anthranilic acid with well-described avian-specific repellency (Kare, '61; Mason et al., '85; Glahn et al., '89). We previously correlated physicochemical properties of anthranilates (e.g., lipophilicity, volatility) to repellency in feeding trials (Mason et al., '89). More recent studies in our laboratory have focused on how molecular structure correlates with drinking behavior. In a comparison of isomers of aminoacetophenone, we found the relative strength of repellency to be related to resonance of lone pairs of electrons and intramolecular hydrogen bonding (Mason et al., '91b). Subsequent studies on isomers of amino-, hydroxy- and methoxyacetophenone showed that the hydrogen bonded ring is not required for repellency, though it may play an ancillary role (Clark and Shah, '91). In a follow-up study, we verified that increased electron donation to the phenyl ring is associated with repellency and showed that decreasing electron withdrawal from the phenyl ring also increases repellency (Shah et al., '91). These studies suggest that factors that act to make the phenyl ring electron rich increase repellency. Here we report experiments designed to determine more precisely the influence of basicity, pi cloud planarity, and electron-donating and withdrawing groups on avian repellency.

MATERIALS AND METHODS

Experiment 1: Water consumption of anthranilates

Adult European starlings (*Sturnus vulgaris*) were decoy trapped in Pilesgrove township, New Jersey, and transported to the laboratory. Birds were captured in September and held for 3 weeks under laboratory conditions prior to testing. This bird species was chosen for use 1) because they show good

chemical-sensing ability (Mason and Silver, '83; Clark and Mason, '87), 2) because comparable data exist concerning the responses of starlings to other avian repellents (anthranilate derivatives; Mason et al., '85, '89; Glahn et al., '89) and mammalian irritants (e.g., capsaicin, zingerone, gingerol, allyl isothiocyanate; Mason and Otis, '90), and 3) because starlings are agricultural pests (Bailey, '66; Besser et al., '67). Upon arrival, the birds were individually caged (61 × 36 × 41 cm) under a 12:12 hr light:dark cycle with light onset at 0700 hr. Food and grit [Purina Flight Bird Conditioner (PBFC) and medicated oyster shells] were available *ad libitum*. Birds were given apples twice per week. Before experiments began, the birds were permitted free access to tap water.

Nine anthranilates were tested. Six concentrations of each compound were prepared: 0.5%, 0.25%, 0.125%, 0.063%, and 0.031% (volume/volume), with 0% deionized distilled water as the control. These compounds were previously examined for repellency in an earlier study of physicochemical correlates of repellency in feeding trials (Mason et al., '89). We reexamined this anthranilate series for repellency in water consumption trials so that a standard bioassay could be used in the context of examining molecular structural correlates to ingestive behavior. In addition, pilot tests had shown that drinking trials were far more sensitive than feeding trials in evaluation of repellency (cf. Kare, '61). Four compounds were obtained from International Flavors and Fragrance (Union Beach, NJ): isobutyl *n,n* dimethyl anthranilate (IBDMA; CAS No. 68480-21-7), linalyl anthranilate (LA; CAS No. 7149-26-0), phenethyl anthranilate (PEA; CAS No. 1333-18-6), and propionyl methyl anthranilate (PMA; CAS No. 25628-84-6). Five compounds were obtained from Firminich (Geneva, Switzerland): dimethyl anthranilate (DMA; CAS No. 85-91-6), ethyl anthranilate (EA; CAS No. 87-25-2), methyl anthranilate (MA; CAS No. 134-20-3), isobutyl anthranilate (IBA; CAS No. 7779-77-), and isobutyl methyl anthranilate (IBMA; CAS No. 65505-24-0).

A one-choice drinking test was used to evaluate repellency. Starlings were given 3 days of pretreatment during which water consumption was measured for 6 hr each day. At the end of this period, individuals whose variance about the 3 day mean consumption was greater than ± 1 SD of the population variance were excluded from the trials. Those birds with stable daily water consumption were ranked according to mean water consumption and assigned to one of the six treatment groups. The bird with the highest water consumption was

assigned to the 0.5% treatment group, the bird with the second highest consumption was assigned to the 0.25% treatment group, and so forth, until all birds were assigned to a group. This ensured that all groups were balanced with respect to drinking when treatment trials began. A total of 36 birds were used for experiments, with six birds per treatment group.

After assignment to a treatment group, a 1-day drinking trial began. Birds had free access to feed and tap water during the night. Beginning at 0930, the tap water was replaced with preassigned concentrations of chemicals in water and consumption was recorded every 2 hr for the next 6 hr. After the test, birds were again provided free access to tap water. Consumption of tap water was monitored overnight and for a 6-hr period the next day. Overnight consumption was monitored to evaluate whether birds made up for any water deficits resulting from experiments. The within group, 6-hr posttreatment water consumption was compared with the mean within group, 6-hr pretreatment water consumption to determine whether consumption had returned to pretreatment levels. If this condition was met, the birds were tested with the next compound, with groups of birds receiving a different concentration of compound as determined by a counter-balanced predetermined schedule. If an individual's intertrial water consumption was within ± 1 SE of its pretreatment value, the bird was used in the next experiment; otherwise, it was replaced with a new bird whose baseline water consumption was matched for the group mean. To test for learning-associated carryover effects due to treatment, we examined the intertreatment deionized, distilled water consumption of all birds using a repeated measures, one-way analysis of variance (ANOVA). We compared each posttreatment day's water consumption by partition, using an a priori contrast with the 3-day pretreatment mean water consumption. The order in which chemicals were tested was arbitrary, being MA, PEA, PMA, LA, EA, IBDMA, DMA, IBA, IBMA. However, the experimental design precluded testing for presentation order effects of compounds. The arbitrary order of presentation with respect to structure and previous experience suggested that this blocking was not critical.

Naïve birds were not used for each test because it was not practical to capture the 324 starlings required for all trials. Birds were checked for health condition after each daily trial, e.g., piloerection. There were no mortalities, and at the end of all the experiments birds were released to the wild.

For each chemical, we tested two *a priori* ques-

tions about consumption of treated water: 1) Did mean water consumption differ among the treatment (i.e., concentration) groups? A one-way ANOVA was used to compare group means, and a Scheffé's posthoc test was used to identify significant ($P < 0.05$) differences among means. 2) Did consumption of treated water differ from a theoretical value of zero consumption? This is of practical interest in that there may be times when a bird must be repelled absolutely from potentially lethal toxic waste water, e.g., cyanide ponds resulting from precious metal extraction in the gold mining industry (McQuivey, '90). The analysis required a slight modification in calculation of the treatment sums of squares, where the grand mean was replaced by zero and the degrees of freedom (df) reflected the number of treatments considered in the experiment (i.e., $k = 6$). Estimates of the error term remained the same as in a standard ANOVA. Posthoc comparisons were made using a modification of Dunnett's *t* test ('55), again using a theoretical value of zero rather than the mean, and comparing the resulting *t* to critical values in Dunnett's calculated distribution with $P < 0.05$.

