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Avian predators taste–reject aposematic prey on the basis of their chemical defence

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Avian predators learn to avoid defended insects on the basis of their conspicuous warning coloration. In many aposematic species, the level of chemical defence varies, with some individuals being more defended than others. Sequestration and production of defence chemicals is often costly and therefore less defended individuals enjoy the benefits of the warning signal without paying the full costs of chemical production. This is a fundamental theoretical problem for the evolutionary stability of aposematism, since less defended individuals appear to be at a selective advantage. However, if predators sample aposematic prey and selectively reject individuals on the basis of their chemical investment, aposematism could become evolutionarily stable. Previous research aimed at testing whether birds can use taste to discriminate between palatable and unpalatable prey has been confounded by other experimental factors. Here, we show that birds can taste and reject prey entirely on the basis of an individual's level of chemical defence and more importantly, they can make decisions on whether or not to consume a defended individual based upon their level of chemical investment. We discuss these results in relation to the evolution of aposematism, mimicry and defence chemistry.

Keywords: aposematism; automimicry;
receiver psychology; insects; toxins

1. INTRODUCTION

Many defended insects advertise their chemical defences using conspicuous aposematic coloration (Edmunds 1974), and visually hunting birds learn to avoid defended insects on the basis of their warning signals (reviewed in Guilford 1990). However, within a species, the level of chemical defence varies, with some individuals being more defended than others (e.g. Brower *et al.* 1967). When the production or storage of defence chemicals is costly (e.g. Cohen 1985; Rowell-Rahier & Pasteels 1986), individuals may benefit by reducing their investment in defences and less defended individuals (automimics) would have a selective advantage over more defended individuals (automodels; Guilford 1994). Therefore, aposematism is unstable as individuals will be selected to have lower chemical defences, and at some point crypsis will become advantageous relative to warning signals.

All attempts to resolve this theoretical problem rely on the assumption that predators selectively reject prey on the basis of their palatability (Leimar *et al.* 1986; Guilford 1994), resulting in higher mortality of automimics than automodels. While it is evident that birds can attack aposematic prey and release them relatively unharmed (e.g. Wiklund & Järvi 1982), it is not clear if they are able to distinguish between visually identical automodels and automimics upon attack.

Despite some support from observations of birds foraging on natural prey (Fink & Brower 1981; Brower & Calvert 1985), there has been no direct experimental test that prey are selected according to their individual chemical investments. In a recent paper, Gamberale-Stille & Guilford (2004) reported data suggesting that birds could discriminate between visually identical palatable automimics and unpalatable automodels upon attack, with fewer automodels being eaten. However, there was a methodological problem with their experiment: the unpalatable prey were both damp and chemically defended while palatable prey were dry and undefended. Since chicks reject damp crumbs more than dry crumbs (J. Skelhorn 2005, unpublished thesis) it is impossible to determine whether the birds used chemical or textural differences for their rejection.

Gamberale-Stille & Guilford's experiment also provided no evidence that birds can discriminate among defended aposematic prey that vary in their degree of defence. This is important for several reasons. First, if birds do selectively reject prey on the basis of their chemical investment, aposematism will be evolutionarily stable. Second, mathematical simulations of both Batesian and Müllerian mimicry (where a palatable and unpalatable species evolve to share the colour pattern of an aposematic species, respectively) assume that models and mimics share the costs of predator education equally (Speed 1993; MacDougall & Dawkins 1998). However, if birds selectively reject prey on the basis of their chemical investment, these simulations may have drastically overestimated the costs of mimics to their models. Finally, if birds are capable of quantifying differences in defence chemistry upon attack, it is possible that receiver psychology could influence the evolution of insects' chemical defences.

Using a laboratory system of domestic chicks (*Gallus gallus domesticus*) foraging on coloured chick crumbs, we specifically ask whether birds can discriminate among visually identical prey with different levels of the same defence chemical. We remove any confounding effects of crumb texture by ensuring that all our crumbs were dry.

