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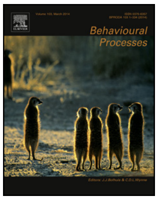

Body size matters for aposematic prey during predator aversion learning

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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).

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 (refs). 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 between

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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, naive predators can also exhibit intrinsic avoidance of larger conspicuous prey, or show enhanced avoidance learning because a larger signal is more salient (Gambarale 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, counteracting 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., 2006; 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

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