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Better the devil you know: avian predators find variation in prey toxicity aversive

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Toxic prey that signal their defences to predators using conspicuous warning signals are called 'aposematic'. Predators learn about the toxic content of aposematic prey and reduce their attacks on them. However, through regulating their toxin intake, predators will include aposematic prey in their diets when the benefits of gaining the nutrients they contain outweigh the costs of ingesting the prey's toxins. Predators face a problem when managing their toxin intake: prey sharing the same warning signal often vary in their toxicities. Given that predators should avoid uncertainty when managing their toxin intake, we tested whether European starlings (*Sturnus vulgaris*) preferred to eat fixed-defence prey (where all prey contained a 2% quinine solution) to mixed-defence prey (where half the prey contained a 4% quinine solution and the other half contained only water). Our results support the idea that predators should be more 'risk-averse' when foraging on variably defended prey and suggest that variation in toxicity levels could be a form of defence.

1. Introduction

Many species, both plants and animals, defend themselves with harmful toxins in order to reduce the chances that they are eaten [1,2]. Toxic insects often advertise their defences to potential predators using warning signals, such as conspicuous colour patterns, sounds and odours [3]. This anti-predator defence strategy is known as 'aposematism' [4] and is effective because predators can readily learn to associate the warning signals with the ingestion of toxins. As a consequence, predators lower their attack rates on aposematic prey in order to reduce and regulate their intake of potentially harmful toxins as they forage [5,6].

One problem for predators regulating their intake of toxins is that individuals within an aposematic prey species often vary in their toxicity [7,8]. 'Automimicry' is characterized by the presence of non-toxic individuals ('automimics') in a population of otherwise aposematic prey ('automodels') [9,10]. Explaining this variability has been a long-standing theoretical challenge in evolutionary biology. The problem is that if possessing toxins is costly, for example, reducing growth or fecundity [11,12], then undefended individuals will benefit from not paying those costs, while at the same time benefitting from the aposematic defence generated by more toxic individuals. This then leads to increasing numbers of automimics in the population, diluting the model's defence and increasing the costs of being conspicuous. Potential solutions to this problem have largely involved the role of secreted defences, which allow predators to taste and selectively reject individuals according to their toxicity [10,13,14]. However, not all aposematic insects have inducible chemical defences that are produced upon attack [15], and alternative mechanisms to explain variability in defences in these species are required.

There are a number of explanations for the variation among prey in their chemical defences, which revolve around individual prey responses to environmental heterogeneity (e.g. host plant availability, predator presence) or intrinsic factors (e.g. sex differences, changes across the life-span) [16]. Here, we propose a radically different solution to this problem: that the ways in which predators avoid toxic prey can actively promote variability in defence levels among prey individuals [17]. Such variability among prey in their chemical defence could be adaptive if predators find variably defended prey (which we term mixed-defence prey) more aversive (e.g. by making it more difficult for predators to manage their toxin burdens [18]). Therefore, it could benefit a female insect to increase the variability in toxicity in her offspring (e.g. by laying eggs on host plants that vary in their toxicity [19]) in order to reduce predation of them. In our experiment, we investigated birds' behaviour towards two populations of insect prey that had the same mean levels of toxin, but differed in whether their defences were variable or not. We tested whether or not variation in toxicity is indeed more aversive to predators, and hence, if predator decisions can promote automimicry.

2. Material and methods

Our experiment used an established protocol where European starlings (*Sturnus vulgaris*) were sequentially given single mealworms (*Tenebrio molitor*) that varied in their defence levels [20–22]. Seven male wild-caught starlings were individually caged in the laboratory (see the electronic supplementary material for full details of capture and housing; see also [20–22]). Initially, birds were trained to flip white paper lids off sequentially presented Petri dishes, each containing a mealworm. We then gave a series of daily sessions where in each session they received: six undefended prey (all injected with 0.02 ml of water); six 'fixed-defence' prey (all injected with 0.02 ml of 2% quinine sulfate solution) and six 'mixed-defence' prey (three injected with 0.02 ml water and three injected with 0.02 ml of 4% quinine sulfate). Therefore, the two defended prey types contained the same mean amount of toxin, but differed in the variation around the mean. The three prey types had distinguishable coloured lids (colours counterbalanced across individuals). Once birds had learned to discriminate between undefended and defended prey, we conducted 10 further sessions on 10 consecutive days. We recorded which mealworms were eaten, allowing us to calculate the proportion of fixed and mixed prey eaten at each presentation number in the sequence (i.e. from 1 to 18) for each bird across the 10 test sessions. We used generalized additive mixed models (GAMMs) to compare the ingestion of fixed and mixed prey within a session (see the electronic supplementary material for further details).

