biology letters

Frequency-dependent taste-rejection by avian predation may select for defence chemical polymorphisms in aposematic prey

John Skelhorn and Candy Rowe

Biol. Lett. 2005 **1**, 500-503
doi: 10.1098/rsbl.2005.0359

biology letters

Biol. Lett. (2005) **1**, 500–503
doi:10.1098/rsbl.2005.0359
Published online 31 August 2005

Frequency-dependent taste-rejection by avian predation may select for defence chemical polymorphisms in aposematic prey

John Skelhorn* and Candy Rowe

School of Biology, University of Newcastle, Henry Wellcome Building for Neuroecology, Framlington Place, Newcastle upon Tyne NE2 4HH, UK
**Author for correspondence (john.skelhorn@ncl.ac.uk)*

Chemically defended insects advertise their unpalatability to avian predators using conspicuous aposematic coloration that predators learn to avoid. Insects utilize a wide variety of different compounds in their defences, and intraspecific variation in defence chemistry is common. We propose that polymorphisms in insect defence chemicals may be beneficial to insects by increasing survival from avian predators. Birds learn to avoid a colour signal faster when individual prey possesses one of two unpalatable chemicals rather than all prey having the same defence chemical. However, for chemical polymorphisms to evolve within a species, there must be benefits that allow rare chemical morphs to increase in frequency. Using domestic chicks as predators and coloured crumbs for prey, we provide evidence that birds taste and reject proportionally more of the individuals with rare defence chemicals than those with common defence chemicals. This indicates that the way in which birds attack and reject prey could enhance the survival of rare chemical morphs and select for chemical polymorphism in aposematic species. This is the first experiment to demonstrate that predators can directly influence the form taken by prey's chemical defences.

Keywords: domestic chick; toxin; insect; warning signal; receiver psychology

1. INTRODUCTION

Aposematic insects gain protection from avian predators by advertising their unpalatability or toxicity using conspicuous coloration (Cott 1940; Edmunds 1974). While insect warning signals have converged on particular colours (Cott 1940), insects have evolved a wide variety of chemicals to ward off predators (reviewed in Blum 1981). There is widespread intraspecific variation in defence chemistry, with individuals differing in the amount (Brower *et al.* 1984; de Jong *et al.* 1991) or the concentration (Eggenberger & Rowell-Rahier 1992) of the defence chemical they possess, in the proportion of different chemicals in their defence secretions (Eggenberger & Rowell-Rahier 1992), or they may simply possess entirely different defence chemicals (Pasteels *et al.* 1995).

Predator psychology is important in the evolution of warning signals (Guilford & Dawkins 1991), but its role in the evolution of defence chemistry has received less attention. We have recently found that birds learn to avoid a population of aposematic prey faster when individuals possess one of two unpalatable chemicals rather than all individuals having the same defence chemical (Skelhorn & Rowe 2005). This study demonstrates a *population* level benefit of chemical polymorphisms when both chemical morphs are equally abundant. Here, we investigate whether *individuals* with rare defence chemicals have a selective advantage within a population of defended prey to test the idea that avian cognition could select for defence chemical polymorphisms in prey.

Birds can use taste to selectively reject prey on the basis of their chemical defences (Gamberale-Stille & Guilford 2004; Skelhorn & Rowe in press), and there is some evidence from insects that predators are more wary of prey with novel toxins than with familiar ones (Pasteels & Gregoire 1984). Taken together, these observations suggest that birds might perceive and reduce their ingestion of rare chemical morphs. Therefore, we measured the post-attack survival of two chemical morphs during predator education when they occurred at different frequencies in an aposematic population. We also determined whether any change from an equal frequency of two morphs slowed avoidance learning, since avoidance rates might be determined by how quickly birds taste both chemical morphs which would occur faster when there were equal frequencies of each morph (Skelhorn & Rowe 2005). This is therefore the first experiment to test whether taste-rejection and learning by avian predators can promote and maintain chemical polymorphisms in aposematic species.

2. MATERIAL AND METHODS

Thirty mixed-sex experimental chicks (*Gallus gallus domesticus*) and 14 'buddy' chicks (see below) were hatched in the laboratory. They were kept in two cages (100×50×50 cm) at 24–25 °C, and subject to a 14L : 10D cycle under uncovered florescent lights with no U.V. component. Water was provided *ad libitum*, as were brown chick starter crumbs except during training and experimenting when food deprivation was necessary (in accordance with Home Office guidelines). After the experiment, chicks were donated to free-range farms.

