



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

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

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16 RUNNING HEADER: Bitter taste and prey selection

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3 18 TITLE: **Bitter taste enhances predatory biases against aggregations of prey with warning**  
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5 19 **coloration**  
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11 21 ABSTRACT  
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14 22 Aposematic prey that possess chemical defences advertise these to potential predators using  
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16 23 conspicuous warning coloration. Aposematism is often associated with group living, which is  
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18 24 hypothesised to enhance the protection of these species. Predators exhibit unlearned biases  
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20 25 against foods with warning coloration, and the presentation of a novel sound or bitter-tasting  
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22 26 toxin augments these biases. Whether these non-visual signal components also cause naïve  
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24 27 predators to more strongly avoid aggregated prey, and whether biases against aggregations are  
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26 28 restricted to situations where aggregated prey possess visual signals typically associated with  
27  
28 29 aposematism, is unknown. We conducted an experiment in which naïve domestic chicks (*Gallus*  
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30 30 *gallus domesticus*) acted as predators and used artificially-colored pastry prey. The experiment had a  
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32 31 2 X 2 design in which naïve birds were offered a drop of either water or bitter-tasting  
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34 32 chloroquine solution before being given the choice between solitary and aggregated prey that  
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36 33 were either both red, a typical aposematic color, or both green (usually associated with crypsis  
37  
38 34 and palatability). We found that birds were warier of red-aggregated prey and attacked  
39  
40 35 significantly more solitary prey before aggregated prey compared to green. After sampling bitter-  
41  
42 36 tasting chloroquine solution the birds showed a bias in their attack decisions, attacking  
43  
44 37 significantly fewer aggregated prey in total compared with those who had sampled water, but  
45  
46 38 only when prey were red. Thus, exposure to a bitter tasting toxin affected predatory preferences.  
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48 39 We discuss our findings in relation to the mechanisms of bias, the benefits of group living and  
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50 40 the evolution of warning coloration and aggregation.  
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42 KEY WORDS: aposematism; warning coloration; aggregation; chemical defence; bitter  
43 taste; domestic chick; *Gallus gallus domesticus*; evolution; innate bias

For Review Only

## 44 INTRODUCTION

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

57       Understanding how prey have evolved aposematic colouration and aggregative behavior  
58 that increases the likelihood of detection by predators (Ruxton & Sherratt 2006) is an intriguing  
59 question for evolutionary biologists, because attacks are likely to be costly, even if prey possess  
60 defences that increase the likelihood of survival (Higginson et al. 2011). The most widely  
61 accepted answer to this question is that naive predators are instinctively averse to the colors and  
62 patterns most commonly associated with toxicity (Mastrota & Mench 1995; Roper & Cook 1989;  
63 Schuler & Roper 1992; Schuler & Hesse 1985; Sillén-Tullberg 1985), and aggregation of  
64 aposematic prey generates unlearned aversions (Gamberale & Tullberg 1996a). Conspicuous  
65 coloration also facilitates faster avoidance learning by predators (Gittleman & Harvey 1980;  
66 Guilford 1992; Roper & Wistow 1986). Similarly, aggregation enhances the speed at which  
67 predators learn to avoid aposematic prey (Gagliardo & Guilford 1993; Riipi et al. 2001; Sillén-  
68 Tullberg 1990; Sillén-Tullberg & Leimar 1988; Tullberg, Leimar & Gamberale-Stille 2000).