3. Results

Birds were less likely to eat a mixed-defence prey than a fixed-defence prey (GAMM: $\chi^2_1 = 7.6114$, $p = 0.0058$, figure 1), and the probability that they ate either type of defended mealworm decreased with increasing presentation number within a session ($\chi^2_1 = 89.862$, $p < 0.0001$, figure 1). The interaction between prey type and presentation number within the daily session was not significant ($\chi^2_1 = 1.3477$, $p = 0.2457$). The difference between the fixed and mixed prey was not due to birds ingesting more quinine following attacks on the mixed prey. There was no difference in the numbers of 4% quinine-

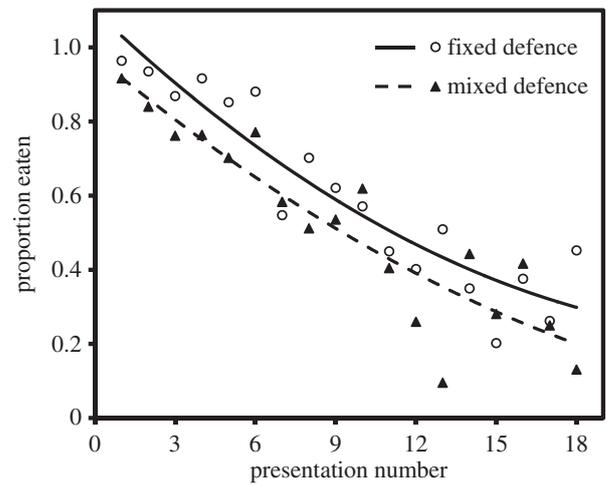


Figure 1. The mean proportions of defended prey eaten throughout the daily sessions. Curves were taken from estimators of the generalized additive mixed model.

injected and water-injected mixed-defence mealworms eaten (GAMM: $\chi^2_1 = 0.4809$, $p = 0.488$; see the electronic supplementary material for further details), meaning that the mean defence level of the mixed-defence prey experienced by the birds was approximately 2%. Therefore, the difference in mean intake of toxin across individuals from mixed-defence and fixed-defence prey was negligible and so could not explain the differences in preferences.

4. Discussion

While the probability of eating a toxic prey decreased with presentation number in a session, due to either the improving nutritional state of the predator or an increasing toxin burden [5,6,21,22], birds were less likely to eat a mixed-defence prey compared with a fixed-defence prey at any point during an experimental session. This clearly shows that birds found mixed-defence prey more aversive than fixed-defence prey, and could use preys' visual signals to reduce their intake of prey with more variable defences. This means that predatory attacks on a toxic prey population are affected not just by the mean toxin content of a prey population [23–25], but also by how variable it is around that mean. Therefore, the foraging decisions of avian predators could promote variation in toxicity in the wild, leading to the intriguing possibility that automimicry itself could be a form of defence.

Our results suggest that predators' behaviour could promote variability in toxicity in aposematic species where predators cannot discriminate between variably defended individuals prior to ingestion, particularly where selective advantages can accrue through living in close proximity to kin [13,15]. For example, a gravid female insect could benefit by laying her eggs on a range of local host plants, or if she has a fixed amount of toxin to distribute among her eggs, differentially provisioning her brood with defensive compounds [19,26]. If her undefended offspring (which have high fecundity because of their lack of investment in toxins [12]) gain sufficient protection from their more toxic brood-mates, the overall fitness of her brood could increase relative to each offspring having a fixed amount of toxin. Of course, whether increasing variability would be a better strategy would depend on the strength of predators' aversion towards variably defended prey in the wild, along with other factors,

such as the availability of other undefended and toxic prey in the environment, and how the costs associated with reduced fecundity change with increasing toxicity. However, it remains a possibility; understanding whether predators' reduced willingness to eat prey with variable defences affects investment in defences across natural prey populations is certainly an intriguing avenue for future research.

Our data also provide to our knowledge the first empirical support for the idea that predators should be less willing to eat prey with variable defences because they are uncertain of the consequences of ingesting any given individual [18]. This means that our findings are also relevant to cases of Batesian mimicry, where a palatable species copies the signal of a more toxic species. Although the Batesian mimic is parasitic on the defence of its toxic model species and dilutes its defence, the uncertainty that the visual mimic generates in relation to the signal may ameliorate these effects. This may be especially pertinent for models in the presence of another aposematic species with a mean toxin level similar to the mimicry complex. However, we cannot fully exclude the possibility that the difference in reinforcement schedules between fixed and mixed prey generated the differences in predator aversion levels between the two prey types. For example, perhaps the sudden ingestion of toxin resulting from eating a 4% quinine-injected mealworm was perceptibly worse than eating two 2% quinine-injected mealworms, leading to a stronger

learned aversion. Results from a previous experiment would argue against this, as we found no evidence that eating a 3% quinine-injected mealworm was more than three times as aversive as eating a 1% quinine-injected mealworm [22]. Therefore, the aversive effects of quinine do not appear to be accelerating with increasing concentration. Moreover, our results are consistent with the literature on risk-sensitive foraging despite using different experimental methods, which indicates that animal subjects prefer rewards that are fixed in pay-off over those that are variable in pay-off [27]. However, further studies could elucidate the cognitive mechanisms underlying predators' decisions to better understand their broader impact on the evolution of defensive strategies.

Ethics statement. All research adhered to the ASAB/ABS Guidelines for the Use of Animals in Research and was approved by the local ethical committee at Newcastle University.

Data accessibility. Data is available in Dryad (<http://datadryad.org/resource/doi:10.5061/dryad.2qk02>).

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