Experiment 2: Water consumption of simple benzoic acid derivatives

Adult European starlings were trapped and maintained as described for experiment 1. Eight compounds were tested: benzoic acid (CAS No. 65-85-0), anthranilic acid (2-aminobenzoic acid; CAS No. 118-92-3), 3-aminobenzoic acid (CAS No. 99-05-8), 4-aminobenzoic acid (CAS No. 150-13-0), o-anisic acid (2-methoxybenzoic acid; CAS No. 579-75-9), m-anisic acid (CAS No. 586-38-9), p-anisic acid (CAS No. 100-09-4), and salicylic acid (2-hydroxybenzoic acid; CAS No. 69-72-7). As in experiment 1, six concentrations of each chemical were prepared: 0.5%, 0.25%, 0.125%, 0.063%, and 0.031% (weight/volume), with 0% deionized, distilled water as the control. Handling, experimental protocol and analyses were identical to those described for experiment 1.

Experiment 3: Water consumption of hetero- and heterocyclic benzoic acid derivatives

Adult European starlings were trapped and maintained as described for experiment 1. Six compounds were evaluated for repellency: 5-nitroanthranilic acid (CAS No. 616-79-5), anthranilamide (CAS No. 88-68-6), benzamide (CAS No. 55-21-0), o-carboethoxybenzene sulfonamide (CAS No. 57683-71-3), isatoic anhydride (CAS No. 118-48-9), and 4-ketobenzotriazine (CAS No. 90-16-4). Six concentrations

for each chemical were tested: 0.5%, 0.1%, 0.05%, 0.01%, 0.005%, and 0.001% (weight/volume).

As in experiment 1, a once-choice drinking test was used to evaluate repellency. Pretreatment monitoring of water consumption, assignment to groups and testing followed protocols outlined in experiment 1.

Because we wished to test a broader range of concentrations in this chemical series, we were forced to eliminate a simultaneously run control group (i.e., 0% concentration). As a result, we restructured the analysis to accommodate the experimental design. Data were transformed using a difference score to control for individuals' pretreatment water consumption, i.e., treatment - pretreatment and posttreatment - pretreatment. Difference scores for water consumption were analyzed using a two-factor repeated-measures ANOVA, with the repeated factor on days which consisted of two levels (treatment, posttreatment). A difference contrast on the repeated measure allowed us to test for treatment effects relative to pretreatment consumption and carryover effects due to consumption of treated water; i.e., did postconsumption return to pretreatment levels. We used Scheffé's test to determine posthoc differences among concentration groups ($P < 0.05$). Evaluation of whether a concentration differed from zero consumption on the treatment day followed the procedure set forth for experiment 1. Unless otherwise indicated, all data were tested and found to be homogeneous using Bartlett's box method.

Experiment 4: Feeding tests

Because drinking tests are more sensitive than feeding tests in evaluating repellency (cf. Kare, '61), we tested only those compounds, previously not tested, that proved to be repellent in drinking trials, e.g., benzoic acid derivatives. Twenty-four adult male European starlings were tested with *o*-anthranilic acid and 4KBT. Birds were captured and handled as described for experiment 1.

Each chemical was mixed with Purina Flight Bird Conditioner (PBFC) to produce the following series of concentrations: 1.0%, 0.5%, 0.1%, and 0.01% (g/g).

The procedures detailed in Mason et al. ('89) for one-cup avian repellency evaluations were followed. Briefly, for each moiety, 24 starlings were randomly selected, weighed, and then assigned to four groups ($n = 6/\text{group}$) as described above. On the day following group assignment, a 4-day pretreatment period began. A 4-day treatment period immediately followed pretreatment, and, during each treatment, each group was presented with 50 g samples of PBFC adulterated with a different amount of

o-anthranilic acid or 4KBT (1.0%, 0.5%, 0.1%, 0.01% for groups 1-4, respectively). Consumption was recorded after 2 hr. Birds had free access to plain PBFC and water during the night. At the end of the fourth treatment trial, all birds were reweighed.

Results were analyzed in using a three-factor ANOVA, with repeated measures on the second (periods) and third (days) factors. Scheffé's posthoc tests were used to isolate significant differences among means ($P < 0.05$).

RESULTS AND DISCUSSION

Experiment 1: Water consumption of anthranilates

We previously found basicity and intramolecular hydrogen bonding to be important features contributing to repellency (Mason et al., '91b). To clarify the contribution of basicity to repellency in an additional series of compounds, we tested MA, DMA and ethyl anthranilate. These compounds represent varying degrees of basicity, while potential contributions of resonance and intramolecular hydrogen bonding are held constant (Fig. 1, top).

Effective elimination of consumption was in direct relation to basicity. MA, DMA and ethyl anthranilate all showed significant repellent effects (Fig. 1, bottom; $F = 16.54$, $df = 5,18$, $P < 0.001$; $F = 15.53$, $df = 5,17$, $P < 0.001$; $F = 16.13$, $df = 5,18$, $P < 0.001$, respectively). However, the more basic compounds, ethyl and DMA, were the better repellents, with concentrations as low as 0.13% differing from controls. Consumption of concentrations as low as 0.25% for the relatively less basic methyl anthranilate were different from controls ($P < 0.05$). For MA, only the highest concentration (0.5%) suppressed consumption to levels statistically indistinguishable from zero ($F = 26.23$, $df = 6,18$, $P < 0.001$). Concentrations as low as 0.13% were effective at suppressing consumption for ethyl anthranilate ($F = 13.09$, $df = 6,17$, $P < 0.001$), while the corresponding effective concentration for DMA was 0.06% ($F = 14.92$, $df = 6,18$, $P < 0.001$).