Quinine and Bitrex are both bitter-tasting chemicals that are unpalatable to chicks (Skelhorn & Rowe 2005). To produce equally aversive crumbs, 150 g of brown chick starter crumbs were either sprayed with 100 ml of 2% quinine sulphate solution or with a Bitrex solution made from one drop of 2% Bitrex in 100 ml of water. Palatable crumbs were sprayed with 100 ml of water. Once dry, the Bitrex- and quinine-flavoured crumbs were sprayed red (2 ml of supercooked red food dye diluted to 90 ml with water), while the palatable crumbs were sprayed green (0.5 ml of sugarflair spruce-green food dye diluted to 90 ml with water). Crumbs were dried and sieved to a similar size.

An experimental cage was divided into two sections by placing a wire screen 25 cm from one end. The smaller section housed two buddy chicks, which had free access to food and water, and provided social contact for experimental chicks. On the first and second days post-hatch, the experimental chicks were trained to eat brown chick crumbs from the green laminated floor of the larger experimental cage section (for details see Skelhorn & Rowe 2005).

After approximately one-and-a-half hours of food deprivation on day 3, chicks were placed individually in the experimental section

Received 13 May 2005
Accepted 30 May 2005

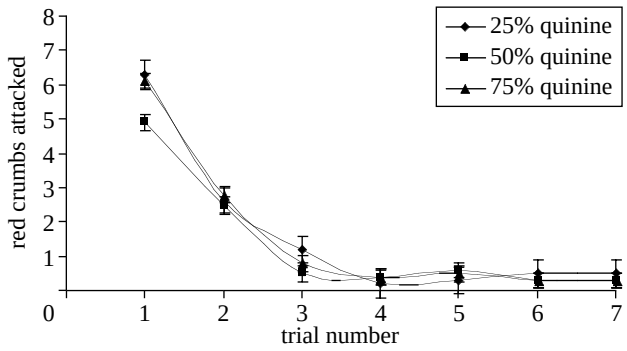

Figure 1. The mean numbers (±s.e.) of red crumbs attacked in trials for each experimental group ($n=10$ for each group).

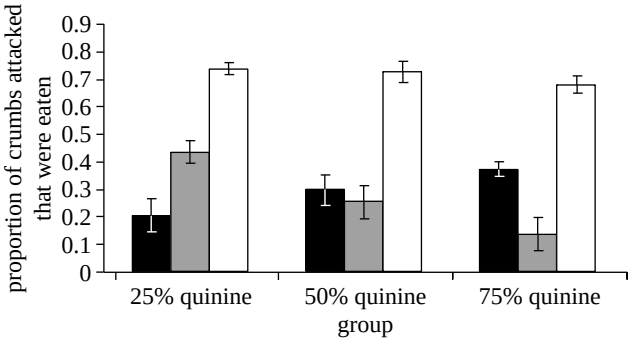

Figure 2. The mean proportion (±s.e.) of red quinine-flavored crumbs (black bars) red Bitrex-flavored crumbs (grey bars), and palatable green crumbs (open bars) that were eaten after attack by each experimental group ($n=10$ for each group).

and given 20 palatable green crumbs and 20 unpalatable red crumbs. Chicks were sexed using the colour of their down, and assigned to one of three groups (balanced for sex) which differed in the types of red crumbs that they received: the 25% quinine group received 5 quinine-flavoured crumbs and 15 Bitrex-flavoured crumbs; the 50% quinine group received 10 quinine-flavoured crumbs and 10 Bitrex-flavoured crumbs; and the 75% quinine group received 15 quinine-flavoured crumbs and 5 Bitrex-flavoured crumbs. Each crumb was randomly placed in one of 80 rectangles drawn on the floor using pre-generated maps. Chicks were allowed to attack 16 crumbs in a trial, and we recorded the number and order of each crumb attacked, and whether it was eaten or rejected. Attacked crumbs were identified by their position on the floor of the arena. All chicks received seven trials in total: two on each of days 3, 4, 5 and one on day 6.

