Avian predators attack aposematic prey more forcefully when they are part of an aggregation

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Defended insects often advertise their unprofitability to potential predators using conspicuous aposematic coloration. Many aposematic insects are also gregarious, and it has been suggested that the aggregation of defended prey may have facilitated the evolution of aposematic coloration. Empirical studies have demonstrated that birds are more wary of aggregated aposematic prey, and learn to avoid them more quickly than solitary prey. However, many aposematic insects survive being attacked by birds, and the effect of aggregation on post-attack survival has not previously been investigated. Using domestic chicks as predators and artificially manipulated mealworms as prey, we provide empirical evidence that predators attack aggregated aposematic prey more forcefully than solitary prey, reducing the likelihood of prey surviving an attack. Hence, we suggest that previous works concluding that aggregation was an important pre-requisite for the evolution of aposematism may have overestimated the fitness benefits of aggregation, since aggregated prey may be attacked less but are also less likely to survive an attack.

Keywords: aposematism; receiver psychology; insects

1. INTRODUCTION

From Wallace & Darwin to the present-day, understanding the evolution of conspicuous signals that warn predators of a defended prey item (aposematic signals) has been an important challenge to evolutionary theory (Darwin 1887; Wallace 1889; Ruxton *et al.* 2004). The dominant present explanations consider aggregation of defended prey to be an important precursor to the evolution of aposematism (Fisher 1930; Harvey *et al.* 1982; Leimar *et al.* 1986), and this has been supported by empirical studies demonstrating that attack rates on novel aposematic individuals can be lower if these individuals are aggregated (Gagliardo & Guilford 1993; Gamberale & Tullberg 1998; Rippi *et al.* 2001). However, there is evidence that defended prey often survive attacks by predators (e.g. Wiklund & Jarvi 1982), and previous studies have not generally considered the effect of aggregation on this chance of survival. This is perhaps surprising because experimental evidence suggests that the

visual signals of a defended prey may well influence the likelihood of it surviving a predatory attack (Sillén-Tullberg 1985).

One study investigating the influence of prey aggregation on the likelihood of being attacked suggested that predators may attack aggregated prey more forcefully than a single prey. However, this was assumed to be an artefact of the experimental design, since only fewer predators were prepared to attack aggregated prey, and these were assumed to be the individuals that were inherently more forceful (Gamberale & Tullberg 1996). Our aim was to perform an experiment that would allow us to quantify any change in forcefulness of attack as a function of prey aggregation, while controlling the potential confounding effect of innately aggressive individuals being more prepared to attack aggregations.

2. MATERIAL AND METHODS

Forty-two domestic chicks (*Gallus gallus domesticus*) of mixed sex were hatched in the laboratory, and housed in two pens. One pen (measuring 160×100 cm) housed the experimental chicks and another (measuring 120×80 cm) housed the buddy chicks (see below). All chicks were subjected to a 14L:10D cycle using uncovered fluorescent lights with no UV component, and temperatures were maintained at 24–25°C. Water was provided *ad libitum*, as were brown chick starter crumbs, except during training and experimenting when food deprivation was necessary. All deprivation periods were in accordance with UK Home Office regulations.

Aversive aposematic worms were produced by painting mealworms with a solution of 3 ml of 0.01% denatonium benzoate (Bitrex: a non-toxic aversive agent added, as an ingestion deterrent, to products that are poisonous to humans) solution mixed with 10 ml of *Ocaldo* ready mixed non-toxic red poster paint (red was chosen as it is a colour typically used in warning displays). Artificial prey items were produced by placing two Petri dishes (5 cm in diameter) on top of each other and taping them together. For solitary prey treatments, a single mealworm (approx. 15 mm in length) was placed in the top Petri dish, and for aggregated prey treatments, seven mealworms were placed in the bottom Petri dish and one in the top dish. This method has been used by previous workers (Gamberale & Tullberg 1996) to standardize both olfactory cues between the two treatments, and the number of prey items that can be taken.

The experimental arena was a cage measuring 100×50×50 cm, with a section measuring 25×50×50 cm partitioned off with mesh to create a separate 'buddy arena'. In all training and experimental trials, two chicks were placed in the buddy arena to reduce any potential distress from placing experimental chicks alone in the arena. These buddy chicks were selected from a stock of six individuals, and were changed every three trials. Buddy chicks had free access to food and water throughout the experiment, but were not given access to mealworms.

On days 1–3 post-hatch, chicks were trained to first eat brown chick starter crumbs, and then to eat unpainted palatable artificial prey items, from the white floor of the experimental arena (see Appendix A). On day 4 post-hatch, chicks were assigned to one of two groups. Each chick was then given a single experimental trial on days 4 and 5. After one and a half hours of food deprivation, all chicks were placed in the experimental arena individually. Chicks in one group were presented with a solitary prey item on day 4, and with an aggregation of prey on day 5. Chicks in the other group were presented with an aggregation of prey on day 4, and with a solitary prey item on day 5. All prey items were placed in the centre of the arena. Chicks were allowed to attack the prey for 5 min before being returned to their home cage. The time taken to attack the worms, the amount of damage the chick inflicted upon the worms, the number of times the chick pecked the worms, whether or not the chicks carried the worms more than 10 cm from the Petri dish before dropping them (birds either attacked and ate the worms at the side of the dish or ran some distance before beginning to handle the worm. These behaviours were obviously very different, and 10 cm was chosen as an empirical way of distinguishing between these two behaviours) and whether or not the chicks struck the worms on the floor of the experimental arena were all recorded.

Figure 1. The mean latency, in seconds ($\pm$ s.e.), for birds to attack both aggregated and solitary prey. Chicks that attacked both prey types are shown to the left of the dotted line and chicks that attacked only the solitary prey are shown to the right of the dotted line. The results are shown for chicks presented with (a) aggregated prey first and (b) solitary prey first.