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3 69 Aposematic prey rarely rely on visual signals only , and make use of additional non-visual  
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5 70 signals of their unpalatability by using for example, sounds (Brown, Boettner & Yack 2007;  
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7 71 Haskell 1966), odours (Moore, Vance Brown & Rothschild 1990; Rothschild, Moore & Vance  
8  
9 72 Brown 1984) and the secretion of bitter-tasting compounds (De Jong et al. 1991). These are  
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11 73 thought to act as ‘go-slow’ signals that cause predators to reduce their attack rates on prey that  
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13 74 are more likely to be defended (Guilford 1994). In line with this theory, it has been found  
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15 75 consistently that the presentation of a novel sound, odour or bitter-tasting compound causes  
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17 76 naïve predators to increase their bias against novel foods or foods with visual traits typically  
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19 77 associated with aposematism, such as conspicuousness or a red or yellow color (Jetz, Rowe &  
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21 78 Guilford 2001; Lindström, Rowe & Guilford 2001; Marples & Roper 1996; Rowe & Guilford  
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23 79 1996, 1999a, b; Rowe & Skelhorn 2005; Siddall & Marples 2008, 2011; Skelhorn, Griksaitis &  
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25 80 Rowe 2008). It is not currently known whether these additional signal components also cause  
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27 81 naïve predators to bias their foraging preferences against aggregated prey, and, if so, whether  
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29 82 biases against aggregations are restricted to situations where aggregated prey possess visual  
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31 83 signals typically associated with aposematism. To answer this question, we examined the foraging  
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33 84 behavior of naïve domestic chicks (*Gallus gallus domesticus*), which served as visually hunting  
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35 85 predators. We produced two ‘species’ of prey which differed in color signal. Prey were either red  
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37 86 (a color typically associated with insect warning patterns) or were green (a color associated with  
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39 87 palatable cryptic prey). We gave separate groups of predators a choice between aggregated and  
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41 88 solitary prey that were either all red or all green. Chicks are known to use taste cues in  
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43 89 conjunction with visual signals to make foraging decisions (Rowe & Skelhorn 2005; Skelhorn,  
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45 90 Griksaitis & Rowe 2008). We therefore used a well-established system of presenting a taste cue  
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47 91 prior to a prey preference test, to determine whether sampling a bitter-tasting toxin prior to  
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49 92 meeting the prey caused birds to bias their attacks away from aggregated prey and towards  
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51 93 solitary prey; and whether any bias to avoid aggregations was present both when prey were red  
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53 94 and when they were green (see Rowe & Skelhorn 2005; Skelhorn, Griksaitis & Rowe 2008;  
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3 95 Skelhorn 2011). This simulates a real ecological scenario that can occur in many predator-prey  
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5 96 interactions. Within a given prey species, both chemical defence and colouration commonly vary  
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7 97 ontogenetically, with aposematism being more common in later life-history stages and crucially  
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9 98 often occurring later in development than the expression of significant chemical defences (e.g.  
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11 99 panic moth caterpillars, *Saucrobotys futilalis*; Grant 2007), giving an ecologically commonplace  
12  
13 100 pathway for predators to experience aversive tastes of cryptic prey before exposure to visual  
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15 101 aposematic signals. Another example occurs in phase-changing desert locust (*Schistocera gregaria*)  
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17 102 which can occur in three different defence conditions: at low densities they exist in a solitary  
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19 103 phase where individuals are palatable and have a cryptic green colour (Sword et al. 2000;  
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21 104 Despland & Simpson 2005); higher densities trigger changes in behaviour in terms of attraction  
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23 105 to conspecifics and host plant preferences leading to an aggregation stage where cryptic  
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25 106 unpalatable animals also sequester bitter-tasting alkaloids (Despland & Simpson 2005); at the  
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27 107 next moult after the density-driven behavioural changes colour change occurs from green to a  
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29 108 conspicuous yellow and black appearance, giving an aggregation stage with aposematic  
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31 109 colouration and chemical defence. Thus during a build-up of these insects over a season  
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33 110 predators could experience unpalatable tastes separately from, and before encountering, an  
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35 111 aposematic visual signal.  
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40 112 We predicted that aggregation would increase the efficacy of red, but not green  
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42 113 coloration and thus chicks in the red groups would be less willing to attack aggregated prey, and  
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44 114 have a lower preference for aggregated prey, than chicks given green prey. Furthermore, we  
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46 115 predicted that chloroquine would enhance biases against red, but not green-colored,  
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48 116 aggregations. We therefore expected chicks in the chloroquine and red prey group to be less  
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50 117 willing to attack aggregated prey, and express a lower preference for aggregated prey, than chicks  
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52 118 in the water and red prey group, whilst we expected to see no differences between chicks given  
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54 119 green prey.  
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121 **METHODS**

## 122 Subjects and housing

123 Fifty-two male chicks (Hyline strain) were obtained from a commercial hatchery on the day of  
124 hatching. Chicks were housed at the University of Glasgow, in white metal cages measuring  
125 120cm x 50cm x 50cm. Two cages housed the experimental chicks (n=20 per cage), and a third  
126 housed the buddy chicks (n=12; buddy chicks serve as visual companions to the experimental  
127 chicks during the foraging experiment, thereby reducing any potential distress from placing  
128 experimental chicks in the arena alone. Buddy chicks never acted as experimental chicks and  
129 never had access to artificial prey). Each cage was heated to 27°C, following guidelines to the  
130 operation of the Animal (Scientific Procedures) Act 1986 (UK 2009), using either one  
131 Interbrooda standard (40cm x 60cm) or two Interbrooda minis (40cm x 40cm)  
132 (<http://www.alphahatch.com/interbrooda-mini-ah630450-104-p.asp>). These brooders, also  
133 known as ‘electric hens’, consist of an electrically heated square or rectangular plate which stands  
134 on four adjustable legs, which enables the adjustment of height and temperature as the chicks  
135 grow. The laboratory was held at a constant temperature of 24 degrees. Temperatures were  
136 monitored and recorded daily. Water was provided *ad libitum* in two one litre jam-jar drinkers.  
137 Brown chick starter crumbs were also provided *ad libitum* in two ceramic food bowls that  
138 contained a clear plastic cylinder, which reduced the tendency of the chicks to sit in the food.  
139 The cages were lined with brown paper cage liners, which were replaced daily. During training  
140 and experimentation, periods of food deprivation were necessary to promote motivation to  
141 forage. During all periods of deprivation, chicks had access to water but not food. All  
142 deprivation periods were in accordance with UK Home Office regulations and guidelines and  
143 were no longer than 1.5 hours.

