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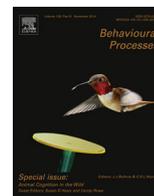
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Body size matters for aposematic prey during predator aversion learning



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ABSTRACT

Aposematic prey advertise their toxicity to predators using conspicuous warning signals, which predators learn to use to reduce their intake of toxic prey. Like other types of prey, aposematic prey often differ in body size, both within and between species. Increasing body size can increase signal size, which make larger aposematic prey more detectable but also gives them a more effective and salient deterrent. However, increasing body size also increases the nutritional value of prey, and larger aposematic prey may make a more profitable meal to predators that are trading off the costs of eating toxins with the benefits of ingesting nutrients. We tested if body size, independent of signal size, affected predation of toxic prey as predators learn to reduce their attacks on them. European starlings (*Sturnus vulgaris*) learned to discriminate between defended (quinine-injected) and undefended (water-injected) mealworm prey (*Tenebrio molitor*) using visual signals. During this process, we found that birds attacked and ate more defended prey the larger they were. Body size does affect the probability that toxic prey are attacked and eaten, which has implications for the evolutionary dynamics of aposematism and mimicry (where species share the same warning pattern).

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1. Introduction

Aposematic insects advertise their defensive toxins to predators using a variety of conspicuous warning signals (Poulton, 1890; Rowe & Guilford, 2001; Mappes et al., 2005; Rowe & Halpin, 2013). Visually hunting predators, including many birds and insect species, learn to associate a conspicuous visual signal with toxicity and the probability that they will attack an aposematic prey declines with repeated encounters (e.g. Gittleman & Harvey, 1980; Riipi et al., 2001; Prudic et al., 2007). The speed with which predators make this association affects how many aposematic prey are killed during this process: the quicker learning is, the fewer individuals are killed. Consequently, how naïve predators learn to avoid toxic prey is important for theories aimed at understanding the evolutionary dynamics of aposematism and mimicry (e.g. Müller, 1879; Brower et al., 1970; Speed, 1993; Yachi & Higashi, 1998; Servidio, 2000; Speed, 2001).

There are two intrinsic properties of aposematic prey that are thought to be crucial in determining the speed of learning and

impact on the overall mortality of prey during the learning process. The first is how toxic the prey are to their predators: for example, predators learn to reduce their attack rates more quickly when prey contain a higher concentration of toxin (Skelhorn & Rowe, 2006a) or multiple defence chemicals (Skelhorn & Rowe, 2005). The second is the salience of the signal, and how much a signal 'stands out' in cognitive terms to a predator (Guilford, 1990; Mappes et al., 2005; Rowe & Halpin, 2013). Factors that could increase signal salience include how novel it is or the degree with which it contrasts with the background (Gittleman & Harvey, 1980; Roper & Redston, 1987), its discriminability from other environmental signals (Sherratt & Beatty, 2003), or the size of the signal itself (Lindstedt et al., 2008). However, birds also learn about the nutritional qualities as well as the toxin content of aposematic prey, and are more likely to include toxic prey in their diets when they are nutritionally enriched (Halpin et al., 2014). Currently we do not know whether the nutrient content of aposematic prey affects mortality during the learning process.

One reason why it is important to know this is to better understand the selection pressures acting on optimal body size in aposematic prey. Body size is an important life history trait, and is related to fecundity and survival in many species (Honěk, 1993). Aposematic prey differ in body size, both within and between species (Cohen, 1984; Brower & Calvert, 1985; de Jong et al., 1991), and consequently there is a growing interest in the interaction

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between body size and predator avoidance behaviour. First, having a larger body could enable aposematic prey to have a larger signal, which has associated costs and benefits. Increasing the size of a conspicuous signal will increase the risk that prey are detected and attacked (Forsman & Merilaita, 1999; Mänd et al., 2007; Sandre et al., 2007; Lindstedt et al., 2008; Remmel & Tammaru, 2009). However, naïve predators can also exhibit intrinsic avoidance of larger conspicuous prey, or show enhanced avoidance learning because a larger signal is more salient (Gamberale and Tullberg, 1998; Lindstedt et al., 2008). Second, having a larger body could enable aposematic prey to be more toxic: there is a tendency for body size and toxin content to be positively correlated both within (Holloway et al., 1993) and across species (Hagman & Forsman, 2003; Phillips & Shine, 2006). Therefore, in terms of mortality during the learning process, a larger body is predicted to be beneficial against predators as both signal size and toxin content can be larger. However, this prediction ignores associated changes in nutritional content. Body size also correlates with nutritional quality (e.g. Wiegert, 1965; Barnard & Brown, 1981; Barnard & Stephens, 1981; Finke, 2002; Lease & Wolf, 2011), and larger bodied prey will be more nutritionally profitable to predators. This could lead to more attacks by predators, counter-acting any benefits associated with having more toxin or a larger signal.