We chose to examine isobutyl, isobutyl methyl, and isobutyl *n,n* dimethyl anthranilate to test for the effects of steric distortion of pi orbital overlap. Each compound in this series is capable of intramolecular hydrogen bonding, except for *n,n* dimethyl anthranilate and is characterized by resonance of lone pairs of electrons just as in the MA series (Fig. 2, top). Isobutyl anthranilate is the least basic of the series, while isobutyl *n,n* dimethyl anthranilate is the most basic. However, the addition of one or two methyl groups to the amine also increases the possibility of distortion of the pi orbital overlap. Thus

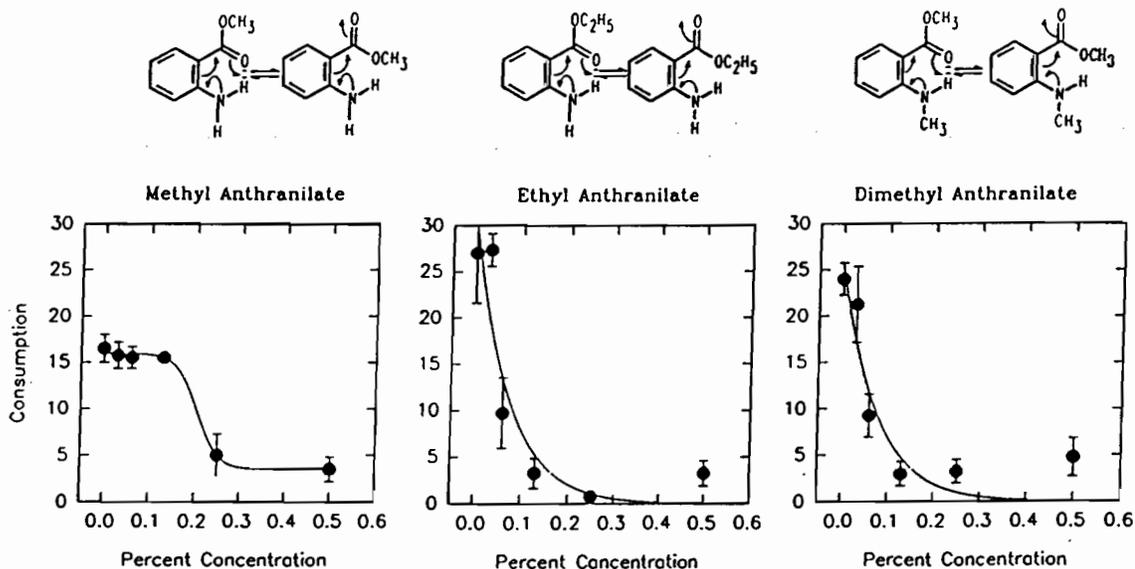


Fig. 1. Top: Chemical structures and electron donation and withdrawal paths (arrows) for methyl, ethyl, and dimethyl anthranilate. The lone pair of electrons associated with nitrogen have been omitted from the figures to reduce clutter. Mol-

ecules are increasingly basic from left to right. Bottom: panel. Dose-response curves for the anthranilate series. Consumption (ml) reflects total consumption during a 6-hr period. Bars are ± 1 SE. $N = 4$ per concentration group.

comparison of the relative water consumption of this series to the first series determines the relative importance of basicity versus planarity for repellency.

Overall, relative to the above anthranilates, isobutyl moieties of anthranilates were weaker repellents (Fig. 2, bottom). Only isobutyl anthranilate

showed any signs of repellency ($F = 3.49$, $df = 5,18$, $P = 0.022$), with only the highest concentration level (0.5%) being different from the untreated control ($P < 0.05$). However, no concentration was sufficiently repellent to suppress water consumption to zero ($F = 67.26$, $df = 6,18$, $P < 0.001$). Neither

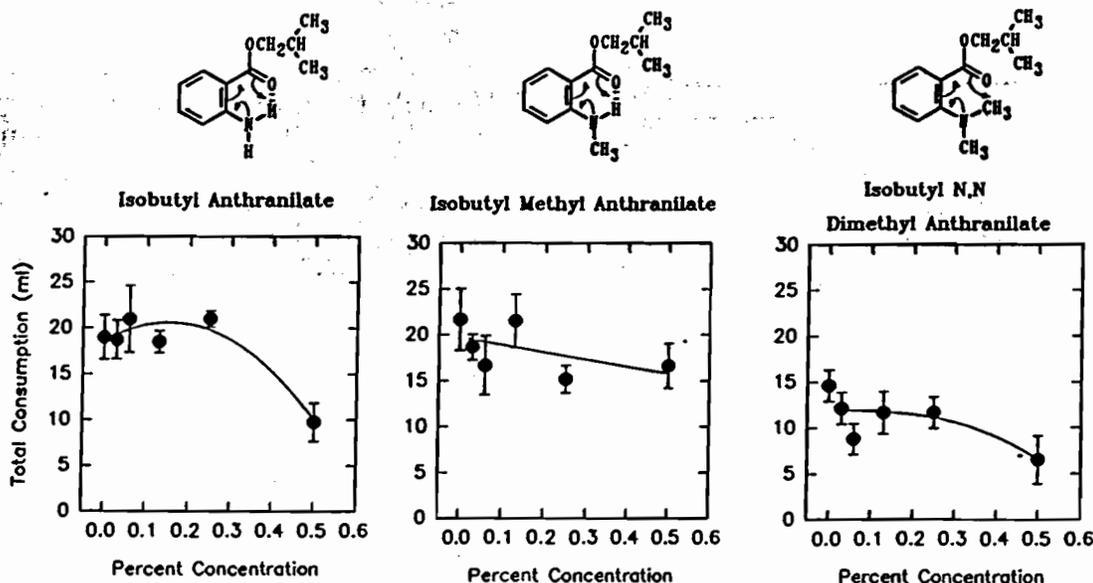


Fig. 2. Top: Chemical structures and electron donation and withdrawal paths (arrows) for isobutyl, isobutyl methyl, and isobutyl dimethyl anthranilate. Molecules are increasingly basic from left to right. Distortion of pi orbital overlap increases from

left to right. Bottom: Dose-response curves for the anthranilate series. Consumption (ml) reflects total consumption during a 6-hr period. Bars are ± 1 SE. $N = 4$ per concentration group.