144 Chicks were subject to a 14:10h light:dark cycle and the lighting had no UV component.

145 All subjects were marked with identifying color codes on the top of their heads using non-toxic



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3 146 Sharpie™ marker pens. Markings did not result in aggressive behavior between individuals.  
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5 147 Weights were monitored for welfare purposes throughout the experiment, with all experimental  
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7 148 chicks gaining as much weight as buddy chicks (who experienced fewer periods of food  
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9 149 deprivation) as the experiment progressed. The experiment was conducted following guidelines  
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11 150 to the operation of the Animal (Scientific Procedures) Act 1986 (UK 2009). The nature of the  
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13 151 study meant we did not require a Home Office Licence (chicks had free food choice, solutions  
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15 152 were offered to the chicks, and deprivation periods less than 1.5 hours). At the end of the  
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17 153 experiment all chicks were euthanized following UK Home Office “schedule one” methods (in  
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19 154 this case we employed cervical dislocation).  
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#### 25 156 Artificial prey

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27 157 Pastry was produced by mixing flour and lard in a 3:1 ratio, into which was mixed 75ml of water  
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29 158 with either 1ml of green food dye (Sugarflair™ spruce green) or 2ml of red food dye (Dr.  
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31 159 Oetker™). The pastry was moulded into worms measuring 10mm x 5mm.  
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#### 36 37 161 Pre training

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39 162 On arrival at the laboratory chicks were allowed to acclimatise for three hours, after which food  
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41 163 was removed from all of the cages for one hour. After one hour of food deprivation, chicks  
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43 164 commenced pre-training which is used to familiarise them with the arena, and to foraging alone.  
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45 165 Without such training, chicks placed in the arena alone become distressed, they call loudly and  
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47 166 do not eat.  
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51 167 Pre-training was conducted in three experimental cages simultaneously. These cages were  
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53 168 identical to the home cages, except that there was a mesh divider separating a buddy arena,  
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55 169 measuring 20cm x 50cm x 50cm, from an experimental arena of 100cm x 50cm x 50cm (see  
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57 170 Skelhorn & Rowe 2006 for a schematic). The floor was covered with the backing paper of sticky-

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3 171 backed plastic (a waxy paper imprinted with a faint red grid whose intersections were at 2.5cm  
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5 172 intervals). All chicks were given six four minute pre-training sessions, during which they were  
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7 173 presented with brown chick starter crumbs scattered on the floor of the experimental arena. In  
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9 174 trials one and two, chicks were placed in the experimental arena in groups of three; in trials three  
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11 175 and four, chicks were placed in the arena in pairs. In trials five and six, lone chicks were placed in  
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13 176 the arena. All training was completed in the presence of two buddies. By the end of pre-training,  
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15 177 all chicks were eating brown starter crumbs from the arena without any signs of distress.  
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20 179 Preference test

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23 180 Prey presentation

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25 181 We used a prey presentation method previously employed by Gamberale and Tullberg (1996a)  
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27 182 and Skelhorn and Ruxton (2006). We taped the up-turned lid of a Petri dish (3 cm diameter) on  
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29 183 top of the base of the dish to create a two-tiered presentation device, so that chicks could only  
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31 184 sample prey placed in the lid and not in the base. This permitted the creation of either solitary or  
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33 185 visually-aggregated prey, whilst controlling olfactory cues and the number of prey that could be  
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35 186 attacked. For solitary treatments one pastry worm was presented in the lid of the dish with none  
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37 187 in the base; for aggregated treatments, one pastry worm was presented in the lid and seven pastry  
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39 188 worms in the base. This allowed us to create four categories of prey: aposematic and solitary (1  
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41 189 red worm on top, none below); aposematic visually-aggregated prey (1 red worm on top, 7 red  
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43 190 below); non aposematic solitary (1 green worm on top, none-below); non-aposematic visually-  
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45 191 aggregated (1 green worm on top, 7 green below).  
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52 193 Experimental procedure

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54 194 On day two, experimental chicks were food deprived (but had water *ad libitum*) for approximately  
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56 195 90 minutes prior to engaging in the task, to promote motivation to forage. Buddy chicks had free  
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3 196 access to food and water in their home cage, but only access to water during the task. Buddy  
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5 197 chicks were used on a rotational basis, and changed every three trials, or after one hour,  
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7 198 whichever came first.