Previous studies of how predators learn to avoid aposematic prey have tightly controlled the size and nutrient content of toxic prey to ensure that it does not affect how predators learn the association between a colour signal and prey toxicity (e.g. Gittleman & Harvey, 1980; Alatalo & Mappes, 1996; Ihalainen et al., 2007; Halpin et al., 2008a; Barnett et al., 2012). In this experiment however, we specifically manipulated body size *per se* to test if it could influence the mortality of aposematic prey during the avoidance learning process. We used an established laboratory system where European starlings (*Sturnus vulgaris*) make foraging decisions on individually presented mealworms (*Tenebrio molitor*). This empirical system allowed us to carefully control and manipulate the size, toxin content and colour signals of each prey presented (e.g. Skelhorn & Rowe, 2006b; Barnett et al., 2007; Halpin et al., 2013). Birds were initially trained with three sizes of undefended prey to ensure that they were familiar with all prey sizes used in the experiment, and also to make sure that they readily ate mealworms in our experimental set-up. Once trained, they received seven sessions where they were given a discrimination task between defended and undefended prey that had different colour signals to make them visually distinguishable. The defended prey differed in size, but had the same toxin content and visual signal. We predicted that larger toxic prey would suffer increased attacks from naïve predators during the learning process.

2. Methods

2.1. Housing and husbandry

16 European starlings (*Sturnus vulgaris*) were caught in Northumberland outside of the breeding season (September/October 2010) using a whoosh net. The birds were immediately transferred by car to laboratories at Newcastle University, where they were weighed, health checked and ringed with a plastic ring for identification purposes. They were then released into an indoor aviary (215 × 340 × 220 cm) that provided ropes, boxes and branches for perches and cover. The birds were kept in the aviary when not used in experiments. Birds were fed *ad libitum* with pheasant breeder pellets supplemented with fruit, and mealworms that were mixed into the bark chippings that covered the floor of the aviary to provide an enriched foraging environment. Drinking water (enriched with vitamins) was available at all times and bathing water was provided daily. Birds were kept under

a 14:10 light cycle and the temperature varied between 17 °C and 21 °C. Birds were regularly weighed and visually inspected by a trained technician to ensure that they remained healthy throughout their time in captivity. At the end of the experiment (June 2011), birds were checked by a vet, BTO ringed and released back to the wild at their site of capture. The experiments were conducted under local ethical approval from Newcastle University and all procedures adhered to ASAB's Guidelines for the Treatment of Animals in Behavioural Research and Teaching.

2.2. Training sessions

We conducted the experiment in two replicates (in May and in June 2011), with 8 starlings in each replicate. Pairs of birds were moved into adjoining cages measuring 75 × 45 × 45 cm that could be separated using an opaque divider during training and learning sessions. On each side of the cage there was a drawer measuring 45 × 75 cm, with a spring-loaded flap facing the front through which prey could be presented. Each cage contained a litter tray filled with bark chips for foraging as well as branches for perching. Birds were given access to bathing water every day. Water and pheasant breeder pellets were provided *ad lib*, except during food deprivation periods prior to training and learning sessions, and during the sessions themselves when mealworms were presented. Birds were kept on the same lighting schedule and within the same temperature range when in cages as they were in the indoor aviary.