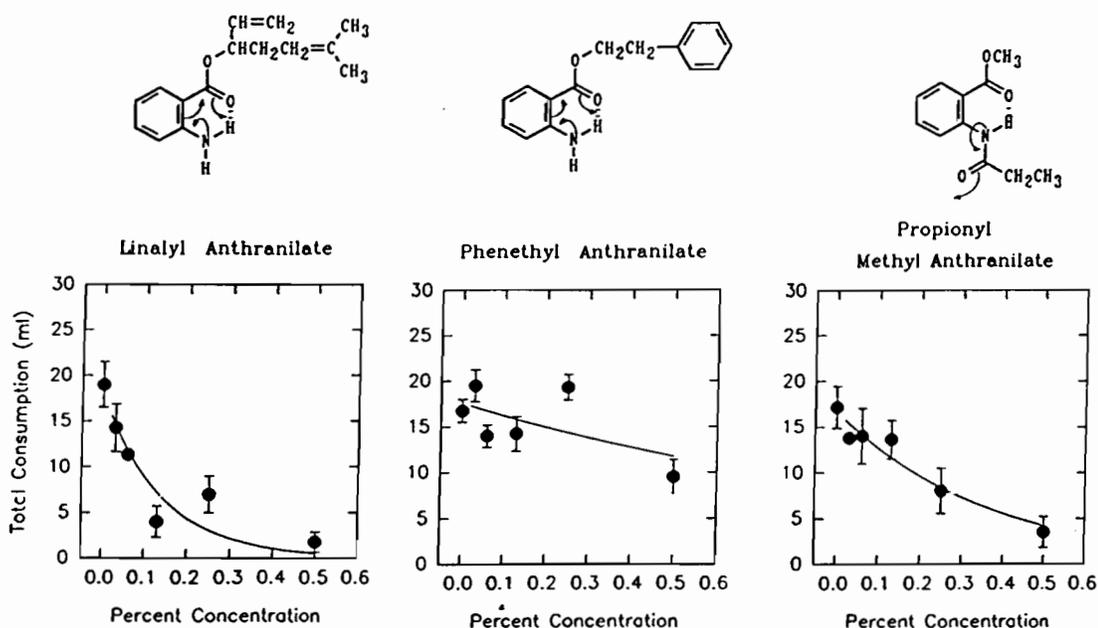


Fig. 3. Top: Chemical structures and electron donation and withdrawal paths (arrows) for linalyl anthranilate, phenethyl anthranilate, and propionyl methyl anthranilate. Bottom:

Dose-response curves for the anthranilate series. Consumption (ml) reflects total consumption during a 6-hr period. Bars are ± 1 SE. $N = 4$ per concentration group.

isobutyl methyl norisobutyl dimethyl anthranilate was an effective repellent ($F = 1.08$, $df = 5,29$, $P = 0.3916$ and $F = 1.95$, $df = 5,30$, $P = 0.12$, respectively). Consumption for all concentrations for both compounds was greater than zero ($F = 29.62$, $df = 6,29$ and $F = 16.5$, $df = 6,30$, $P < 0.001$, respectively).

Linalyl and phenethyl anthranilate were selected because they represented low-basicity molecules with a capacity for intramolecular hydrogen bonding and lone-pair electron resonance similar to moieties tested in the previous two series. Linalyl anthranilate has a large ester side chain (eight carbons) as in the isobutyl series, but, because of the double bonds, planar distortion of the pi orbital overlap is minimized (Fig. 3, top). Phenethyl anthranilate is of the same molecular weight as linalyl anthranilate. However, a benzene ring is attached at the side chain without a double bond, allowing for the possibility of greater steric effects (Fig. 3, top). Though the structure of propionyl methyl anthranilate does not result in planar distortion of pi orbital overlap, it does not allow electron donation to the phenyl ring (Fig. 3, top).

Linalyl anthranilate was a good repellent (Fig. 3, bottom; $F = 10.65$, $df = 5,16$, $P < 0.001$). Concentrations as low as 0.13% suppressed consumption to zero, while all other concentrations were significantly higher ($F = 17.90$, $df = 6,16$, $P <$

0.001). Propionyl methyl anthranilate was a weak repellent (Fig. 3, bottom; $F = 4.98$, $df = 5,17$, $P = 0.005$), with only the highest concentration (0.5%) being different from control consumption ($P < 0.05$). However, recorded consumptions at all concentration levels were greater than a theoretical level of zero ($F = 38.12$, $df = 6,17$, $P < 0.001$). Phenethyl anthranilate was not repellent to starlings ($P < 0.923$), with all concentrations tested being different from zero ($F = 19.77$, $df = 6,30$, $P < 0.001$).

Experiment 2: Consumption of simple benzoic acid derivatives

Benzoic acid derivatives were selected to determine the effects of increasing the acidity of molecules relative to the structurally similar but more basic acetophenone moieties (see, e.g., Clark and Shah, '91). Isomers of anthranilic acid were the most basic of the benzoic acid derivatives tested. The amino substituent in the ortho position allows for electron donation via resonance and intramolecular hydrogen bonding (Fig. 4, top). The para isomer has no such intramolecular bonding capacity but does allow resonance of the lone-pair electrons. The meta isomer is not capable of either. Anthranilic acid was moderately aversive to starlings (Fig. 4, bottom; $F = 24.29$, $df = 5,18$, $P < 0.001$), with concentrations of 0.5%, 0.25%, and 0.13% all differing from the water control ($P < 0.05$). Para aminoben-

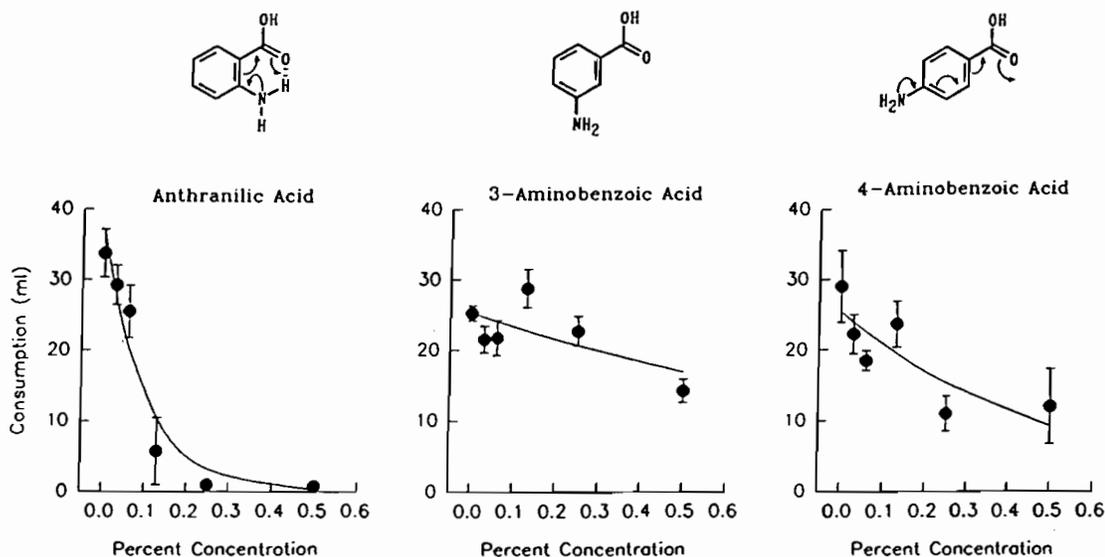


Fig. 4. Top: Chemical structures of isomers of anthranilic acid. Arrows indicate electron donation and withdrawal paths. Bottom: Dose-response curves for isomers of anthranilic acid.