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10 199 An experimental chick was chosen at random after the deprivation period and allocated  
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12 200 to receive a drop of either 0.4% chloroquine phosphate solution (Chloroquine Group) or  
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14 201 distilled water (Control Group) from a 20-100 ml micropipette. Previous work suggests that  
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16 202 domestic chicks find this concentration of chloroquine phosphate solution aversive, and that this  
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18 203 method of tastant delivery has exactly the same effect as allowing predators to sample toxic prey  
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20 204 prior to a choice test (Rowe and Skelhorn 2005). The benefit of using a solution over experience  
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22 205 of a toxic prey is that the possibility of generalisation of color signals of the toxic prey to the test  
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24 206 prey is reduced to virtually zero.

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27 207 Directly before being placed into the experimental cage, we offered each chick the  
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29 208 allocated taste solution from the end of micropipette. If chicks did not drink this drop then the  
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31 209 solution was dropped on the tip of the beak, which they could shake and wipe off if they wanted.  
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33 210 All chicks consumed some of the solution. The experimental chick was then immediately placed  
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35 211 in the experimental arena. Two buddy chicks occupied the buddy arena of the same cage. Inside  
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37 212 the experimental arena, the experimental chick encountered 24 Petri dishes (3cm diameter), 12 of  
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39 213 which contained solitary prey and the other 12, visually-aggregated prey. For half of the chicks,  
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41 214 the prey were all red; for the remaining chicks, the prey were all green.

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44 215 Chicks were required to attack (peck or eat) 12 of the 24 available prey before being  
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46 216 removed from the arena. All chicks attacked 12 prey items. The order of attacks was recorded.

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## 49 50 51 218 STATISTICAL ANALYSES

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54 219 We calculated the number of solitary prey attacked before the first aggregated prey as a measure  
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56 220 of wariness in the chicks. This data was positively skewed and included zero-counts. We

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3 221 therefore tested whether a GLM with a standard negative binomial regression model provided a  
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5 222 better fit than a zero-inflated model using a Vuong test in R (UCLA: Academic Technology  
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7 223 Services). The Vuong test showed that a zero-inflated model did not provide a significant  
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9 224 improvement ( $P = 0.144$ ), so we tested our predictions that (i) red colouration would increase  
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11 225 wariness towards aggregations, so that chicks given red prey would attack significantly more  
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13 226 solitary prey before attacking an aggregated prey than chicks given green prey; (ii) that  
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15 227 chloroquine would enhance wariness against red-aggregated prey more than water and (iii) that  
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17 228 there would be no difference in wariness towards green-aggregated and green-solitary prey after  
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19 229 experience of chloroquine or water. We tested this using a standard negative binomial regression  
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21 230 model with the two predictor variables of colour and solution type and the interaction between  
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23 231 the two in R (version 2.14; 2012).

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27 232 We calculated the total number of aggregated prey attacked by the chicks as a measure of  
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29 233 preference. The data satisfied the requirements for parametric statistics. With three degrees of  
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31 234 freedom amongst our four experimental groups, we used orthogonal contrasts to test our *a priori*  
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33 235 predictions (following Ruxton and Beauchamp 2008) within GLM ANOVA with the two  
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35 236 predictor variables of colour and solution type and the interaction between the two using R. By  
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37 237 only testing the comparisons of interest, we simplify our analyses and reduce the risk of type I  
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39 238 errors (Ruxton and Beauchamp 2008). We tested the predictions that (i) aggregation would  
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41 239 increase the efficacy of the red signal, so that chicks in the red group would have a lower  
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43 240 preference for aggregated prey than chicks in the green group, we compared [water and red prey  
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45 241 + chloroquine and red prey] versus [water and green prey + chloroquine and green prey]; (ii)  
46  
47 242 that chloroquine would enhance biases against red-aggregated prey, we compared water and red-  
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49 243 aggregated prey versus chloroquine and red-aggregated prey; and (iii) that chloroquine would not  
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51 244 enhance biases against green-aggregated prey, we compared water and green-aggregated prey  
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56 245 versus chloroquine and green-aggregated prey.

