Bitter taste enhances predatory biases against aggregations of prey with warning coloration

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Title: Toxin-incited biases against aggregations of prey with warning colouration

Authors: Hannah M. Rowland a, Graeme D. Ruxton b, John Skelhorn c

a. Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK
b. School of Biology, Dyers Brae, University of St Andrews, St Andrews, Fife, KY16 9TH, UK
c. Centre for Research in Animal Behaviour, College of Life and Environmental Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter EX4 4QG, UK

Corresponding Author: Hannah M. Rowland.
Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK.
hr325@cam.ac.uk
Tel: +44 (0) 1223 331 759
Fax: +44 (0) 1223 336 676

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ABSTRACT

Aposematic prey that possess chemical defences advertise these to potential predators using conspicuous warning coloration. Aposematism is often associated with group living, which is hypothesised to enhance the protection of these species. Predators exhibit unlearned biases against foods with warning coloration, and the presentation of a novel sound or bitter-tasting toxin augments these biases. Whether these non-visual signal components also cause naïve predators to more strongly avoid aggregated prey, and whether biases against aggregations are restricted to situations where aggregated prey possess visual signals typically associated with aposematism, is unknown. We conducted an experiment in which naïve domestic chicks (*Gallus gallus domesticus*) acted as predators and used artificially-colored pastry prey. The experiment had a 2 X 2 design in which naïve birds were offered a drop of either water or bitter-tasting chloroquine solution before being given the choice between solitary and aggregated prey that were either both red, a typical aposematic color, or both green (usually associated with crypsis and palatability). We found that birds were warier of red-aggregated prey and attacked significantly more solitary prey before aggregated prey compared to green. After sampling bitter-tasting chloroquine solution the birds showed a bias in their attack decisions, attacking significantly fewer aggregated prey in total compared with those who had sampled water, but only when prey were red. Thus, exposure to a bitter tasting toxin affected predatory preferences. We discuss our findings in relation to the mechanisms of bias, the benefits of group living and the evolution of warning coloration and aggregation.
KEY WORDS: aposematism; warning coloration; aggregation; chemical defence; bitter taste; domestic chick; Gallus gallus domesticus; evolution; innate bias
INTRODUCTION

Aposematism, the combination of a repellent physical or chemical defence, such as a toxin, with conspicuous coloration (Cott 1940; Poulton 1890), is taxonomically and geographically widespread (including birds, Dumbacher et al. 1993; marine animals, Edmunds 1991; insects, Schmidt 2008; and amphibia, Summers & and Clough 2001). Aposematism has been particularly well studied in the insects: the Monarch Butterfly (*Danaus plexippus* L.) sequesters toxic cardenolides from its host plant and signals its chemical defence using a highly conspicuous orange-and-black pattern (Brower et al. 1968); the Seven Spot Ladybird (*Coccinella septempunctata*) synthesizes the toxin coccinelline, and advertises using black spots on a red background (Marple, Brakefield & Cowie 1989); and the yellow-and-black stripes of the Common Wasp (*Vespula vulgaris*) are an indicator of its venomous sting (Schmidt 2008). These species have in common a tendency to live, migrate or hibernate in groups, which is hypothesised to enhance their conspicuousness and the power of their advertisements (Sillén-Tullberg & Leimar 1988).