Consumption values reflect total consumption during a 6-hr period. Bars are \pm SE.

zoic acid was also aversive to starlings ($F = 3.59$, $df = 5,17$, $P = 0.021$), with concentrations as low as 0.25% differing from water controls. Meta aminobenzoic acid was aversive ($F = 5.549$, $df = 5,17$, $P = 0.004$) but only slightly so. Posthoc analysis showed only the 0.5% concentration differing from the water control.

While all three isomers showed some repellency relative to water controls, they differed in the degree of absolute repellency (Fig. 4, bottom). Anthranilic acid was the most potent repellent of the isomeric series ($F = 37.2$, $df = 6,18$, $P < 0.001$). Posthoc tests showed that concentrations as low as 0.13% reduced consumption to zero. All concentrations of meta and para aminobenzoic acid differed from zero consumption ($F = 117.53$, $df = 6,17$, $P < 0.001$; $F = 29.48$, $df = 6,17$, $P < 0.001$, respectively).

Methoxy substitution (i.e., anisic acids) of the electron-donating substituent results in a less basic molecule relative to aminobenzoic acid isomers. Positional changes of the electron-donating substituent yield similar changes in resonance as described above (Fig. 5, top). None of the anisic acid isomers is capable of intramolecular hydrogen bonding. The increase in acidity relative to anthranilic acid was sufficient to overcome any resonance effects on repellency. None of the anisic acid isomers was repellent (Fig. 5, bottom; $P = 0.812$, 0.368, 0.475, $df = 5,18$ for the ortho, meta, and para isomers, respectively). All treatment concentrations for the three isomers differed from zero consumption ($F = 21.53$, $df = 6,18$, $F = 22.22$, $df = 6,18$, $F = 23.31$

$df = 6,18$, $P < 0.001$ for ortho, meta, and para methoxybenzoic acid, respectively).

Increasing acidity even further by substituting the electron donating group with a hydroxyl group, as in salicylic acid, made the treated solutions less aversive relative to anthranilic acids (Fig. 6, bottom), even in the presence of intramolecular hydrogen bonding. There were no differences among concentrations tested, including the water control, for salicylic acid ($F = 2.52$, $df = 5,18$, $P = 0.0671$). All concentrations tested differed from zero consumption ($F = 25.48$, $df = 6,18$, $P = 0.001$).

Elimination of the electron-donating group, as with benzoic acid, resulted in poor repellency (Fig. 6, bottom, $F = 1.5925$, $df = 5,18$, $P = 0.213$). All concentrations tested differed from zero consumption levels ($F = 14.70$, $df = 6,18$, $P < 0.001$).

Experiment 3: Consumption of hetero- and heterocyclic benzoic acid derivatives

Elimination of electron withdrawal enhances repellency, suggesting that electron richness of the phenyl ring is an important feature of repellency (Shah et al., '91). We tested this hypothesis by increasing the electron withdrawal on an otherwise good repellent (i.e., presence of a basic amine and intramolecular hydrogen bonding) (Fig. 7, top). Addition of a second withdrawing group, as seen with 5-nitro-anthranilic acid, reduced repellency relative to anthranilic acid. The profiles of concentration group by treatment day were similar (Fig. 7, middle; $P = 0.991$), though there was a tendency

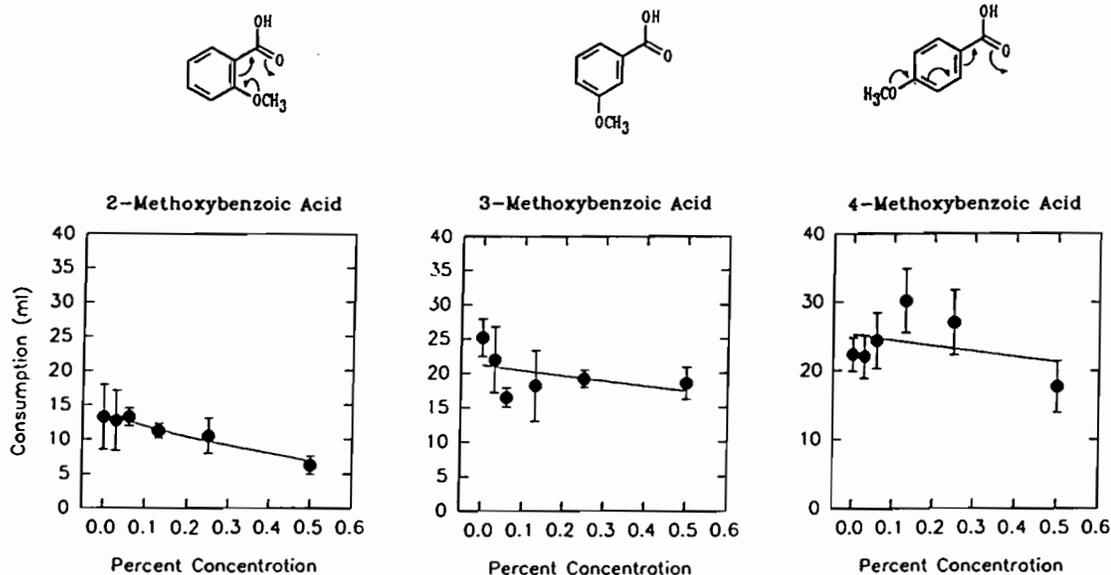


Fig. 5. Top: Chemical structures of isomers of anisic acid. Arrows depict electron donation and withdrawal paths. Bottom: Dose-response curves of methoxybenzoic acid. Consump-

tion reflects total amount of water consumed over a 6-hr trial period. Bars are ± 1 SE.

for relative water consumption to be lower on the treatment day and to return to normal on the post-treatment day ($F = 5.81$, $df = 1,30$, $P = 0.022$). Despite differences in relative consumption among concentration groups, there was no systematic pattern for which concentrations differed from the pre-treatment water consumption ($F = 2.70$, $df = 5,30$, $P = 0.04$). Nonetheless, 5-nitro anthranilic acid was not a particularly effective repellent (Fig. 7, bottom). Total consumption remained high, and all con-

centrations tested on the treatment day differed from zero consumption ($F = 7.22$, $df = 6,30$, $P < 0.001$).