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## 6 247 RESULTS

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8 248 There was a significant interaction between the colour of the prey and the solution type offered  
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10 249 to the chicks on the number of solitary prey attacked before an aggregated prey – from hereon  
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12 250 termed ‘wariness’ (likelihood ratio test:  $\chi^2_1 = 40.71$ ,  $P = 0.014$ ; Figure 1). Chicks given red prey  
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14 251 attacked significantly more solitary prey before attacking an aggregated prey than chicks given  
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16 252 green prey ( $z = -2.031$ ,  $P = 0.042$ ). However, chicks’ wariness of aggregated prey did not differ  
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18 253 as a function of solution type when prey were red, ( $z = 1.701$ ,  $P = 0.089$ ). In line with our  
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20 254 predictions, when prey were green there was no significant difference in wariness of aggregated  
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22 255 prey as a function of solution type ( $z = -1.659$ ,  $P = 0.097$ ).  
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27 256 As predicted, chicks given red prey attacked significantly fewer aggregated prey than chicks  
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29 257 given green prey ( $t = -7.368$ ,  $P < 0.001$ ). Furthermore, when prey were red, chicks attacked  
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31 258 significantly fewer aggregated prey after sampling chloroquine than after sampling water ( $t =$   
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33 259  $2.558$ ,  $P = 0.020$ ); when prey were green, there was no significant difference in the number of  
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35 260 aggregated prey attacked as a function of solution type ( $t = -0.624$ ,  $P = 0.540$ ). This suggests that  
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37 261 birds showed a bias against red aggregations, and chloroquine enhanced biases against red, but  
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39 262 not green aggregations. However, the interaction between the colour of the prey and the solution  
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41 263 type offered on the total number of aggregated prey attacked by the chicks was non-significant  
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43 264 ( $F_{1,40} = 2.831$ ,  $P = 0.09$ ).  
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47 265 Avian predators have been shown to possess unlearned aversions to particular colors and  
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49 266 patterns associated with warning signals. We measured whether chicks in each experimental  
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51 267 group had preferences for solitary or aggregated prey by comparing the numbers of chicks in  
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53 268 each group that attacked more aggregated than solitary prey with the number of chicks that  
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55 269 attacked more solitary than aggregated prey and analysed this data with a binomial test. We  
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57 270 found that chicks given red prey showed a significant preference for solitary prey over  
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3 271 aggregated prey both when given water (binomial test;  $P = 0.002$ ,  $n = 10$ ) and when given  
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5 272 chloroquine (binomial test;  $P = 0.002$ ,  $n = 10$ ) prior to the preference test. However, birds given  
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7 273 green prey showed no significant preference for either solitary or aggregated prey (binomial test;  
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9 274 water group,  $P = 0.754$ ,  $n=10$ ; chloroquine group,  $P = 0.344$ ,  $n=10$ ).

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15 276 DISCUSSION

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18 277 The main finding from our experiment was that aggregation of prey was more effective at  
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20 278 deterring predation when prey were a colour typically associated with aposematism (red) than  
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22 279 when they were green (usually associated with crypsis and palatability), and that experience of a  
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24 280 bitter-tasting toxin caused naïve predators to more strongly avoid red-aggregated prey but not  
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26 281 green-aggregated prey. This is the first evidence that a non-visual component of prey's defence (a  
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28 282 bitter taste) causes biases against aggregations, and that this is restricted to situations where  
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30 283 aggregated prey possess visual signals typically associated with aposematism.

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34 284 Our findings are consistent with those of Gamberale and Tullberg (1998), who  
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36 285 demonstrated that the probability of naïve domestic chicks attacking live larvae of the  
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38 286 aposematic bug *Tropidothorax leucopterus* decreased with increasing prey group size, whereas the  
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40 287 probability of chicks attacking larvae of the non-aposematic bug *Graptostethus servus* was  
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42 288 unaffected by group size. Because we controlled for factors other than color (e.g. shape and  
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44 289 movement) that may have differed between the two species used in Gamberale and Tullberg's  
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46 290 experiment, our findings represent stronger support for the conclusion that patterns of  
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48 291 preference were due to naïve predators being disinclined to attack aggregations of prey with an  
49  
50 292 aposematic visual trait but not prey that have a color typically associated with palatability (first  
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52 293 hypothesised by Poulton 1890, and subsequently by Beddard 1895; Cott 1940; and Edmunds  
53  
54 294 1974).

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3 295 It is clear that aggregation itself was not the important stimulus promoting avoidance,  
4  
5 296 since aggregated and solitary green prey were attacked at similar rates (see also Gamberale &  
6  
7 297 Tullberg 1998). It is therefore reasonable to conclude that aggregation enhances the repellence of  
8  
9 298 prey visual signals if these signals are of a color associated with aposematism. We know that such  
10  
11 299 coloration is more effective at prompting avoidance learning when the prey's body size or color  
12  
13 300 patch is larger (Gamberale & Tullberg 1996b, 1998; Lindström et al. 1999; Mand, Tammaru &  
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15 301 Mappes 2007), and, in a similar fashion, aggregation might increase the salience of a color signal.  
16  
17 302 Alternatively, aggregation may simply increase the conspicuousness of the signal (again by  
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19 303 increasing its size): a factor known to generate unconditioned aversions in birds (Gamberale-  
20  
21 304 Stille 2000; Gamberale & Tullberg 1996a; Rimmel & Tammaru 2011).