Understanding how prey have evolved aposematic colouration and aggregative behavior that increases the likelihood of detection by predators (Ruxton & Sherratt 2006) is an intriguing question for evolutionary biologists, because attacks are likely to be costly, even if prey possess defences that increase the likelihood of survival (Higginson et al. 2011). The most widely accepted answer to this question is that naive predators are instinctively averse to the colors and patterns most commonly associated with toxicity (Mastrota & Mench 1995; Roper & Cook 1989; Schuler & Roper 1992; Schuler & Hesse 1985; Sillén-Tullberg 1985), and aggregation of aposematic prey generates unlearned aversions (Gamberale & Tullberg 1996a). Conspicuous coloration also facilitates faster avoidance learning by predators (Gittleman & Harvey 1980; Guilford 1992; Roper & Wistow 1986). Similarly, aggregation enhances the speed at which predators learn to avoid aposematic prey (Gagliardo & Guilford 1993; Riipi et al. 2001; Sillén-Tullberg 1990; Sillén-Tullberg & Leimar 1988; Tullberg, Leimar & Gamberale-Stille 2000).
Aposematic prey rarely rely on visual signals only, and make use of additional non-visual signals of their unpalatability by using for example, sounds (Brown, Boettner & Yack 2007; Haskell 1966), odours (Moore, Vance Brown & Rothschild 1990; Rothschild, Moore & Vance Brown 1984) and the secretion of bitter-tasting compounds (De Jong et al. 1991). These are thought to act as ‘go-slow’ signals that cause predators to reduce their attack rates on prey that are more likely to be defended (Guilford 1994). In line with this theory, it has been found consistently that the presentation of a novel sound, odour or bitter-tasting compound causes naïve predators to increase their bias against novel foods or foods with visual traits typically associated with aposematism, such as conspicuousness or a red or yellow color (Jetz, Rowe & Guilford 2001; Lindström, Rowe & Guilford 2001; Marples & Roper 1996; Rowe & Guilford 1996, 1999a, b; Rowe & Skelhorn 2005; Siddall & Marples 2008, 2011; Skelhorn, Griksaitis & Rowe 2008). It is not currently known whether these additional signal components also cause naïve predators to bias their foraging preferences against aggregated prey, and, if so, whether biases against aggregations are restricted to situations where aggregated prey possess visual signals typically associated with aposematism. To answer this question, we examined the foraging behavior of naïve domestic chicks (*Gallus gallus domesticus*), which served as visually hunting predators. We produced two ‘species’ of prey which differed in color signal. Prey were either red (a color typically associated with insect warning patterns) or were green (a color associated with palatable cryptic prey). We gave separate groups of predators a choice between aggregated and solitary prey that were either all red or all green. Chicks are known to use taste cues in conjunction with visual signals to make foraging decisions (Rowe & Skelhorn 2005; Skelhorn, Griksaitis & Rowe 2008). We therefore used a well-established system of presenting a taste cue prior to a prey preference test, to determine whether sampling a bitter-tasting toxin prior to meeting the prey caused birds to bias their attacks away from aggregated prey and towards solitary prey; and whether any bias to avoid aggregations was present both when prey were red and when they were green (see Rowe & Skelhorn 2005; Skelhorn, Griksaitis & Rowe 2008;
Skelhorn 2011). This simulates a real ecological scenario that can occur in many predator-prey
interactions. Within a given prey species, both chemical defence and colouration commonly vary
ontogenetically, with aposematism being more common in later life-history stages and crucially
often occurring later in development than the expression of significant chemical defences (e.g.
panic moth caterpillars, *Saucrobotys futilalis*; Grant 2007), giving an ecologically commonplace
pathway for predators to experience aversive tastes of cryptic prey before exposure to visual
aposematic signals. Another example occurs in phase-changing desert locust (*Schistocerca gregaria*)
which can occur in three different defence conditions: at low densities they exist in a solitary
phase where individuals are palatable and have a cryptic green colour (Sword et al. 2000;
Despland & Simpson 2005); higher densities trigger changes in behaviour in terms of attraction
to conspecifics and host plant preferences leading to an aggregation stage where cryptic
unpalatable animals also sequester bitter-tasting alkaloids (Despland & Simpson 2005); at the
next moult after the density-driven behavioural changes colour change occurs from green to a
conspicuous yellow and black appearance, giving an aggregation stage with aposematic
colouration and chemical defence. Thus during a build-up of these insects over a season
predators could experience unpalatable tastes separately from, and before encountering, an
aposematic visual signal.