2. MATERIAL AND METHODS

Thirty-two domestic chicks *G. gallus domesticus* of mixed sex were hatched in the laboratory. They were assigned to one of two groups: there were 20 experimental chicks and 12 'companion' chicks (see below). Each group was housed in a separate cage ($100 \times 50 \times 50$ cm) in a laboratory maintained at 25 °C and subject to a 14L : 10D cycle using fluorescent lights. Water was available *ad libitum* as were brown chick starter crumbs (nutritionally balanced pellets approximately 1 mm in diameter) except when food deprivation was necessary. After the experiment, chicks were donated to free range smallholdings.

We produced palatable, moderately defended and highly defended crumbs by spraying 150 g of brown starter crumbs with 100 ml of either water, 1 or 4% quinine sulphate solution,

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respectively. Quinine is bitter-tasting and at higher doses than used in this experiment, has been used to cause emesis in birds (Alcock 1970). These concentrations were chosen because chicks of the same age and strain can perceive these concentrations upon attack and readily learn to avoid crumbs flavoured with either 1 or 4% quinine solutions (J. Skelhorn 2005, unpublished thesis). The palatable crumbs were then sprayed with 0.5 ml of sugarflair spruce-green food dye diluted to 90 ml with water and both types of defended crumbs were sprayed with 2 ml of supercook red food dye diluted to 90 ml with water. Crumbs were then dried, before being sieved to ensure they were of a similar size.

We used a cage identical to those used for housing as an experimental arena. It was divided into two sections separated by a wire mesh screen placed 25 cm from one end of the cage (Skelhorn & Rowe 2005). The main section was the experimental arena, the floor of which was green laminated cardboard divided into 80 equally sized rectangles. As a result, green crumbs appeared cryptic and visually identical crumbs that differed in flavour could be identified by their position on the grid. The smaller section was used for two 'companion' chicks, which had free access to food and water during trials and prevented experimental chicks becoming distressed by being alone in the arena. On days 1 and 2 post-hatch, experimental chicks were trained to eat brown chick starter crumbs in the experimental arena (Skelhorn & Rowe 2005). On day 3, after approximately 1 h of food deprivation, chicks were placed in the experimental arena individually, and given 20 palatable green crumbs, 10 moderately defended red crumbs and 10 highly defended red crumbs. The crumbs were positioned using randomly generated maps, with no more than one crumb per rectangle (visually identical crumbs with different levels of quinine could therefore, be identified by their position in the arena). Each chick was allowed to attack (peck or eat) 16 crumbs before being removed from the arena. Each chick received two of these trials per day on each of four consecutive days.

3. RESULTS

Initial analysis revealed that our data were normally distributed and that the variances were homogeneous, so we analysed them using parametric tests. All birds learned to avoid unpalatable red crumbs (see figure 1). However, birds could not visually discriminate between the moderately and highly defended red crumbs since there was no difference in the proportions attacked across the eight trials (proportion of highly unpalatable red crumbs attacked compared to random expectation (0.5); one sample t-test, $t = 0.109$, d.f. $= 19$, n.s.).

Since the number of each crumb type attacked in a single trial was small, we calculated the total number of each crumb type attacked across all eight trials. In the critical test of our hypothesis, we compared the proportion of crumbs that were eaten once they had been attacked for each crumb type (see figure 2). Chicks ate a higher proportion of the palatable green crumbs attacked than defended red crumbs (paired t-test, $t = 5.25$, d.f. $= 19$, $p < 0.001$). However, chicks ate proportionally fewer of the highly defended crumbs attacked compared to the moderately defended crumbs attacked (paired t-test, $t = 12.55$, d.f. $= 19$, $p < 0.001$). Indeed, this effect was so strong that the pattern was repeated in the behaviour of every bird.