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24  
25 305 Aversions towards prey that possess colors typically associated with insect warning  
26  
27 306 patterns have previously been reported in a variety of avian species (Caldwell 1983; Mastrotta &  
28  
29 307 Mench 1995; Roper & Cook 1989; Schuler & Hesse 1985; Sillén-Tullberg 1985), but the results  
30  
31 308 of studies assessing color aversions are not always consistent (Roper & and Marples 1997; Roper  
32  
33 309 & Wistow 1986). Furthermore, the idea that predators possess unconditioned aversions to  
34  
35 310 aggregations of aposematic prey has received mixed support (Gamberale & Tullberg 1996a,  
36  
37 311 1998; Sillén-Tullberg 1990). Whilst this may be due to the fact that some species possess only  
38  
39 312 weak aposematic signals that are insufficient to generate unlearned aversions (Lindstedt et al.  
40  
41 313 2011), an alternate explanation is that predators may sample potentially valuable novel prey items  
42  
43 314 when their expectation of risk of the prey being defended is perceived to be low, but avoid novel  
44  
45 315 or brightly colored prey items when the risk of their being toxic is perceived to be high  
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47 316 (Gamberale-Stille & Tullberg 2001; Rowe & Guilford 1999b). This appears to be the case when  
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49 317 predators experience a bitter tasting toxin prior to encountering brightly colored prey for the first  
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51 318 time, as in our experiment.  
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3 319 Our study is the first to show that bitter taste enhances biases against attacking prey that  
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5 320 are aggregated in favour of solitary prey when these possess coloration typically associated with  
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7 321 aposematism (red), but not with crypsis and palatability (green). Our findings are consistent with  
8  
9 322 other studies that have found additional signal components such as a novel sound, odour, bitter-  
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11 323 tasting toxin or a conspecific's disgust response, can increase bias against single food items with  
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13 324 visual traits typically associated with aposematism, including conspicuousness or a red or yellow  
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15 325 color (Jetz et al. 2001; Lindström et al. 2001; Marples & Roper 1996; Rowe & Guilford 1996,  
16  
17 326 1999b; Rowe & Skelhorn 2005; Siddall & Marples 2011; Skelhorn et al. 2008; Skelhorn 2011).  
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19 327 Because the interaction term (colour x solution type) in our main ANOVA for the number of  
20  
21 328 aggregated prey attacked (Fig. 2) was non-significant, we must treat our conclusions with some  
22  
23 329 caution. However, we note that the contrasts within ANOVA (a more appropriate form of  
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25 330 analysis: see Ruxton and Beauchamp 2008) confirmed our *a priori* predictions, and therefore we  
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27 331 feel justified to discuss the effects of taste on unconditioned colour biases.  
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31  
32 332 A likely mechanism that would explain the avoidance of novel and brightly colored  
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34 333 aggregated prey items is that bitter taste increases a predator's perceived risk of prey being toxic  
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36 334 by inducing an aversive state that results in altered perception of and responses to stimuli in  
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38 335 other modalities (for example see Nitschke 2006). Peyrot des Gachons et al (2011) found that a  
39  
40 336 bitter tasting stimulus induces nausea in human subjects up to 30 minutes after exposure,  
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42 337 showing that the body not only detects potential toxins but anticipates and prevents their  
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44 338 ingestion by inducing a prophylactic aversive state. Previous work suggests that domestic chicks  
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46 339 find the concentration of chloroquine phosphate solution we used aversive (Rowe and Skelhorn  
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48 340 2005), and that at high doses chloroquine is emetic (Alcock 1970). There is a link therefore,  
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50 341 between the experience of a bitter tasting compound in the chick's mouth and exhibiting an  
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52 342 aversive state.  
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3 343 These taste-related attack biases against red aggregations were only evident when  
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5 344 preferences were measured by the total number of each prey attacked in the whole trial, and were  
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7 345 not evident in chicks' wariness, as measured by the number of solitary prey attacked before an  
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9 346 aggregated prey. There are several potential explanations for this, and understanding differences  
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11 347 between wariness and longer-term foraging preferences could help us to understand the  
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13 348 mechanisms underlying 'unlearned' color biases. The difference could simply be due to sampling  