We predicted that aggregation would increase the efficacy of red, but not green
coloration and thus chicks in the red groups would be less willing to attack aggregated prey, and
have a lower preference for aggregated prey, than chicks given green prey. Furthermore, we
predicted that chloroquine would enhance biases against red, but not green-colored,
aggregations. We therefore expected chicks in the chloroquine and red prey group to be less
willing to attack aggregated prey, and express a lower preference for aggregated prey, than chicks
in the water and red prey group, whilst we expected to see no differences between chicks given
green prey.
METHODS

Subjects and housing

Fifty-two male chicks (Hyline strain) were obtained from a commercial hatchery on the day of hatching. Chicks were housed at the University of Glasgow, in white metal cages measuring 120 cm x 50 cm x 50 cm. Two cages housed the experimental chicks (n=20 per cage), and a third housed the buddy chicks (n=12; buddy chicks serve as visual companions to the experimental chicks during the foraging experiment, thereby reducing any potential distress from placing experimental chicks in the arena alone. Buddy chicks never acted as experimental chicks and never had access to artificial prey). Each cage was heated to 27°C, following guidelines to the operation of the Animal (Scientific Procedures) Act 1986 (UK 2009), using either one Interbrooda standard (40 cm x 60 cm) or two Interbrooda minis (40 cm x 40 cm) (http://www.alphahatch.com/interbrooda-mini-ah630450-104-p.asp). These brooders, also known as ‘electric hens’, consist of an electrically heated square or rectangular plate which stands on four adjustable legs, which enables the adjustment of height and temperature as the chicks grow. The laboratory was held at a constant temperature of 24 degrees. Temperatures were monitored and recorded daily. Water was provided *ad libitum* in two one litre jam-jar drinkers. Brown chick starter crumbs were also provided *ad libitum* in two ceramic food bowls that contained a clear plastic cylinder, which reduced the tendency of the chicks to sit in the food. The cages were lined with brown paper cage liners, which were replaced daily. During training and experimentation, periods of food deprivation were necessary to promote motivation to forage. During all periods of deprivation, chicks had access to water but not food. All deprivation periods were in accordance with UK Home Office regulations and guidelines and were no longer than 1.5 hours.

Chicks were subject to a 14:10h light:dark cycle and the lighting had no UV component. All subjects were marked with identifying color codes on the top of their heads using non-toxic
Sharpie™ marker pens. Markings did not result in aggressive behavior between individuals.

Weights were monitored for welfare purposes throughout the experiment, with all experimental chicks gaining as much weight as buddy chicks (who experienced fewer periods of food deprivation) as the experiment progressed. The experiment was conducted following guidelines to the operation of the Animal (Scientific Procedures) Act 1986 (UK 2009). The nature of the study meant we did not require a Home Office Licence (chicks had free food choice, solutions were offered to the chicks, and deprivation periods less than 1.5 hours). At the end of the experiment all chicks were euthanized following UK Home Office “schedule one” methods (in this case we employed cervical dislocation).

Artificial prey

Pastry was produced by mixing flour and lard in a 3:1 ratio, into which was mixed 75ml of water with either 1ml of green food dye (Sugarflair™ spruce green) or 2ml of red food dye (Dr. Oetker™). The pastry was moulded into worms measuring 10mm x 5mm.

Pre training

On arrival at the laboratory chicks were allowed to acclimatise for three hours, after which food was removed from all of the cages for one hour. After one hour of food deprivation, chicks commenced pre-training which is used to familiarise them with the arena, and to foraging alone. Without such training, chicks placed in the arena alone become distressed, they call loudly and do not eat.

Pre-training was conducted in three experimental cages simultaneously. These cages were identical to the home cages, except that there was a mesh divider separating a buddy arena, measuring 20cm x 50cm x 50cm, from an experimental arena of 100cm x 50cm x 50cm (see Skelhorn & Rowe 2006 for a schematic). The floor was covered with the backing paper of sticky-
backed plastic (a waxy paper imprinted with a faint red grid whose intersections were at 2.5 cm intervals). All chicks were given six four minute pre-training sessions, during which they were presented with brown chick starter crumbs scattered on the floor of the experimental arena. In trials one and two, chicks were placed in the experimental arena in groups of three; in trials three and four, chicks were placed in the arena in pairs. In trials five and six, lone chicks were placed in the arena. All training was completed in the presence of two buddies. By the end of pre-training, all chicks were eating brown starter crumbs from the arena without any signs of distress.