4. DISCUSSION

These data provide the first evidence that birds can quantify different levels of prey defence chemicals and discriminate between visually identical prey on the level of their chemical investment. The probability of avian predators releasing unpalatable prey after the initial attack increases with increasing quinine content
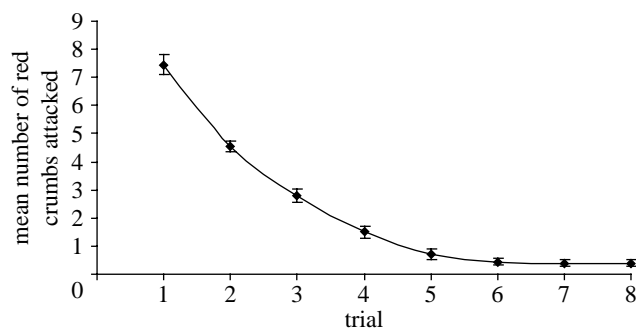
Figure 1. The mean number ($\pm$ standard error) of red crumbs attacked in each trial ($n = 20$).
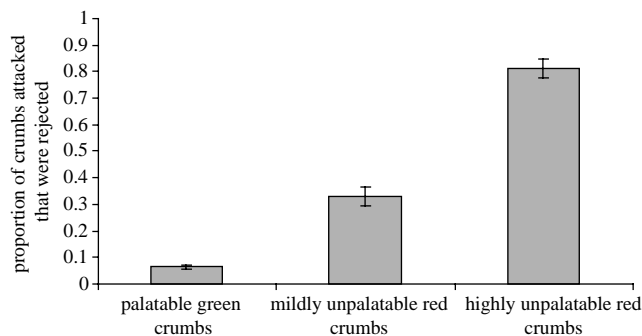

Figure 2. The mean proportion ($\pm$ standard error) of the total number of palatable green crumbs, mildly, and highly unpalatable red crumbs attacked that were rejected ($n = 20$).

and since it is well established that predators can release aposematic prey relatively unharmed (e.g. Wiklund & Järvi 1982), investment in chemical defences will increase an individual's survival chances, provided the defence chemical is perceptible upon attack. Therefore, by sampling and taste–rejecting prey on the basis of their unpalatability, birds can maintain the reliability of their prey's aposematic signal and render aposematism evolutionarily stable.

While Gamberale-Stille & Guilford (2004) showed that rejection following attack could select for the stability of aposematic systems, they did not show conclusively that birds were rejecting defended crumbs on the basis of their chemical investment. Our results support the findings of Gamberale-Stille & Guilford, but, more importantly, they show that birds can discriminate between different levels of the same defence chemical, preferentially eating prey with lower defence levels. We have demonstrated that, food need not be wet to be tasted by birds. Taken together, these data imply that avian taste is better than some authors have suggested (e.g. Kassarov 1999), and could potentially allow for discrimination among defended prey in order to reduce their toxin intake, provided that birds respond in similar ways to defended insects and our artificial prey.

While it is now well established that the psychology of avian predators influences the design of the visual signals of their prey, our results raise the intriguing possibility that it may also have played an important role in the evolution of a prey's chemical defences. Many insects secrete some of their chemical defences upon attack (Eisner & Meinwald 1966) or store defence chemicals in areas of the body most likely to

be attacked (Nishida 2002). Provided that taste is a reliable indicator of toxicity, this would allow birds to reject prey according to perceived toxin levels. Our findings might also explain why females of some aposematic species choose mates on the basis of their toxin contents (Eisner & Meinwald 1995). Females could gain direct benefits by choosing males that provide nuptial gifts with the highest toxin content (Eisner & Meinwald 1995), either to increase their own fitness or that of their offspring.

Perhaps most importantly, we have demonstrated that prey selection by birds is a two-step process. First, birds use visual cues to decide whether or not to attack an individual, which will depend primarily upon both unlearned biases and learned associations (Guilford 1990), but may also be influenced by other factors, such as hunger or availability of alternative palatable prey (Sherratt *et al.* 2004). Second, once an individual has been attacked, birds use chemical cues to decide whether or not to eat the prey according to how defended it is perceived to be. This two-step model can explain the maintenance of aposematism within a species and also has implications for under-standing the evolution of visual mimicry between species of different palatabilities. Recent mathematical simulations of Müllerian mimicry predict that because mimics share the cost of predator education, more palatable mimics can raise predation on their more unpalatable models (Speed 1993; MacDougall & Dawkins 1998). However, our data show that the costs are determined by palatability and are higher for more palatable mimics and these simulations may have drastically overestimated the costs of mimics to their models. Future simulations of the evolution of defensive coloration and mimicry should therefore incorporate this mechanism of prey selection in order to fully understand the evolutionary process.