Birds received a single training session at the same time each day. Birds were food deprived for 75 min prior to the start of each session. Five minutes before the start of a session, a white curtain was put up in front of the cage to visually isolate the subject from the experimenter (KS) and other birds in the room. Foraging decisions were monitored by the experimenter using a video camera connected to a viewing screen. A training session consisted of 24 individual presentations of single mealworms in a petri dish (38 mm diameter) on a white background. A mealworm was presented once every three minutes, and the birds were given one minute in which to eat the prey before the dish was removed. Eight mealworms were presented of each of three different mealworm sizes: small (0.15–0.17 g), medium (0.22–0.24 g) and large (0.31–0.33 g). Once birds ate at least 75% of the mealworms in a training session, they started the learning sessions (birds took between 3 and 7 sessions to reach criterion). Three birds (1 female, 2 males) failed to meet this criterion after seven training sessions and were excluded from the learning sessions.

2.3. Learning sessions

The learning sessions followed the same basic protocol as in training, except that birds ($N = 13$) now received 25 sequential presentations of undefended (10) and defended (15) mealworms in each session. Since the body size of undefended prey eaten affects the number of defended prey that starlings eat (smaller undefended prey increase predation on defended prey; Halpin et al., 2013), it was important that we controlled the size of our undefended prey. Therefore, all our undefended prey were small mealworms injected with 0.02 mL water which ensured that the birds did not acquire all their nutrients from undefended prey and would continue to attack defended prey during a session. In addition, if birds learned that small prey were undefended and generalised this association to defended prey, we would expect to see small defended prey being attacked more during the learning process than large defended prey. Therefore, if we found that large defended prey were attacked more than small defended prey, we could be confident that this was not because of a size preference generated by our undefended prey.

We made small, medium and large defended prey by injecting mealworms of the three different sizes with 0.02 mL 4% quinine

sulfate solution (Sigma–Aldrich Q0132). The solutions were injected through the mouthparts of the mealworms, immediately prior to a presentation, using a hypodermic needle. From our own experiments and those of others, we know that quinine is aversive to a range of bird species (for example: black-capped chickadees, *Parus atricapillus* (Alcock, 1970); great tits, *Parus major* (e.g. Alatalo & Mappes, 1996); European starlings (e.g. Skelhorn & Rowe, 2007); domestic chickens, *Gallus gallus domesticus* (e.g. Halpin et al., 2008a)). Previous experiments have shown that quinine cannot be detected prior to ingestion when injected in this manner (Skelhorn & Rowe, 2009, 2010).

Undefended and defended prey were made visually distinguishable to the birds by being placed on paper disks of two different shades of grey (15% and 65% grey). The two shades were counterbalanced across birds to ensure that any preference was not due to a bias for different shades of grey. Each daily trial consisted of 10 undefended prey, and five small (0.15–0.17 g), five medium (0.22–0.24 g) and five large (0.31–0.33 g) defended prey. We selected these sizes to ensure that the prey differed in their nutritional content (Finke, 2002), and to ensure that the differences were large enough for the birds to discriminate between the prey (Marples, 1993; Halpin et al., 2013). We randomized the presentation of these prey types within each block of five presentations so that there were two undefended, one small defended, one medium defended, and one large defended mealworm presented in each block. Balancing the presentations in blocks across the session was important because we know that predator state can change according to the amount of nutrients and toxins in the prey, and this in turn affects foraging behaviour (Skelhorn & Rowe, 2007; Barnett et al., 2012; Halpin et al., 2013). Therefore, by presenting each prey type equally across each session, changes in state could not bias our results. Birds received a single learning session per day for seven sequential days, and the numbers of mealworms that were attacked and eaten of each prey type were recorded.

2.4. Statistical analysis

There was no effect of replicate (May or June) on the numbers of any prey type eaten during training (independent *t*-tests; small unmanipulated prey: $t_{11} = 1.376$, $p = 0.196$; medium unmanipulated prey: $t_{11} = 1.217$, $p = 0.249$; large unmanipulated prey: $t_{11} = 1.298$, $p = 0.221$), or during the learning sessions (undefended prey, Mann–Whitney *U* test: $Z = 0.293$, $p = 0.769$, $n = 13$; small defended prey, *t*-test: $t_{11} = 0.276$, $p = 0.787$; medium defended prey, *t*-test: $t_{11} = 0.893$, $p = 0.391$; large defended prey, *t*-test: $t_{11} = 0.796$, $p = 0.443$). Therefore, we pooled data from across replicates in our analyses.