Preference test

Prey presentation

We used a prey presentation method previously employed by Gamberale and Tullberg (1996a) and Skelhorn and Ruxton (2006). We taped the up-turned lid of a Petri dish (3 cm diameter) on top of the base of the dish to create a two-tiered presentation device, so that chicks could only sample prey placed in the lid and not in the base. This permitted the creation of either solitary or visually-aggregated prey, whilst controlling olfactory cues and the number of prey that could be attacked. For solitary treatments one pastry worm was presented in the lid of the dish with none in the base; for aggregated treatments, one pastry worm was presented in the lid and seven pastry worms in the base. This allowed us to create four categories of prey: aposematic and solitary (1 red worm on top, none below); aposematic visually-aggregated prey (1 red worm on top, 7 red below); non-apeosmatic solitary (1 green worm on top, none-below); non-apeosmatic visually-aggregated (1 green worm on top, 7 green below).

Experimental procedure

On day two, experimental chicks were food deprived (but had water ad libitum) for approximately 90 minutes prior to engaging in the task, to promote motivation to forage. Buddy chicks had free
access to food and water in their home cage, but only access to water during the task. Buddy chicks were used on a rotational basis, and changed every three trials, or after one hour, whichever came first.

An experimental chick was chosen at random after the deprivation period and allocated to receive a drop of either 0.4% chloroquine phosphate solution (Chloroquine Group) or distilled water (Control Group) from a 20-100 ml micropipette. Previous work suggests that domestic chicks find this concentration of chloroquine phosphate solution aversive, and that this method of tastant delivery has exactly the same effect as allowing predators to sample toxic prey prior to a choice test (Rowe and Skelhorn 2005). The benefit of using a solution over experience of a toxic prey is that the possibility of generalisation of color signals of the toxic prey to the test prey is reduced to virtually zero.

Directly before being placed into the experimental cage, we offered each chick the allocated taste solution from the end of micropipette. If chicks did not drink this drop then the solution was dropped on the tip of the beak, which they could shake and wipe off if they wanted. All chicks consumed some of the solution. The experimental chick was then immediately placed in the experimental arena. Two buddy chicks occupied the buddy arena of the same cage. Inside the experimental arena, the experimental chick encountered 24 Petri dishes (3cm diameter), 12 of which contained solitary prey and the other 12, visually-aggregated prey. For half of the chicks, the prey were all red; for the remaining chicks, the prey were all green.

Chicks were required to attack (peck or eat) 12 of the 24 available prey before being removed from the arena. All chicks attacked 12 prey items. The order of attacks was recorded.

STATISTICAL ANALYSES

We calculated the number of solitary prey attacked before the first aggregated prey as a measure of wariness in the chicks. This data was positively skewed and included zero-counts. We
therefore tested whether a GLM with a standard negative binomial regression model provided a
better fit than a zero-inflated model using a Vuong test in R (UCLA: Academic Technology
Services). The Vuong test showed that a zero-inflated model did not provide a significant
improvement ($P = 0.144$), so we tested our predictions that (i) red colouration would increase
wariness towards aggregations, so that chicks given red prey would attack significantly more
solitary prey before attacking an aggregated prey than chicks given green prey; (ii) that
chloroquine would enhance wariness against red-aggregated prey more than water and (iii) that
there would be no difference in wariness towards green-aggregated and green-solitary prey after
experience of chloroquine or water. We tested this using a standard negative binomial regression
model with the two predictor variables of colour and solution type and the interaction between
the two in R (version 2.14; 2012).