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15 349 noise. Alternatively, another explanation is that our measure of wariness is subject to an  
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17 350 increased level of variance in the red and chloroquine group: six out of ten chicks attacked an  
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19 351 average of 0.83 (+/- 0.98 s.d) solitary prey before an aggregated, and four of the ten chicks  
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21 352 attacked 9.5 (+/- 1.00 s.d) solitary prey before an aggregated prey. This heavily influences the  
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23 353 variance of our measurements of wariness, but has less effect on measures of overall preference.  
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25 354 This difference could be a result of cognitive or perceptual differences in the predators. For  
26  
27 355 example, birds may not use rules like 'avoid scary prey', but instead 'sample scary prey less often'  
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29 356 or 'eat smaller meals when faced with scary prey'. Alternatively, this difference in predator  
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31 357 behavior could also be explained by individual differences in the birds' perception of the bitter  
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33 358 taste which affects their expectation of risk and expression of wariness (see Davis et al 2010 who  
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35 359 suggest that polymorphisms exist in bitter taste receptor genes of white-throated sparrows,  
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37 360 which could result in differences in perception and behaviour). Finally, the number of  
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39 361 aggregated/solitary prey attacked across the entire trial may not be a measure of preference *per se*,  
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41 362 but may instead reflect differences in the way that birds learn about different types of prey. We  
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43 363 know that predators learn more quickly to avoid aggregations of aposematic prey than solitary  
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45 364 aposematic prey (Gagliardo & Guilford 1993; Gamberale & Tullberg 1996a, 1998; Tullberg,  
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47 365 Gamberale-Stille & Solbreck 2000), but we do not know how aggregation influences the way in  
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49 366 which birds learn to associate visual signals with rewards. If aggregation only facilitates aversion  
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51 367 learning then it is possible that it also makes it more difficult for birds to learn to associate  
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53 368 aposematic aggregations with positive experiences. Therefore, our measure of preference could  
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3 369 simply reflect the fact that birds are learning to associate solitary prey with food rewards more  
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5 370 quickly than aggregated aposematic prey.  
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8 371 Irrespective of the exact mechanisms via which color and aggregation influence prey  
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10 372 selection, our data suggest an alternative route for the evolution of aggregation behavior. The  
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12 373 experience of bitter-tasting toxins of both aggregated green mutants and their solitary  
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14 374 conspecifics (and potentially even the toxins of visually distinct aposematic prey) could cause  
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16 375 predators to become more wary of other aggregated aposematic mutants, thus allowing them to  
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18 376 reproduce and spread through the population. Alternatively, biases against aposematic  
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20 377 aggregations may have evolved in response to the presence of aggregated aposematic prey. If this  
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22 378 is the case, enhanced biases may not have influenced the initial evolution of aggregation  
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24 379 behavior. However, they could certainly increase the benefit of aggregation in existing systems,  
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26 380 which could potentially make it more evolutionarily stable amongst aposematic species.  
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30 381 In conclusion, we have shown that aggregation enhances chicks' foraging biases against  
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32 382 prey with coloration that is typically associated with aposematism (red), but not with crypsis and  
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34 383 edibility (green), and that sampling a bitter-tasting toxin enhances this bias further by altering  
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36 384 expectation of risk in some individuals. Our findings help to explain why the evolution of  
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38 385 aposematic coloration may facilitate the evolution of aggregation behavior, and the evolution of  
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40 386 complex aposematic signals involving multiple sensory modalities and associated behavioral  
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42 387 traits.  
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55 392 Cambridge.  
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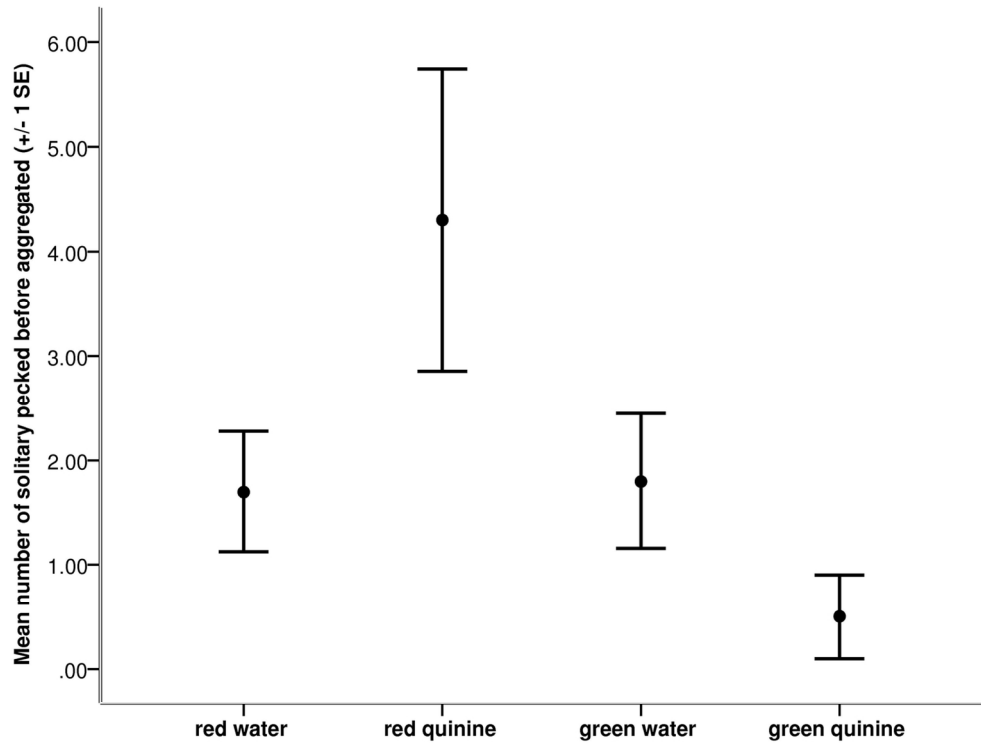