2.5. Training sessions

To investigate the effect of body size on birds' decisions to eat unmanipulated mealworms during the training sessions, we first calculated the mean number of small, medium and large mealworms that each bird ate per session across the training trials as the dependent variable. We tested whether birds preferred to eat larger prey using an ANOVA with the mean size of each prey type as a covariate (i.e. 0.16 g for small, 0.23 g for medium and 0.32 g for large prey) and subject as a random factor (see also Barnett et al., 2007, 2012).

2.6. Learning sessions: visual discrimination between undefended and defended prey

To test if birds were learning to use the visual signals to discriminate between defended and undefended prey, we compared the proportions of undefended and defended prey that were attacked

during each of the learning sessions. We calculated the proportion of defended and undefended prey attacked, rather than using numbers, because we used different numbers of each prey type in a session (10 undefended and 15 defended). These values did not conform to the assumptions of parametric tests, mainly because there were a large number of birds that attacked all of the undefended prey presented to them in a given session. Therefore, in order to test visual discrimination learning, we compared the proportion of undefended and defended prey attacked in sessions 1 and 7 using a Wilcoxon signed rank test.

In previous experiments, birds have reduced the number of defended prey that they attack in each session before reaching a stable asymptotic level. To test whether the birds in this experiment had also reached an asymptote or were still learning, we ran a series of repeated measures ANOVAs on the data from the learning sessions, with session number as the repeated measure and the number of defended prey attacked as our dependent variable. Our first analysis included all the sessions, after which we then removed the first session and ran the analysis again, then we removed the second session and ran it again, and so on, until we no longer found an effect of session which would indicate an asymptotic ingestion of defended prey (Halpin et al., 2012, 2014).

2.7. Learning sessions: effect of size on predation of defended prey

Finally, we compared the numbers of small, medium and large defended prey that were attacked and eaten during the learning sessions. Data from individual sessions were not normally distributed and could not be transformed for parametric tests. Therefore, we analysed the data from the first and final sessions using non-parametric statistics to look for effects of size at the start and end of the sessions (Friedman and post-hoc Wilcoxon signed ranks tests). We also calculated the total number of each defended prey type attacked and eaten across all seven sessions. Once pooled across trials, the data for the numbers of defended prey attacked and eaten conformed to the assumptions of parametric tests. Therefore, we conducted two ANOVAs with the mean size of each defended prey type as a covariate (i.e. 0.16 for small, 0.23 for medium and 0.32 g for large defended prey) and subject as a random factor (Barnett et al., 2007, 2012) to test for the effect of size on the number of defended prey attacked and eaten by the birds.

3. Results

3.1. Training sessions

During the training sessions, when all prey were undefended and unmanipulated, body size did not affect predators' decisions to eat mealworms (ANOVA, $F_{1,25} = 3.23$, $p = 0.080$). This lack of effect of prey size on birds' foraging decisions could not be attributable to any ceiling effect, since the mean number of each prey type eaten was less than the eight prey that were offered in each session (Fig. 1).

3.2. Learning sessions: visual discrimination between undefended and defended prey

Over the course of the seven learning sessions, birds learned to discriminate between undefended and defended prey using the greyscale signals associated with each prey type. Although there was no difference between the proportion of undefended and defended prey attacked in session 1 (Wilcoxon signed ranks test: $Z = 31.0$, $p = 0.72$, $n = 13$; Fig. 2), birds attacked more undefended than defended prey in session 7 ($Z = 7.0$, $p = 0.007$, $n = 13$; Fig. 2).

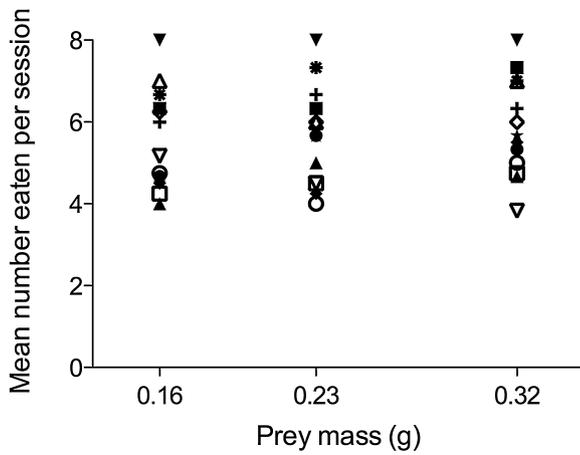


Fig. 1. The mean number of small (0.16 g), medium (0.23 g) and large (0.32 g) unmanipulated prey eaten in each session by individual birds during training.