The effect of electron withdrawal can be internally compensated. In benzamide, electrons are withdrawn from the amine group rather than the phenyl ring. However, benzamide does not possess other attributes of previously identified repellents, e.g., an electron-donating group (Fig. 7, top). Thus benzamide provides a test for the effects of electron withdrawal on repellency. The profile of the treat-

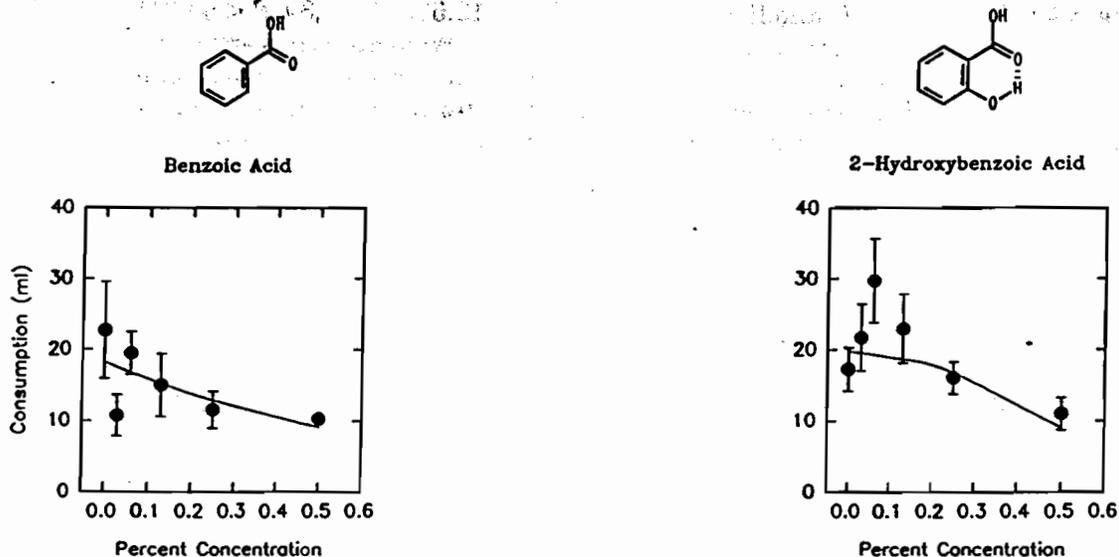


Fig. 6. Top: Chemical structures of 2-hydroxy benzoic acid (salicylic acid) and benzoic acid. Bottom: Dose-response

curves for total water consumption during a 6-hr period. Bars are ± 1 SE.

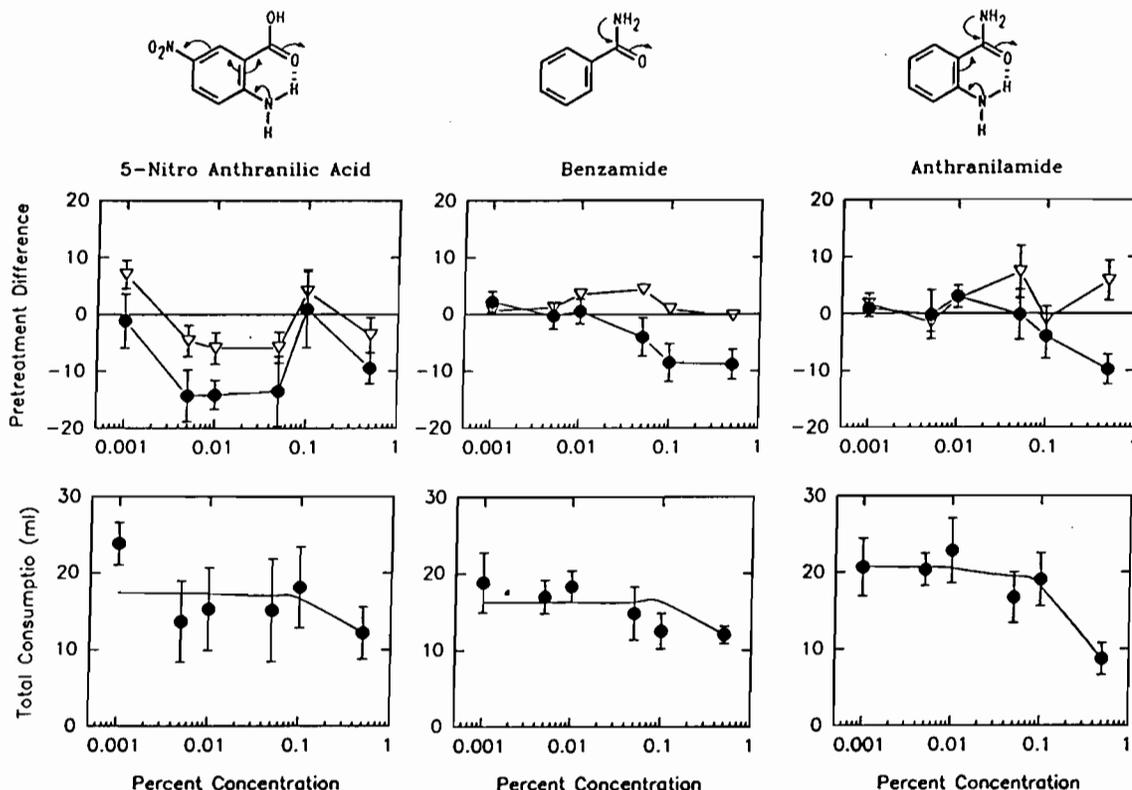


Fig. 7. Top: Chemical structure for 5-nitro anthranilic acid, benzamide, and anthranilamide and electron donation and withdrawal paths (arrows). Middle: Consumption of water during the treatment (circles) and posttreatment (triangles) days, relative to pretreatment consumption. A value of zero indicates

no difference between pretreatment and treatment or posttreatment consumption. Negative scores reflect repellency, while positive scores reflect preference. Bottom: Dose-response curve for water consumption during the day of treatment for a 6-hr period. Bars are ± 1 SE. $N = 6$ /concentration group.

ment by concentration interaction shows that birds receiving higher concentrations of benzamide reduced water consumption relative to their pretreatment levels (Fig. 7, middle; $F = 2.46$, $df = 10.60$, $P = 0.015$). Posttreatment consumption returned to pretreatment levels. However, inhibition of water consumption during the day of treatment was poor because no concentration was able to suppress consumption entirely (Fig. 7, bottom; $F = 21.29$, $df = 6,30$, $P < 0.001$).

We tested anthranilamide because the amine at the 2-carbon position donates electrons to the phenyl ring. As is the case for benzamide, electron withdrawal is internally compensated by the amide, thus increasing the electron richness of the phenyl ring. Additionally, this molecule is capable of intramolecular hydrogen bonding (Fig. 7, top). Together, these features should act to restore repellency. Inspection of the day by concentration group profile showed that the highest concentration effectively decreased relative water consumption (Fig. 7, middle; $F = 6.22$, $df = 5,30$, $P < 0.001$). Posttreatment consumption was similar to pretreatment consump-

tion. Even though a 0.5% concentration was an effective repellent consumption was not reduced to zero for any concentration tested (Fig. 7, bottom; $F = 19.56$, $df = 6,30$, $P < 0.001$).