We calculated the total number of aggregated prey attacked by the chicks as a measure of
preference. The data satisfied the requirements for parametric statistics. With three degrees of
freedom amongst our four experimental groups, we used orthogonal contrasts to test our \textit{apriori}
predictions (following Ruxton and Beauchamp 2008) within GLM ANOVA with the two
predictor variables of colour and solution type and the interaction between the two using R. By
only testing the comparisons of interest, we simplify our analyses and reduce the risk of type I
errors (Ruxton and Beauchamp 2008). We tested the predictions that (i) aggregation would
increase the efficacy of the red signal, so that chicks in the red group would have a lower
preference for aggregated prey than chicks in the green group, we compared [water and red prey
+ chloroquine and red prey] versus [water and green prey + chloroquine and green prey]; (ii)
that chloroquine would enhance biases against red-aggregated prey, we compared water and red-aggregated prey versus chloroquine and red-aggregated prey; and (iii) that chloroquine would not
enhance biases against green-aggregated prey, we compared water and green-aggregated prey
versus chloroquine and green-aggregated prey.
RESULTS

There was a significant interaction between the colour of the prey and the solution type offered to the chicks on the number of solitary prey attacked before an aggregated prey – from hereon termed ‘wariness’ (likelihood ratio test: $\chi^2_{1} = 40.71, P = 0.014$; Figure 1). Chicks given red prey attacked significantly more solitary prey before attacking an aggregated prey than chicks given green prey ($z = -2.031, P = 0.042$). However, chicks’ wariness of aggregated prey did not differ as a function of solution type when prey were red, ($z = 1.701, P = 0.089$). In line with our predictions, when prey were green there was no significant difference in wariness of aggregated prey as a function of solution type ($z = -1.659, P = 0.097$).

As predicted, chicks given red prey attacked significantly fewer aggregated prey than chicks given green prey ($t = -7.368, P < 0.001$). Furthermore, when prey were red, chicks attacked significantly fewer aggregated prey after sampling chloroquine than after sampling water ($t = 2.558, P = 0.020$); when prey were green, there was no significant difference in the number of aggregated prey attacked as a function of solution type ($t = -0.624, P = 0.540$). This suggests that birds showed a bias against red aggregations, and chloroquine enhanced biases against red, but not green aggregations. However, the interaction between the colour of the prey and the solution type offered on the total number of aggregated prey attacked by the chicks was non-significant ($F_{1,40} = 2.831, P = 0.09$).

Avian predators have been shown to possess unlearned aversions to particular colors and patterns associated with warning signals. We measured whether chicks in each experimental group had preferences for solitary or aggregated prey by comparing the numbers of chicks in each group that attacked more aggregated than solitary prey with the number of chicks that attacked more solitary than aggregated prey and analysed this data with a binomial test. We found that chicks given red prey showed a significant preference for solitary prey over
aggregated prey both when given water (binomial test; \( P = 0.002, n = 10 \)) and when given chloroquine (binomial test; \( P = 0.002, n = 10 \)) prior to the preference test. However, birds given green prey showed no significant preference for either solitary or aggregated prey (binomial test; water group, \( P = 0.754, n=10 \); chloroquine group, \( P = 0.344, n=10 \)).

DISCUSSION

The main finding from our experiment was that aggregation of prey was more effective at deterring predation when prey were a colour typically associated with aposematism (red) than when they were green (usually associated with crypsis and palatability), and that experience of a bitter-tasting toxin caused naïve predators to more strongly avoid red-aggregated prey but not green-aggregated prey. This is the first evidence that a non-visual component of prey’s defence (a bitter taste) causes biases against aggregations, and that this is restricted to situations where aggregated prey possess visual signals typically associated with aposematism.