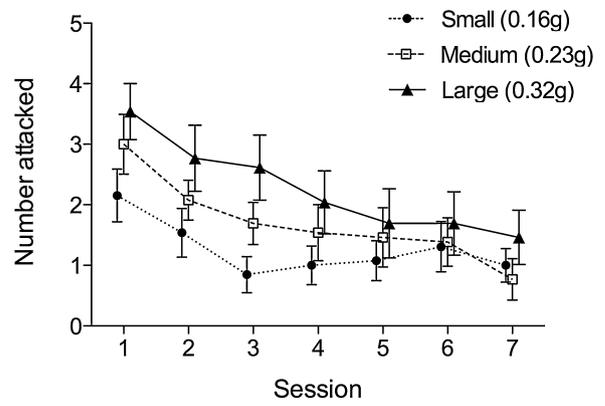


Fig. 3. The mean (\pm se) number of small, medium and large defended prey attacked across the seven learning sessions (note that the bars are slightly offset for ease of viewing).

numbers of defended prey attacked (Friedman test; $\chi^2 = 7.66$, $p = 0.024$, $df = 2$; Fig. 3). Post-hoc tests revealed a difference in the numbers of small and large defended prey attacked (Wilcoxon signed rank test: $Z = 2.63$, $p = 0.009$, $n = 13$), but no difference between the numbers of medium and small ($Z = 1.70$, $p = 0.089$, $n = 13$) or medium and large ($Z = 1.33$, $p = 0.184$, $n = 13$) mealworms attacked. There was also an effect of body size on predators' behaviour across the seven learning sessions. As expected, as the mass of the defended prey increased, individuals were attacked (ANOVA: $F_{1,25} = 14.64$, $p = 0.001$; Fig. 4) and eaten more often ($F_{1,25} = 13.43$, $p = 0.001$; Fig. 4). Finally, when we compared the numbers of defended prey attacked in the final session, there was

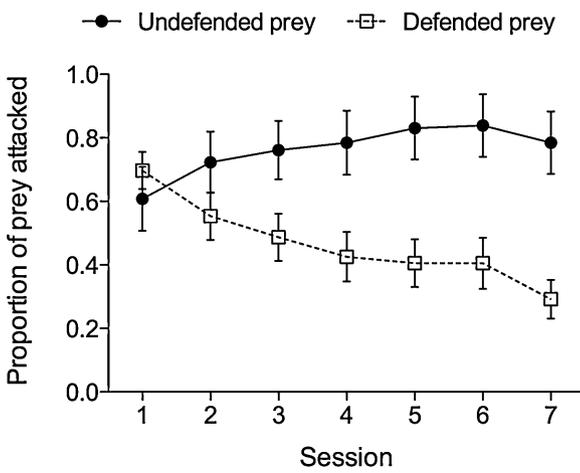


Fig. 2. The mean (\pm se) proportion of undefended and defended prey attacked over the seven learning sessions.

Table 1
Results from a series of repeated measures ANOVAs testing for an asymptotic attack rate on defended prey.

Sessions included in the analysis	F	P
1–7	49.85	<0.001
2–7	20.07	0.001
3–7	12.96	0.004
4–7	7.74	0.017
5–7	7.87	0.016
6–7	8.16	0.014

Birds clearly learned to use the visual cues placed under the mealworms to reduce their intake of quinine-injected prey. However, the birds did not appear to have reached a stable asymptotic attack rate on defended prey. Using a series of repeated measures ANOVAs (see statistics section for full explanation), we found an effect of session on the number of defended prey attacked in all of our analyses (Table 1). This demonstrates that the birds were still acquiring the task and had not reached an asymptotic phase of the learning process (Fig. 2).