We tested orthocarboethoxybenzene sulfonamide because it represents a benzoic acid derivative with two electron-withdrawing groups, one of them being a heteroatom functionality (Fig. 8, top). Internal compensation of electron donation, as seen for *o*-carboethoxybenzene sulfonamide, eliminated repellency. There were no differences in relative consumption across concentration groups for the treatment and posttreatment days (Fig. 8, middle; $P = 0.91$), with consumption in both cases equalling pretreatment consumption. Consumption for all the concentration groups differed from zero consumption (Fig. 8, bottom; $F = 40.24$, $df = 6,30$, $P < 0.001$).

Restoration of an attached ring structure within the same plane as the phenyl ring, as in isatoic anhydride (Fig. 8, top), but with a lower electron richness, also failed to yield repellency. Neither posttreatment nor treatment periods differed for relative water consumption across concentration groups (Fig. 8,

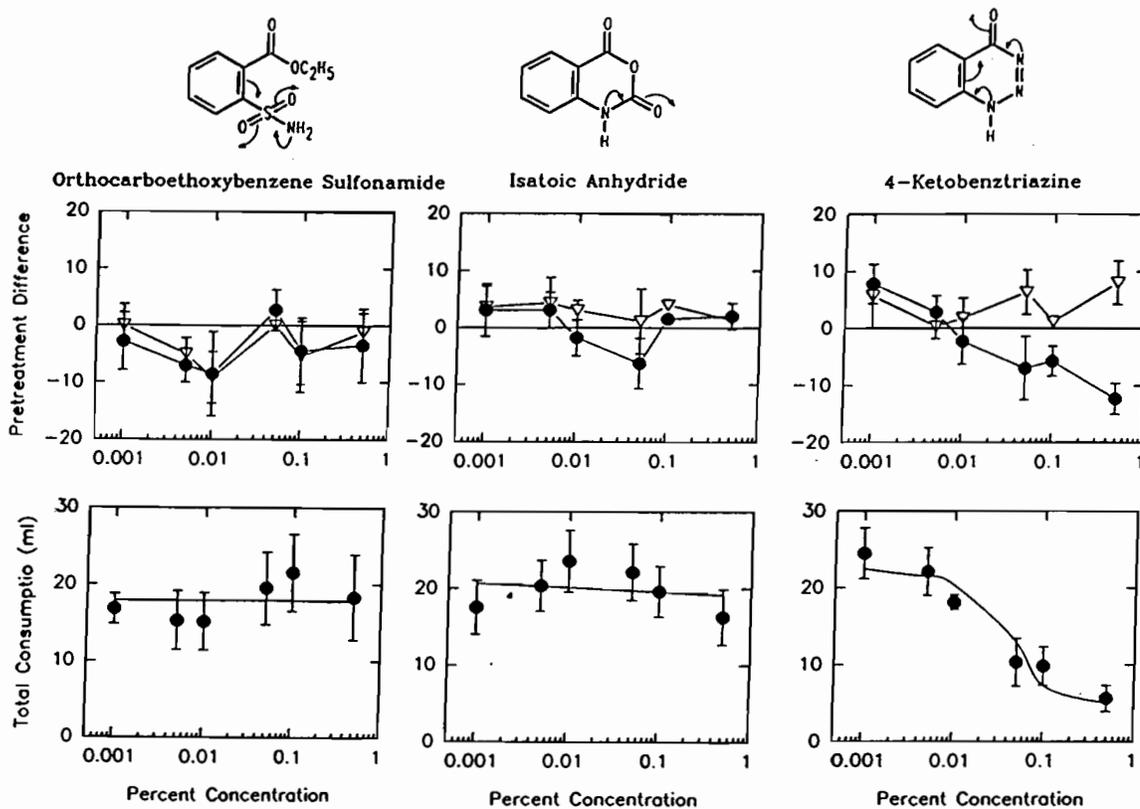


Fig. 8. Top: Chemical structure for *o*-carboethoxybenzene sulfonamide, isaloic anhydride, and 4-ketobenztriazine and electron donation and withdrawal paths (arrows). Middle: Relative water consumption for the treatment (circles) and post-

treatment (triangles) days. Definition of scores are the same as for Figure 4. Bottom: Dose-response curve for the 6-hr consumption period on the day of treatment. Bars reflect ± 1 SE. $N = 6/\text{concentration group}$.

middle; $P = 0.683$), being similar to pretreatment consumption levels. All concentrations tested on the day of treatment differed from zero consumption (Fig. 8, bottom; $F = 51.66$; $df = 6,30$, $P < 0.001$).

Examination of 4KBT shows that the electron withdrawal is internally compensated within the carboxyl group, while the phenyl ring is kept electron rich through donation via the amine (Fig. 8, top). Because of the covalent bonds, the heteroring structure kept the electron-donating and -withdrawing groups in the same pi orbital. We hypothesized that because 4KBT possessed all the salient features it should be a good avian repellent. Moderate to high concentrations reduce consumption relative to the pretreatment period ($F = 4.35$, $df = 5,30$, $P = 0.004$); then consumption returns to pretreatment levels during the posttreatment period (Fig. 8, middle). Posthoc tests show that concentrations as low as 0.05% differ from the lowest concentration tested for total water consumption (Fig. 8, bottom). 4KBT can be considered a strong repellent, with concentrations as low as 0.05% effectively equalling zero consumption. Thus the combination

of basicity, resonance, electron richness of the phenyl ring, and pi cloud planarity are necessary and sufficient attributes for an avian repellent.

Experiment 4: Feeding experiments on anthranilic acid and 4KBT

Previous experiments examined effective anthranilates and acetophenones in a feeding context (Mason et al., '89, '91b), and were largely consistent with the water consumption results reported above. For comparative purposes only those compounds found to be good repellents in the benzoic acid series were examined. In one-cup feeding trials, anthranilic acid proved to be a moderately good repellent (Fig. 9, top; concentration \times treatment period interaction, $F = 8.59$, $df = 5,30$, $P < 0.001$). Posthoc tests showed that only 1.5% and 2.0% concentrations were effective at decreasing consumption levels. 4-KBT was a better repellent (Fig. 9, bottom; concentration \times period interaction, $F = 8.17$, $df = 5,30$, $P = 0.001$). Concentrations as low as 0.1% were effective at inhibiting food consumption.