Our findings are consistent with those of Gamberale and Tullberg (1998), who demonstrated that the probability of naïve domestic chicks attacking live larvae of the aposematic bug *Tropidothorax leucopterus* decreased with increasing prey group size, whereas the probability of chicks attacking larvae of the non-aposematic bug *Graptostethus servus* was unaffected by group size. Because we controlled for factors other than color (e.g. shape and movement) that may have differed between the two species used in Gamberale and Tullberg’s experiment, our findings represent stronger support for the conclusion that patterns of preference were due to naïve predators being disinclined to attack aggregations of prey with an aposematic visual trait but not prey that have a color typically associated with palatability (first hypothesised by Poulton 1890, and subsequently by Beddard 1895; Cott 1940; and Edmunds 1974).
It is clear that aggregation itself was not the important stimulus promoting avoidance, since aggregated and solitary green prey were attacked at similar rates (see also Gamberale & Tullberg 1998). It is therefore reasonable to conclude that aggregation enhances the repellence of prey visual signals if these signals are of a color associated with aposematism. We know that such coloration is more effective at prompting avoidance learning when the prey’s body size or color patch is larger (Gamberale & Tullberg 1996b, 1998; Lindström et al. 1999; Mand, Tammaru & Mappes 2007), and, in a similar fashion, aggregation might increase the salience of a color signal. Alternatively, aggregation may simply increase the conspicuousness of the signal (again by increasing its size): a factor known to generate unconditioned aversions in birds (Gamberale-Stille 2000; Gamberale & Tullberg 1996a; Remmel & Tammaru 2011).

Aversions towards prey that possess colors typically associated with insect warning patterns have previously been reported in a variety of avian species (Caldwell 1983; Mastrota & Mench 1995; Roper & Cook 1989; Schuler & Hesse 1985; Sillén-Tullberg 1985), but the results of studies assessing color aversions are not always consistent (Roper & and Marples 1997; Roper & Wistow 1986). Furthermore, the idea that predators possess unconditioned aversions to aggregations of aposematic prey has received mixed support (Gamberale & Tullberg 1996a, 1998; Sillén-Tullberg 1990). Whilst this may be due to the fact that some species possess only weak aposematic signals that are insufficient to generate unlearned aversions (Lindstedt et al. 2011), an alternate explanation is that predators may sample potentially valuable novel prey items when their expectation of risk of the prey being defended is perceived to be low, but avoid novel or brightly colored prey items when the risk of their being toxic is perceived to be high (Gamberale-Stille & Tullberg 2001; Rowe & Guilford 1999b). This appears to be the case when predators experience a bitter tasting toxin prior to encountering brightly colored prey for the first time, as in our experiment.
Our study is the first to show that bitter taste enhances biases against attacking prey that are aggregated in favour of solitary prey when these possess coloration typically associated with aposematism (red), but not with crypsis and palatability (green). Our finding are consistent with other studies that have found additional signal components such as a novel sound, odour, bitter-tasting toxin or a conspecific’s disgust response, can increase bias against single food items with visual traits typically associated with aposematism, including conspicuousness or a red or yellow color (Jetz et al. 2001; Lindström et al. 2001; Marples & Roper 1996; Rowe & Guilford 1996, 1999b; Rowe & Skelhorn 2005; Siddall & Marples 2011; Skelhorn et al. 2008; Skelhorn 2011). Because the interaction term (colour x solution type) in our main ANOVA for the number of aggregated prey attacked (Fig. 2) was non-significant, we must treat our conclusions with some caution. However, we note that the contrasts within ANOVA (a more appropriate form of analysis: see Ruxton and Beauchamp 2008) confirmed our a priori predictions, and therefore we feel justified to discuss the effects of taste on unconditioned colour biases.