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3 529 Figure 1. The mean number ( $\pm$  1SE) of solitary prey attacked before aggregated prey by birds  
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5 530 in each of our four experimental groups (n=10 chicks for each group).  
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8 531 Figure 2. The mean number ( $\pm$  1SE) of aggregated prey attacked by birds in each of our four  
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10 532 experimental groups (n=10 chicks for each group).  
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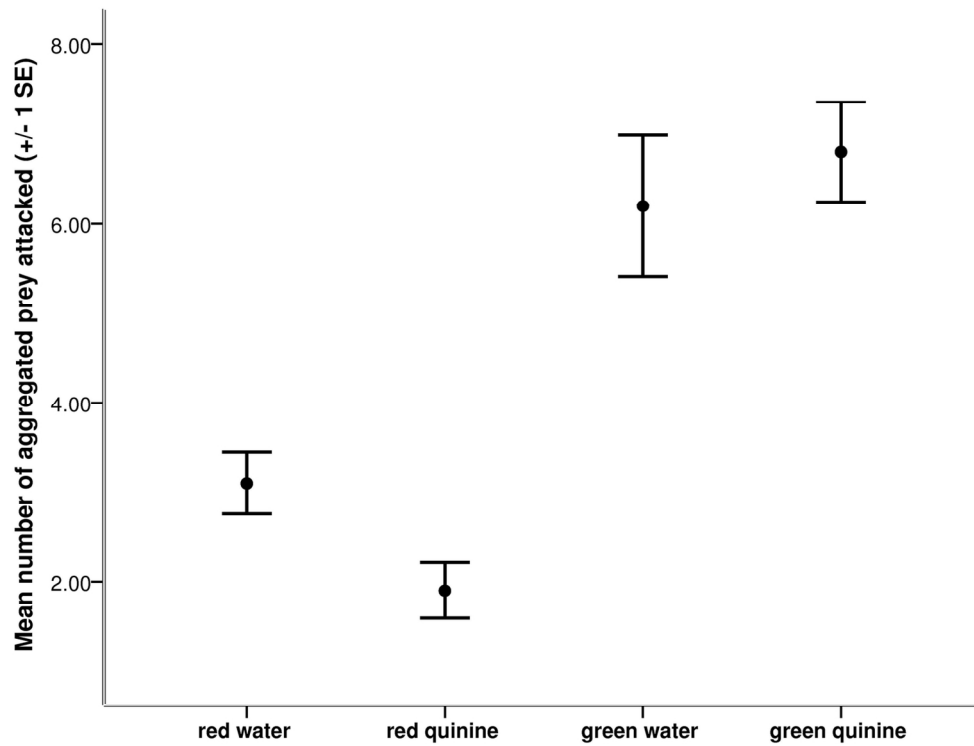
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The mean number (+/- 1SE) of solitary prey attacked before aggregated prey by birds in each of our four experimental groups (n=10 for each group).  
131x105mm (300 x 300 DPI)

Only



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)

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3 Predators who experience a bitter tasting toxin more strongly avoid groups of warningly-  
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5 coloured prey than groups of prey with a colour typically associated with palatability. Birds are  
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7 known to use taste cues in conjunction with visual signals to make foraging decisions. But this is  
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9 the first evidence that a bitter taste causes biases against aggregations, and that this is restricted  
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11 to situations where aggregated prey possess visual signals typically associated with aposematism.  
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For Review Only