Finally, although experimental studies dealing with natural insect prey often distinguish between those attacked by predators and those killed (e.g. Wiklund & Järvi 1982), laboratory experiments using artificial prey have used a variety of different behaviours to measure predation pressure (Guilford 1990). Since we have shown that attack rates do not necessarily predict survival rates, it may be appropriate to reassess the results of these studies in light of these new data.

Alcock, J. 1970 Punishment levels and the response of black-capped chickadees (*Parus atricapillus*) to three kinds of artificial seeds. *Anim. Behav.* **18**, 592–599. (doi:10.1016/0003-3472(70)90057-6)

Brower, L. P. & Calvert, W. H. 1985 Foraging dynamics of bird predators on overwintering monarch butterflies in Mexico. *Evolution* **39**, 852–868.

Brower, L. P., Brower, J. V. Z. & Corvino, J. M. 1967 Plant poisons in a terrestrial food chain. *Proc. Natl Acad. Sci. USA* **57**, 893–898.

Cohen, J. A. 1985 Differences and similarities in cardenolide contents of Queen and Monarch butterflies in Florida and their ecological and evolutionary implications. *J. Chem. Ecol.* **11**, 85–103. (doi:10.1007/BF00987608)

Edmunds, M. 1974 *Defence in animals*. Harlow, UK: Longman.

Eisner, T. & Meinwald, J. 1966 Defensive secretions of arthropods. *Science* **153**, 1341–1350.

Eisner, T. & Meinwald, J. 1995 The chemistry of sexual selection. *Proc. Natl Acad. Sci. USA* **92**, 50–55.

Fink, L. S. & Brower, L. P. 1981 Birds can overcome the cardenolide defence of monarch butterflies in Mexico. *Nature* **291**, 67–70. (doi:10.1038/291067a0)

Gamberale-Stille, G. & Guilford, T. 2004 Automimicry destabilizes aposematism: predator sample-and-reject behaviour may provide a solution. *Proc. R. Soc. B* **271**, 2621–2625. (doi:10.1098/rspb.2004.2893)

Guilford, T. 1990 The evolution of aposematism. In *Insect defences: adaptive mechanisms and strategies of prey and predators* (ed. D. L. Evans & J. O. Schmidt). Albany, NY: State of New York Press.

Guilford, T. 1994 "Go-slow" signalling and the problem of automimicry. *J. Theor. Biol.* **170**, 311–316. (doi:10.1006/jtbi.1994.1192)

Kassarov, L. 1999 Are birds able to taste and reject butterflies based on 'beak mark tasting'? A different point of view. *Behaviour* **136**, 965–981. (doi:10.1163/156853999501676)

Leimar, O., Enquist, M. & Sillén-Tullberg, B. 1986 Evolutionary stability of aposematic coloration and prey unprofitability: a theoretical analysis. *Am. Nat.* **128**, 469–490. (doi:10.1086/284581)

MacDougall, A. & Dawkins, M. S. 1998 Predator discrimination error and the benefits of Müllerian mimicry. *Anim. Behav.* **55**, 1281–1288. (doi:10.1006/anbe.1997.0702)

Nishida, R. 2002 Sequestration of defensive substances from plants by Lepidoptera. *Annu. Rev. Entomol.* **47**, 57–92. (doi:10.1146/annurev.ento.47.091201.145121)

Rowell-Rahier, M. & Pasteels, J. M. 1986 Economics of chemical defense in Chrysomelinae. *J. Chem. Ecol.* **12**, 1189–1203. (doi:10.1007/BF01639004)

Sherratt, T. N., Speed, M. P. & Ruxton, G. D. 2004 Natural selection on unpalatable species imposed by state-dependent foraging behaviour. *J. Theor. Biol.* **228**, 217–226. (doi:10.1016/j.jtbi.2003.12.009)

Skelhorn, J. & Rowe, C. 2005 Tasting the difference: do multiple defence chemicals interact in Müllerian mimicry? *Proc. R. Soc. B* **272**, 339–345. (doi:10.1098/rspb.2004.2953)

Speed, M. P. 1993 Müllerian mimicry and the psychology of predation. *Anim. Behav.* **45**, 571–580. (doi:10.1006/anbe.1993.1067)

Wiklund, C. & Järvi, T. 1982 Survival of distasteful insects after being attacked by naïve birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution* **36**, 998–1002.