3.3. Learning sessions: effect of size on predation of defended prey

Finally, we analysed predators' behaviour towards each defended prey type across the seven learning sessions (Fig. 3). In the very first session, we found an effect of prey body size on the

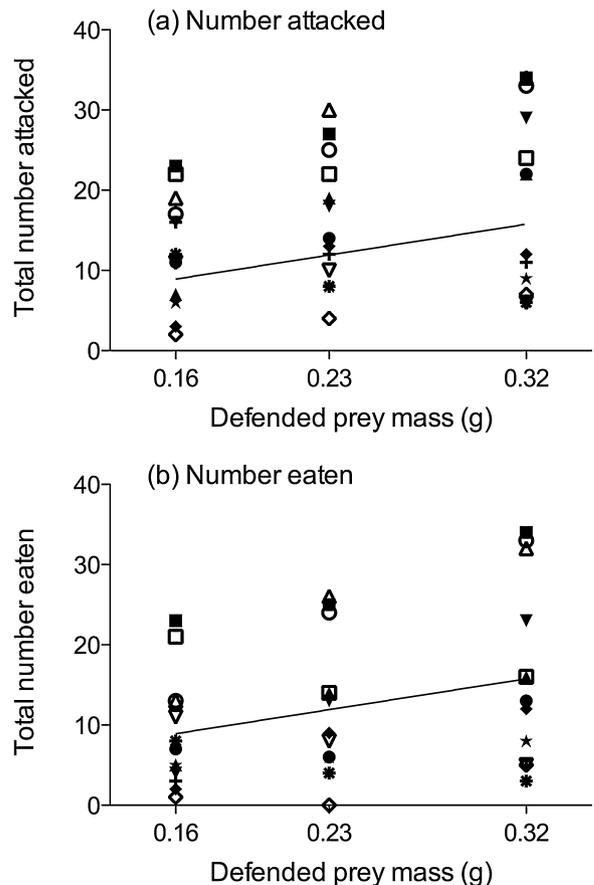


Fig. 4. Increase in the number of defended prey (a) attacked and (b) eaten across the seven learning trials as the size of defended prey increased.

no effect of size (Friedman test; $\chi^2 = 3.08$, $p = 0.214$, $df = 2$; Fig. 3), suggesting that the effect of body size was less pronounced at the end of the sessions compared to at the start.

4. Discussion

Prey body size clearly affected birds' decisions to attack and eat defended prey during the learning process. Large defended prey were more likely to be attacked compared to smaller prey sizes even in the very first learning session. In addition, defended prey were attacked and eaten more often across the seven learning sessions with increasing body size, although differences could not be detected in the final session. This shows that body size *per se*, independent of colour signal, can alter predatory behaviour and increase the costs to larger-bodied aposematic prey during the learning process.

At first glance, this result may not seem that surprising since body size is a good indicator of nutrient content (Wiegert, 1965; Barnard & Brown, 1981; Barnard & Stephens, 1981; Finke, 2002; Lease & Wolf, 2011), and it is well established that predators show preferences for non-toxic foods that are energetically or nutritionally more profitable (e.g. Charnov, 1976; Pyke et al., 1977, and references therein; Mayntz et al., 2005; Jensen et al., 2012). Although we did not find an effect of body size on predation of undefended prey in our experiment, this could be due to our protocol. When all three sizes were undefended, there was no opportunity cost for birds to eating smaller prey, since they quickly processed and eat mealworms of all sizes before the next presentation. However, when prey are defended, it pays a bird to be more discriminatory since eating a small toxic mealworm will increase its toxin burden, which may prevent it from being able to eat a larger toxic mealworm later in the session. Our results are the first demonstration that prey body size affects predation on toxic prey when they are known to contain equal amounts of toxin. Intriguingly, in a previous experiment with starlings, we found that the body size of defended prey had no detectable effect on birds' decisions to eat mealworms that were injected with the same amount of quinine (Halpin et al., 2013). However, the mealworms used in that experiment were equivalent to the small and medium-sized mealworms that we used here, which may have limited our ability to detect an effect (exacerbated by the fact that we used a between-subject rather than a within-subject design as we did here). The body size effect that we have now detected is novel in the context of aposematism and mimicry because we show that: (1) mortality during the avoidance learning process may not simply result from how birds associate a colour cue with a given toxin level; and (2) large body size can be costly for aposematic prey in terms of predation. We discuss each of these points in turn.