3. RESULTS

Since the aim of our experiment was to quantify any change in aggressiveness of attack as a function of prey aggregation, while controlling the potentially confounding effect of innately aggressive individuals being more prepared to attack aggregations, we only included the data of chicks that attacked both prey types in our analysis: 11 out of the 18 chicks that were offered the aggregated treatment first, and 14 out of the 18 chicks that were offered the solitary treatment first. In addition, since only the data from the first trial is truly independent, we restricted the analysis to the data collected in this trial.

All birds dropped the unpalatable worms upon attack, meaning no coloured mealworms were actually eaten. In line with several previous works, birds presented with aggregated prey showed a greater latency to attack the focal worm than birds presented with solitary prey (t -test: $t=2.73$, d.f. = 23, $p=0.012$; figure 1). No bird in the aggregated treatment attempted to attack a non-focal worm at any point during the experiment. This indicates that birds were more reluctant to attack the aggregated prey than the solitary prey.

In order to determine whether the likelihood of surviving a predatory attack differed between aggregated and solitary prey, we compared the number of solitary and aggregated prey killed in the first trial, again only using data from birds that attacked worms in both trials. We found that aggregated prey were significantly more likely to be killed by a predatory attack than solitary prey (Fisher's exact test: $p=0.017$; figure 2), indicating that there may be survival costs, as well as benefits, associated with aggregation.

Figure 2. The number of each prey type attacked that survived (open bars) or were killed (filled bars) by chicks that attacked both prey types (to the left of the dotted line) and by those that attacked only the solitary prey (to the right of the dotted line). The results are shown for chicks presented with (a) aggregated prey first and (b) solitary prey first.

Birds also appeared to differ in the way they attacked solitary and aggregated prey, so we compared the behaviour of chicks that attacked solitary prey in trial 1 with the behaviour of chicks that attacked aggregated prey in trial 1, again only using data from birds that attacked worms in both trials. We found that birds were more likely to grab aggregated worms and run to a distance before rejecting them than they were with solitary worms (Fisher's exact test; $p=0.017$). However, birds did not differ in the number of times that they pecked aggregated and solitary worms (t -test: $t=0.90$, d.f. = 23, $p=0.38$), or in the likelihood of striking the worms on the floor of the experimental arena (Fisher's exact test: $p=0.133$).

4. DISCUSSION

Our results clearly demonstrated that aggregation has a strong influence on the outcome of attacks, with an attack on an aggregation being much more likely to end in the death of the prey. However, it is unclear why birds attacked aggregated worms more forcefully than solitary worms. One potential explanation is that birds attacked aggregated prey more forcefully in an attempt to peck the worms in the lower Petri dish. However, birds' first pecks were always successfully directed at the focal worm, and no bird attempted to return to the aggregated prey to attack a second worm. Alternatively, differences in the behaviour of mealworms between the treatments may have caused chicks to attack aggregated worms more aggressively.

Again, this seems unlikely because all worms appeared to behave in a similar manner: they moved to the edge of the Petri dish and remained motionless. Therefore, it seems unlikely that our findings were an artefact of experimental design.

Interestingly, fear and aggression seem to be closely linked: humans faced with a potentially dangerous task react with reluctance to initiate, but if they do initiate, then they do so with high levels of aggression and vigour (Potegal & Knutson 1994). The birds in our experiment were much more hesitant to approach the aggregated prey than the solitary prey, indicating that they were more afraid of them, and, as a result, may have approached their foraging task with higher levels of aggression.

Irrespective of the exact mechanisms, birds were more likely to kill aggregated prey than solitary prey, indicating that there is a previously neglected cost involved in being part of an aggregation. Whether this cost is large enough to overcome many benefits of being part of an aggregation (Gagliardo & Guilford 1993; Rippi *et al.* 2001) is questionable, but our results suggest that the benefit of being part of an aggregation has been overestimated, and that mathematical models of the evolution of aposematism should take post-attack foraging decisions into account.

Birds presented with aggregated prey were more likely to grab the prey and run to a distance before rejecting it than those presented with single prey. Although there is a possibility that this behaviour is limited to domestic chicks, if this behaviour is seen in natural situations, it may benefit birds by allowing them to avoid the defences of other individuals in the aggregation.

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APPENDIX A: DETAILS OF PRE-TRAINING

On day 1 post-hatch, chicks were trained to eat brown chick starter crumbs from the white floor of the experimental arena. Chicks were given two 5 min trials in which they were presented with brown chick starter crumbs scattered on the white floor of the experimental arena. In the first trial, chicks were placed in the experimental arena in groups of three, and in the second trial, chicks were placed in the arena in pairs. On day 2 post-hatch, chicks were given a further two training trials. In the first trial, chicks were placed in the arena individually, where they were again presented with brown chick starter crumbs scattered on the floor of the arena. In the second trial on day 2, and in two further trials on day 3, chicks were trained to eat palatable artificial prey items in the experimental arena. They were food deprived for approximately one and a half hours before each of the three training trials. In each of these trials, chicks

were placed in the arena individually for 5 min, where they were presented with one solitary palatable prey and one aggregation of palatable prey. The Petri dishes containing the experimental prey were placed at 20 cm from the mesh that divided the experimental and buddy arenas, and the dishes were placed 20 cm apart. In each trial, the dish containing the solitary prey item was placed on the left-hand side of the arena for half of the chicks and on the right-hand side for the other half. No chick received two consecutive trials in which the dish containing the solitary worm was placed on the same side of the experimental arena. Every chick ate all of the worms presented in the training phase of the experiment.

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