3. RESULTS

All groups learned to avoid the unpalatable red crumbs (figure 1). Given the small number of red crumbs attacked in each trial, we compared the total numbers of each chemical morph attacked pooled across all trials. There was no evidence that birds could visually discriminate between the chemical morphs. Birds' attack rates on the quinine-flavoured crumbs did not differ from the expected value for any group: 25% quinine group (one-sample *t*-test; $t=0.22$, $p>0.05$, d.f.$=9$; mean proportion of red crumbs that were quinine-flavoured$=0.26$; s.e.$=0.040$); 50% quinine group ($t=0.74$, $p>0.05$, d.f.$=9$; mean proportion of red crumbs that were quinine-flavoured$=0.47$; s.e.$=0.049$); 75% quinine group ($t=0.64$, $p>0.05$, d.f.$=9$; mean proportion of red crumbs that were quinine-flavoured$=0.77$; s.e.$=0.029$). However, the proportions of crumbs eaten post-attack were different for each crumb type (figure 2). Unsurprisingly, chicks ate a higher proportion of the palatable green crumbs than the unpalatable red crumbs attacked (paired *t*-tests: 25% quinine group, $t=9.63$, $p<0.001$, d.f.$=9$; 50% quinine group $t=9.38$, $p<0.001$, d.f.$=9$; 75% quinine group, $t=7.40$, $p<0.001$, d.f.$=9$). Crucially, the groups differed in their consumption of the two unpalatable chemical morphs. The 25% quinine group ate a significantly higher proportion of the Bitrex-flavoured than the quinine-flavoured crumbs attacked (paired *t*-test; $t=3.05$, $p<0.05$, d.f.$=9$); the 50% quinine group ate similar proportions of the quinine-flavoured and Bitrex-flavoured crumbs attacked ($t=0.55$, $p>0.05$, d.f.$=9$); and the 75% quinine group ate a significantly higher proportion of the quinine-flavoured than the Bitrex-flavoured crumbs attacked ($t=3.64$, $p<0.01$, d.f.$=9$).

This indicates that the post-attack survival of an aposematic individual depends upon the frequency of its defence chemical in the population. We used regression analyses to investigate the effect of the percentage of quinine-flavoured crumbs on post-attack survival of each chemical morph. (Initial analyses showed that chick sex had no effect, and since sex was also balanced across groups, we did not include it as a factor in our final analyses.) The percentage of quinine-flavoured crumbs in each treatment correlated positively with the proportion of quinine crumbs eaten post-attack ($F_{1,28}=4.99$, $p<0.05$; $y=0.003x+0.14$), and correlated negatively with the proportion of Bitrex crumbs that were eaten post-attack ($F_{1,28}=21.06$, $p<0.001$; $y=-0.0069x+0.63$). Therefore, the rarer a chemical morph was in the population, the more likely it was to survive an attack.

We also compared birds' avoidance learning rates, since we expected learning rates to depend upon the relative frequencies of each chemical morph (Skelhorn & Rowe 2005). We used *a priori* contrasts within ANOVAs to test for differences between our groups in the total number of red crumbs attacked. We made two orthogonal contrasts: (i) the responses of chicks in the 25% and 75% quinine groups compared to those in the 50% quinine group, where we expected that the 50% quinine group would attack fewer red prey; (ii) the responses of chicks in the 25% group compared to those in the 75% group, where we expected no difference in the number of red prey attacked. There were significant differences amongst the groups in the total numbers of unpalatable prey attacked ($F_{2,27}=3.55$, $p<0.05$), with chicks in the 50% quinine group attacking significantly fewer red crumbs than chicks in the 25% and 75% quinine groups combined (contrast $F_{1,28}=7.07$, $p<0.05$). There was no difference in the total number of red prey attacked between the 25% and 75% quinine groups (contrast $F_{1,18}=0.19$, $p>0.05$). However, it seems likely that the difference in the total number of unpalatable prey attacked between the 50% group and the other two groups may have been caused by behaviour measured in the first trial (see figure 1).

The numbers of red crumbs attacked in the first trial differed amongst groups ($F_{2,27}=6.48$, $p<0.01$), and chicks in the 50% quinine group attacked fewer red crumbs than the 25% and 75% quinine groups combined (contrast $F_{1,28}=13.09$, $p<0.01$), but there was no significant difference between the 25% and 75% quinine groups (contrast $F_{1,18}=0.19$, $p>0.05$).

We also looked for differences in asymptotic attack rates amongst the groups (the dependent variable could not be normalized by transformation and we used non-parametric ANOVAs, i.e. Kruskal–Wallis tests). We found no evidence that the relative frequencies of each chemical morph affected asymptotic avoidance rates, and the number of red crumbs attacked in Trial 7 was the same for all groups (Kruskal–Wallis test, $\chi^2=1.11$, $p>0.05$; figure 1). The number of green crumbs attacked before a red crumb in Trial 7 (which can also be an indicator of asymptotic performance—see Skelhorn & Rowe 2005, in press) also did not differ between the groups (Kruskal–Wallis test, $\chi^2=2.19$, $p>0.05$).