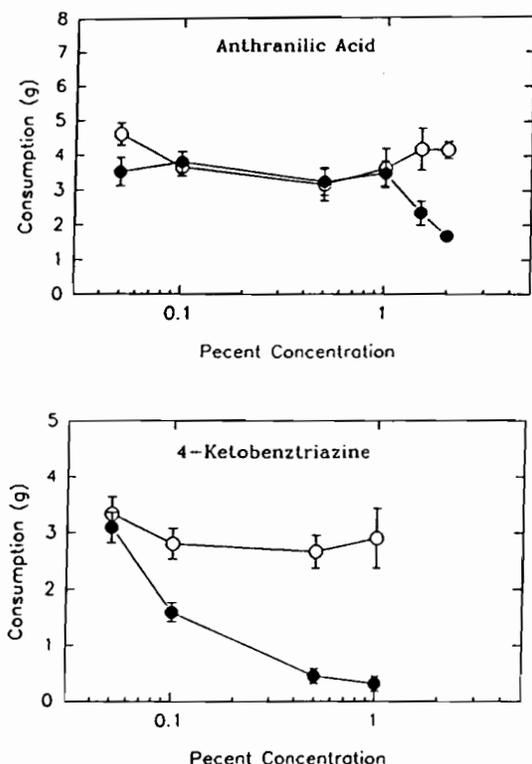


Fig. 9. Top: Profiles for the one-cup feeding trials for anthranilic acid. Bottom: Profiles for the one-cup feeding trials for 4-ketobenztriazine. Open circles depict 4-day average food consumption during the pretreatment period. Solid circles depict 4-day average of food treated with chemical. Bars reflect ± 1 SE.

GENERAL DISCUSSION

Aminoacetophenones are potent avian repellents (Mason et al., '91b). We speculated that this effectiveness could be due to resonance of lone pairs of electrons and a capacity to form a ring structure via intramolecular hydrogen bonding. Subsequently, we showed that basicity and resonance of lone pairs of electrons are critical for repellency (Clark and Shah, '91). The well-described anthranilate repellents (Kare, '61), e.g., ethyl anthranilate, MA, and DMA, also have these features, though they are slightly less basic than aminoacetophenones. The least basic of this series, MA, is not as effective a repellent as the other two at lower concentrations, although it is equally effective at concentrations between 0.5% and 1.0% (g/g) (Mason et al., '91a). These data are consistent with the hypothesis that basicity is important for avian repellency.

Tests of compounds such as those in the isobutyl series showed that steric distortion could decrease repellency despite the presence of resonance, basicity, and intramolecular hydrogen bonding. If side

chains attached to the withdrawing group are free to rotate, they may cause the electron-donating group to move out of plane with the electron-withdrawing group. Thus, the two phenyl rings of the ineffective compound, phenethyl anthranilate, were free to rotate with respect to one another causing steric hinderance of electron donation, whereas the 8-carbon ester of linalyl anthranilate, a good repellent, was kept in the same plane as the electron-donating group by double bonds, and hence was less likely to hinder resonance effects. These data suggest that steric effects can reduce repellency even when optimal conditions of basicity and resonance are present.

It is also unlikely that molecular weight *per se* was a major factor influencing repellency. Certainly with the aminoacetophenones isomers and moieties tested earlier molecular weight had no effect on repellency (Clark and Shah, '91). For example, linalyl anthranilate, with a basic structure capable of intramolecular hydrogen bonding and resonance, had twice the number of carbons ($n = 8$) in the ester group as isobutyl anthranilate ($n = 4$), yet linalyl anthranilate was a much more effective repellent. Furthermore, linalyl and phenethyl anthranilate have identical molecular weights, but phenethyl anthranilate was a weak repellent at best.

When the ester of molecules such as MA and DMA is replaced with a hydroxy group to yield anthranilic acid, basicity is reduced and repellency is significantly lowered. Effectiveness of the repellent is further reduced if resonance is eliminated as in the meta isomer of anthranilic acid. The less basic methoxy and hydroxy substitutions at the 2-carbon site eliminate repellency, even in the presence of resonance and intramolecular hydrogen bonding.

Donation of lone pairs of electrons to the phenyl ring is also an important factor influencing repellency. When a second withdrawing group is added, as in 5-nitro anthranilic acid, repellency is reduced relative to anthranilic acid. Only the highest concentration of 5-nitro anthranilic acid differed from pretreatment levels. Internal compensation of the withdrawing group most likely did not enhance repellency by itself as was the case for benzamide. Repellency was only marginally improved when electrons were donated to the phenyl ring in the presence of internal compensation of electron withdrawal in anthranilamide. We concluded that, while electron withdrawal can decrease repellency, compensation of electron withdrawal, or in combination with electron donation to the phenyl ring, was not by itself sufficient factors to improve repellency dramatically.

The ester ethyl anthranilate was by all measures a good repellent for the reasons stated above. However, when a sulfonyl group was placed between the phenyl ring and the amine, electron donation and intramolecular hydrogen bonds were no longer possible. Also, the distortion of the pi cloud was increased. The result was total elimination of repellency.

Even planar structures such as isatoic anhydride were ineffective repellents. We suspect this to be the case primarily because there was no electron donation to the phenyl ring. We predicted that only when 1) electron withdrawal is internally compensated, 2) donation to the phenyl ring is allowed, and 3) the heteroatoms are kept in the same plane should repellency be restored. This was the case for 4-KBT.

In summary, we believe the essential features of an avian repellent incorporate some combination of the following attributes into a molecule: high basicity, capacity for a heteroatom ring structure whether by covalent or intramolecular hydrogen bond, and increased electron richness of the phenyl ring either by direct donation of lone pairs of electrons and/or with internal compensation or elimination of electron withdrawal. These features may be general attributes of avian repellents. Independent work on cinnamic acids shows that cinnamamide is a stronger repellent relative to derivatives where methoxy and hydroxy groups were substituted as the electron donating groups (Crocker and Perry, '90).

It is clear from the data that no single simple relationship can predict repellency (Beets, '78; Graedel, '84). Interactions are likely to occur among the identified features which will influence repellency (McGill and Kowalski, '77). Nonetheless, the heuristic approach toward structure activity relationships used here is a first step toward a more quantitative model and a step that may prove to be of great value in the identification and screening of new repellents and in achieving a better understanding of structural differences of bird and mammal irritants. Our current understanding of structure-activity relationship allows us to predict qualitatively whether a compound will be repellent to a bird. We cannot, at present, predict the shape of the dose-response curve. However, we currently are modelling the topographic, conformational, and electronic aspects of compounds for use in a canonical correlative analysis for structure activity. While we do not know how such variables interact with receptors of the chemosensory systems involved, we will be able to draw relationships between multi-dimensional chemical attributes and the behavioral dose-response curve.

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