A likely mechanism that would explain the avoidance of novel and brightly colored aggregated prey items is that bitter taste increases a predators’ perceived risk of prey being toxic by inducing an aversive state that results in altered perception of and responses to stimuli in other modalities (for example see Nitschke 2006). Peyrot des Gachons et al (2011) found that a bitter tasting stimulus induces nausea in human subjects up to 30 minutes after exposure, showing that the body not only detects potential toxins but anticipates and prevents their ingestion by inducing a prophylactic aversive state. Previous work suggests that domestic chicks find the concentration of chloroquine phosphate solution we used aversive (Rowe and Skelhorn 2005), and that at high doses chloroquine is emetic (Alcock 1970). There is a link therefore, between the experience of a bitter tasting compound in the chick’s mouth and exhibiting an aversive state.
These taste-related attack biases against red aggregations were only evident when preferences were measured by the total number of each prey attacked in the whole trial, and were not evident in chicks’ wariness, as measured by the number of solitary prey attacked before an aggregated prey. There are several potential explanations for this, and understanding differences between wariness and longer-term foraging preferences could help us to understand the mechanisms underlying ‘unlearned’ color biases. The difference could simply be due to sampling noise. Alternatively, another explanation is that our measure of wariness is subject to an increased level of variance in the red and chloroquine group: six out of ten chicks attacked an average of 0.83 (+/- 0.98 s.d) solitary prey before an aggregated, and four of the ten chicks attacked 9.5 (+/- 1.00 s.d) solitary prey before an aggregated prey. This heavily influences the variance of our measurements of wariness, but has less effect on measures of overall preference. This difference could be a result of cognitive or perceptual differences in the predators. For example, birds may not use rules like ‘avoid scary prey’, but instead ‘sample scary prey less often’ or ‘eat smaller meals when faced with scary prey’. Alternatively, this difference in predator behavior could also be explained by individual differences in the birds’ perception of the bitter taste which affects their expectation of risk and expression of wariness (see Davis et al 2010 who suggest that polymorphisms exist in bitter taste receptor genes of white-throated sparrows, which could result in differences in perception and behaviour). Finally, the number of aggregated/solitary prey attacked across the entire trial may not be a measure of preference per se, but may instead reflect differences in the way that birds learn about different types of prey. We know that predators learn more quickly to avoid aggregations of aposematic prey than solitary aposematic prey (Gagliardo & Guilford 1993; Gamberale & Tullberg 1996a, 1998; Tullberg, Gamberale-Stille & Solbreck 2000), but we do not know how aggregation influences the way in which birds learn to associate visual signals with rewards. If aggregation only facilitates aversion learning then it is possible that it also makes it more difficult for birds to learn to associate aposematic aggregations with positive experiences. Therefore, our measure of preference could
simply reflect the fact that birds are learning to associate solitary prey with food rewards more quickly than aggregated aposematic prey.

Irrespective of the exact mechanisms via which color and aggregation influence prey selection, our data suggest an alternative route for the evolution of aggregation behavior. The experience of bitter-tasting toxins of both aggregated green mutants and their solitary conspecifics (and potentially even the toxins of visually distinct aposematic prey) could cause predators to become more wary of other aggregated aposematic mutants, thus allowing them to reproduce and spread through the population. Alternatively, biases against aposematic aggregations may have evolved in response to the presence of aggregated aposematic prey. If this is the case, enhanced biases may not have influenced the initial evolution of aggregation behavior. However, they could certainly increase the benefit of aggregation in existing systems, which could potentially make it more evolutionarily stable amongst aposematic species.

In conclusion, we have shown that aggregation enhances chicks’ foraging biases against prey with coloration that is typically associated with aposematism (red), but not with crypsis and edibility (green), and that sampling a bitter-tasting toxin enhances this bias further by altering expectation of risk in some individuals. Our findings help to explain why the evolution of aposematic coloration may facilitate the evolution of aggregation behavior, and the evolution of complex aposematic signals involving multiple sensory modalities and associated behavioral traits.

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In: ScotPIL manual - avian species.
Figure 1. The mean number (+/- 1SE) of solitary prey attacked before aggregated prey by birds in each of our four experimental groups (n=10 chicks for each group).

Figure 2. The mean number (+/- 1SE) of aggregated prey attacked by birds in each of our four experimental groups (n=10 chicks for each group).
The mean number (+/- 1SE) of solitary prey attacked before aggregated prey by birds in each of our four experimental groups (n=10 for each group).

131x105mm (300 x 300 DPI)
The mean number (+/- 1SE) of aggregated prey attacked by birds in each of our four experimental groups (n=10 for each group).

131x105mm (300 x 300 DPI)
Predators who experience a bitter tasting toxin more strongly avoid groups of warningly-coloured prey than groups of prey with a colour typically associated with palatability. Birds are known to use taste cues in conjunction with visual signals to make foraging decisions. But this is the first evidence that a bitter taste causes biases against aggregations, and that this is restricted to situations where aggregated prey possess visual signals typically associated with aposematism.