4. DISCUSSION

Our experiment clearly shows that within a population of defended prey, individuals benefit from possessing a relatively rare defence chemical since they are less likely to be eaten post-attack than individuals of the more common morph. As a result, rare chemical morphs would be at a selective advantage, and increase in the population. Therefore, avian predators may select for defence chemical polymorphisms in aposematic prey. The same may be true in Müllerian mimicry, where two defended species share a warning pattern and, as a result, avian predators may select for interspecific variation in defence chemicals.

Of course, this conclusion depends upon prey being released relatively unharmed. Experiments have demonstrated that the chances of unpalatable insects surviving an attack by an avian predator can be between 80% and 100% (Wiklund & Järvi 1982; Sillén-Tullberg 1985; Marples *et al*. 1994). As a novel chemical morph spreads through a population, avian predators would reduce their attack rates on the aposematic species as a whole (Skelhorn & Rowe 2005), but more importantly, the benefit at the individual level would decrease as the frequency of the novel chemical morph increased. This could potentially result in the different chemical morphs reaching stable frequencies.

It is impossible to make strong conclusions about the effect of the frequency of chemical morphs on aversion learning. Although the 50% quinine group attacked fewer red crumbs in total than both the 25% and 75% quinine groups, this seemed to be attributable to the difference in the numbers of red crumbs attacked within the first trial. The differences in the numbers of red crumbs attacked in the first trial could indicate that the benefit of chemical morphs being equally abundant happens within the first few encounters, perhaps caused either by initial differences in learning, or by differences in attack biases elicited against warningly coloured crumbs by the different tastes (Skelhorn & Rowe 2005). However, we found no other measurable differences between our groups in learning rates or asymptotic avoidance.

Our results demonstrate that predation plays an important role in the evolution of defence chemicals. With all else being equal, rare chemical morphs will enjoy an initial selective advantage, but once their frequency increases to beyond 50%, the other morph will benefit from rarity. This may lead to a balanced polymorphism that could potentially be stabilized by reduced attack rates during predator education when morph frequencies are equal. In natural aposematic systems, whether chemical polymorphisms are stable, and at what frequency the morphs coexist, will be determined by a number of additional factors including the relative costs of chemical production and/or storage, and how aversive each chemical is.

We thank Michelle Waddle and Lin Hedgecock for looking after our animals. J.S. is supported by a School of Biology studentship, and C.R. holds a Royal Society Dorothy Hodgkin Research Fellowship. This work was supported by a BBSRC Research Grant and a Wellcome JIF Award.

Blum, M. S. 1981 *Chemical defenses of arthropods*. New York: Academic Press.

Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P. & Holland, M. M. 1984 Plant-determined variation in the cardenolide content, thin layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexipus* L. reared on milkweed plants in California. *J. Chem. Ecol.* **10**, 601–639. (doi:10.1007/BF00994224.)

Cott, H. B. 1940 *Adaptive coloration in animals*. London: Methuen.

Edmunds, M. 1974 *Defense in animals*. Harlow: Longman.

Eggenberger, F. & Rowell-Rahier, M. 1992 Genetic component of variation in chemical defense of *Oreina gloriosa* (coleopteran, chrysomelidae). *J. Chem. Ecol.* **18**, 1375–1404. (doi:10.1007/BF00994363.)

de Jong, P. W., Holloway, G. J., Brakefield, P. M. & Vos, H. 1991 Chemical defense in the ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defense in 2-spot ladybirds (*Adalia bipunctata*). *Chemoecology* **2**, 15–19. (doi:10.1007/BF01240661.)

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. & Dawkins, M. S. 1991 Receiver psychology and the evolution of animal signals. *Anim. Behav.* **42**, 1–14.

Marples, N. M., van Veelen, W. & Brakefield, P. M. 1994 The relative importance of colour, taste and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Anim. Behav.* **48**, 967–974. (doi:10.1006/anbe.1994.1322.)

Pasteels, J. M. & Gregoire, J. C. 1984 Selective predation on chemically defended Chrysomelid larvae—a conditioning process. *J. Chem. Ecol.* **10**, 1693–1700. (doi:10.1007/BF00987355.)

Pasteels, J. M., Dobler, S., Rowell-Rahier, M., Ehmke, A. & Hartmann, T. 1995 Distribution of autogenous and host-derived chemical defences in *Oreina* leaf beetles (Coleoptera: Chrysomelidae). *J. Chem. Ecol.* **21**, 1163–1179.

Sillén-Tullberg, B. 1985 Higher survival of an aposematic than of a cryptic form of distasteful bug. *Oecologia* **67**, 411–415. (doi:10.1007/BF00384948.)

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

Skelhorn, J. & Rowe, C. In press. Prey palatability influences predator learning and memory. *Anim. Behav.*

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.