It is well established that many predatory species can readily learn to associate a prey's warning signal with its defence, and reduce their attack rates on prey carrying that warning signal (including birds (e.g. Riipi et al., 2001), fish (e.g. Miller & Pawlik, 2013), mammals (e.g. Barber & Conner, 2007), and insects (e.g. Berenbaum & Miliczky, 1984)). The way that predators learn this association is thought to follow the rules of associative learning theory where a conditioned stimulus or CS (in this case, the warning signal) becomes associated with an unconditioned stimulus or US (such as the presence of toxins or physical defences) (Rescorla & Wagner, 1972). This view of avoidance learning has led researchers to vary only the signal and/or the toxicity of prey in their experiments, and widely used laboratory systems tend to tightly control the size of their prey (e.g. Gittleman & Harvey, 1980; Rowland et al., 2007; Halpin et al., 2008b; Barnett et al., 2012). However, of course, aposematic prey in the wild do differ in size, both within and across species (Brower & Calvert, 1985; Cohen, 1984; de Jong et al., 1991),

so by controlling body size, we have missed out on understanding how prey body size affects the learning process in predators under more naturalistic conditions.

Based on our findings, we are now not convinced that prey mortality during the learning process is solely driven by how quickly predators make the association between colouration and toxicity. In our experiment, all our defended prey contained the same amount of toxin and carried the same visual signal, so why the different mortality rates? One mechanistic explanation is that the birds could have had intrinsic preferences for larger prey, which were either unlearned or acquired prior to the start of the experiment. Starlings do show preferences for larger palatable prey in this size range (Whitehead et al., 1995), presumably because they are more nutritionally profitable (Whitehead et al., 1995; Finke, 2002; Halpin et al., 2014). However, in this experiment, we found no effect of prey body size on the starlings' decisions to eat unmanipulated prey during the training trials (Fig. 1), although as already discussed, this could have been due to there being no opportunity costs to eating prey of all sizes. But this does suggest that there is no strong intrinsic preference that can readily explain our findings.

An alternative mechanism is that body size could have influenced what the birds were actually learning about the defended prey. Whilst quinine is a negative reinforcer and causes a learned aversion, nutrients are of course positively rewarding. Since larger mealworms contain more nutrients (Finke, 2002), and starlings can detect and learn about the nutrient content of toxic prey (Halpin et al., 2014), the positive reinforcement from the additional nutrients in larger bodied mealworms could have countered the negative effects of the quinine during the learning process. How this might be achieved is not known, since the cognitive and neural mechanisms that integrate information about rewards and punishments are not known. This is because, to our knowledge, there has been no interest in the psychological literature to investigate how positive and negative reinforcers affect behaviour when they are both outcomes from the same action, as in the case of eating a toxic prey which contains both nutrients and toxins. Since predators can learn about both the nutrient and toxin content of their prey and use both sources of information in their foraging decisions (Halpin et al., 2014), the enhanced rewards associated with eating larger prey could explain our data.

If this was the case, learning certainly appeared to occur very quickly, since birds showed a higher rate of ingestion of large prey in the very first session (Fig. 3). At first glance, this appears to conflict with our previous work, which found that birds take several sessions to learn about variation in the nutrient value of mealworm prey (Halpin et al., 2014). However, it could be that learning may have been quicker in this experiment because the nutritional differences were more detectable to the birds. The range of nutritional rewards used in this experiment was larger than those that we have previously used which could have made variation more detectable (e.g. Halpin et al., 2013, 2014). It could also have been easier for the birds to learn to visually discriminate among prey that differed nutritionally because of the differences in body size (e.g. Marples, 1993). Knowing more about how predators detect and use information about nutrient content in their foraging decisions on toxic prey will be important for understanding the specific mechanisms involved, but our data can clearly be interpreted in the context of the birds learning about both nutrient and toxin content of aposematic prey.

However, there is one caveat, since there is another mechanistic explanation that does not involve birds learning about nutritional value of prey, and is consistent with the idea that mortality during the learning process is based upon how predators learn the toxin-colour association. Although birds could have learned simultaneously about nutrients and toxins, it could also be that increasing amounts of nutrients reduced the impact of the toxin

on the birds. For example, the post-ingestive efficacy of the toxin could have been reduced because of there being a larger volume of nutrients to digest (Levine, 1970). If longer digestion times mean a slower release of the toxin, this could potentially mean that the birds perceived the larger mealworms to be less toxic, leading to slower aversion learning. Currently, we cannot rule out this mechanism, although future experiments that increase prey size without increasing prey nutrient content, could test this idea.

In our study, learning was incomplete, and it is therefore difficult to say whether or not the different prey types would have had the same or different asymptotic attack rates. Intriguingly, the difference in attacks on the three prey types was no longer present in the final session. Whilst this suggests that the attack rates on the three defended prey types could be tending towards the same asymptote, we urge some caution in this interpretation. This is because the attack rates are also tending towards zero, and so the variation in our data may not make the differences detectable with our sample sizes. It would certainly be interesting to know where the asymptotic attack rates lie in terms of understanding selection pressures from predators on toxic prey of different sizes beyond the learning process. However, our study is unable to provide conclusive data on this, and this will need to be addressed in future research.

Overall, although we are unable to fully identify the cognitive mechanism underpinning our finding, the fact that body size *per se* affects predation during the learning process has implications for the study of aposematic prey and their mimics. First and foremost, we have identified an additional selection pressure that will select against increasing body size in aposematic prey. Having a larger body is considered to be beneficial in predator learning, since toxin content may be greater and the signal also larger: our results challenge this view. Clearly, the interactions between nutritional value, toxicity and signal size are likely to be complex as body size changes. For example, signal size will be subject to other life history trade-offs, and is not necessarily linked to body size (e.g. Ojala et al., 2007; Hegna et al., 2013). In addition, ingesting toxins for defence can reduce body size (Pasteels et al., 1983; Rowell-Rahier & Pasteels 1986), and toxic prey may trade-off growth that can increase fecundity with increased predator avoidance and survival. Empirical studies that explicitly measure the costs and benefits of increasing body size for aposematic prey are crucial, particularly for understanding the selection pressures acting on optimal body size in aposematic prey (Mänd et al., 2007; Rimmel & Tammaru, 2009) and ontogenetic changes in their defence strategy (Higginson & Ruxton, 2010).

Our results also have implications for the evolutionary dynamics of mimicry, where species often differ in their body size (Cohen, 1984; Marples, 1993; Penney et al., 2012). The relationship between mimics, whether it is beneficial to both (Müllerian) or one species benefits at the expense of the other (Batesian or quasi-Batesian), is thought to depend upon the relative toxin content of each species which affect how quickly predators learn to reduce their rates of attack on models and mimics (Bates, 1862; Müller, 1879; Speed, 1993; Turner & Speed, 2001; Rowland et al., 2007). However, our results show that this may be an overly simplistic view, and that differences in body size could also affect the dynamics. Body size could affect the way that the mimicry complex is sampled, and how predators learn about the toxin content and profitability of each species. It could also be used by predators to discriminate between models and mimics if it is worth doing so, i.e. if the time invested in distinguishing between model and mimic is outweighed by the benefits of identifying more profitable individuals (Holen, 2013). Making specific predictions is again difficult, simply because we do not know enough about what predators are learning about aposematic prey and how that acquired information affects predatory decisions. We need to know more about what cues are important for predator learning, and how what predators learn influences their

decisions on different-sized aposematic prey that can vary in their signals, toxicity and nutritional value (see also Turner and Speed, 2001). These data could then be used to refine theoretical models of mimicry to make clear predictions about the relationships between mimics that differ in body size, signal size and toxin content in the wild.

Of course, trying to understand how cognitive processes affect evolutionary processes in the wild underpins our whole research endeavour. Our studies, like those of others (Alatalo & Mappes, 1996; Rowland et al., 2007; Prokopova et al., 2010), use wild-caught avian predators in carefully controlled laboratory experiments to measure selection pressures acting on aposematic prey. Our experiments with starlings also carefully control prey presentation; we know the numbers of mealworms eaten and the amount of toxin ingested which allows us to better understand the cognitive mechanism underpinning both learning and decision-making. This level of control over stimulus presentation and measurement of cognitive processes has not yet been achieved in the wild, and consequently, these laboratory studies make a valuable contribution to knowing how selection is likely to act on natural prey populations. A challenge for the future is whether we can actually measure the cognitive processes underlying natural predation events in the wild, and identify which are the most important for the evolution of aposematism and mimicry.

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