Predators are less likely to misclassify masquerading prey when their models are present

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Masquerading animals have evolved striking visual resemblances to inanimate objects. These animals gain protection from their predators not simply by avoiding detection, but by causing their predators to misclassify them as the 'models' that they appear to resemble. Using domestic chicks as predators and twig-mimicking caterpillars as prey, we demonstrated that masquerading prey were more likely to be misclassified as their models when viewed in isolation from their models than when viewed alongside examples of their model, although they benefitted from masquerade to some extent in both conditions. From this, we predict a selection pressure on masqueraders to use microhabitats that reduce the risk of them being viewed simultaneously with examples of their model, and/or to more closely resemble their model in situations where simultaneous viewing is commonplace.

Keywords: masquerade; camouflage; predation; predator–prey; detection; classification

1. INTRODUCTION

Masquerading animals have evolved striking visual resemblances to inanimate objects found in the same locality (Endler 1981; Allen & Cooper 1985; Skelhorn *et al.* 2010*a*). For example, many species of caterpillar resemble twigs or bird-droppings, and many species of mantid resemble leaves or flowers (see Edmunds 1974; Cott 1940; Caro 2005 for further examples). These animals gain protection from their predators not simply by avoiding detection (crypsis: see definitions in Stevens & Merilaita 2009), but also by causing their predators to misclassify them as the 'models' that they appear to resemble (Skelhorn *et al.* 2010*b*).

Hailman (1977) divided masquerade into two separate phenomena: 'element imitation' and 'object imitation'. He stated that element imitators resemble some specific and common object that is one element of the environmental pattern (for example, twigs or leaves), whereas object imitators resemble some specific object that is not necessarily common and is not an element of a regular environmental pattern (such as bird droppings). According to his definition, the essential difference is that element imitators are viewed against a background that includes examples of the type of item that they masquerade as (their models), whereas object imitators are viewed in isolation from examples of the model. Hailman suggested that element imitators benefit from crypsis (they are difficult to detect as discrete items against a background containing many models, in the same way it would be difficult to detect a single leaf in a canopy), whereas object imitators benefit from masquerade (predators detect them, but misclassify them as something inedible).

Hailman's suggestion is not entirely correct: we recently demonstrated that predators misclassify element imitators as the models that they appear to resemble, and they therefore benefit from masquerade (Skelhorn *et al.* 2010*b*). However, we feel that making the distinction between element and object imitation is still useful. Since element imitators and their models are viewed simultaneously by a predator, we would predict that they are under stronger selection to resemble their model than object imitators (which are viewed in isolation from their models). This is because, when viewing element imitators, predators have a 'frame of reference' for what the model should look like.

Using domestic chicks as predators and twig-mimicking caterpillars as prey, we tested whether masquerading prey were more likely to be misclassified as their models when viewed in isolation from their models than when viewed alongside their models. Thus, extending our previous work by asking how the environment in which prey is found influences the benefit of masquerade.

2. MATERIAL AND METHODS

(a) Experience manipulation trials

Eighty domestic chicks, trained to eat chick crumbs in the experimental arena served as predators, and twig-mimicking larvae of the Earthly thorn moth *Selenia dentaria* served as masquerading prey (see the electronic supplementary material for details of caterpillars, housing, arena and training; for photograph of Early Thorn, see Skelhorn *et al.* 2010*b*). On day 2 of life, trained chicks were divided into 10 groups, each containing eight individuals. Birds in all groups received four, 2 min trials, in which they were placed in the experimental arena individually. The items placed in the experimental arena during these trials differed among groups. Five groups encountered a Hawthorn branch complete with leaves; and five groups encountered a manipulated Hawthorn branch that had been bound in purple cotton thread to change its visual appearance while minimizing changes to its odour or physical structure. All branches measured approximately 20 cm in length, containing between 8 and 12 twigs. The branch was placed in the centre of the arena, and the latency to peck the branch and the number of times the branch was pecked were recorded. Chicks were trained in a random order. Chicks were equally reluctant to attack branches, and manipulated branches and learned to stop pecking them at similar rates (see electronic supplementary material figures S1 and S2).

(b) Testing

On day 3, birds received a single testing trial: they were deprived of food for 30 min and then placed in the experimental arena where they encountered the test stimulus/stimuli. The test stimuli differed among our 10 groups: two groups were given a single twig; two groups were given two twigs; two groups were given a single caterpillar; two groups were given two caterpillars; and two groups were given one caterpillar and one twig. One group given each of the five different stimuli had previously encountered manipulated branches, and the other had previously encountered unmanipulated branches (table 1). In all cases, stimuli were placed 15 cm from the buddy arena: if only one stimulus was presented, it was placed 25 cm from each of the arena walls; if two stimuli were presented, one was placed 22.5 cm from one wall and one 22.5 cm from the other wall, so that they were 5 cm apart. Half the birds given one caterpillar and one twig received the caterpillar on the left and the twig on the right, for the other birds the reverse was true. Caterpillars

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Table 1. Overview of experimental design.

group	experience manipulation stimulus	testing stimulus/ stimuli
Br-1T	branch	1 twig
Br-2T	branch	2 twigs
Br-1C	branch	1 caterpillar
Br-2C	branch	2 caterpillars
Br-T+C	branch	1 twig + 1 caterpillar
ManBr-1T	manipulated branch	1 twig
ManBr-2T	manipulated branch	2 twigs
ManBr-1C	manipulated branch	1 caterpillar
ManBr-2C	manipulated branch	2 caterpillars
ManBr-T+C	manipulated branch	1 twig + 1 caterpillar

were refrigerated before use which prevented them from moving during the experiment. Twigs were also refrigerated to control for stimuli temperature, and different twigs were used for each chick. Chicks remained in the arena until they had pecked a caterpillar/twig, and the latency to pecking the caterpillar/twig was recorded.

(c) Statistical analysis

We used Kruskal–Wallis tests to test the following *a priori* predictions:

— birds trained on unmanipulated branches would take longer to attack twigs/caterpillars than those trained on manipulated branches. That is, caterpillars will benefit more from masquerade when chicks have previous experience of normal-looking twigs;

— birds trained on unmanipulated branches would take longer to attack twigs than caterpillars. That is, the resemblance between masqueraders and their model is not perfect;

— birds trained on unmanipulated branches would take longer to attack caterpillars presented in the absence of twigs than caterpillars presented alongside twigs. That is, simultaneously viewed models reduce the probability of masqueraders being misclassified as their model; and

— birds trained on manipulated branches would take a similar amount of time to attack twigs and caterpillars; and would take a similar amount of time to attack caterpillars presented in the absence of twigs and caterpillars presented alongside twigs. That is, in the absence of masquerade, the presence of other stimuli in the chick's field of view does not inhibit its willingness to attack caterpillars.

3. RESULTS

The time taken to attack the first stimulus differed significantly among the experimental groups (Kruskal–Wallis test: $\chi^2 = 60.76$, $p < 0.001$, d.f. = 9; figure 1). As predicted, birds trained on unmanipulated branches took significantly longer to attack the test stimuli than those trained on manipulated branches (Kruskal–Wallis test: $\chi^2 = 50.73$, $p < 0.001$, d.f. = 1). This is consistent with the findings of our previous experiment (Skelhorn *et al.* 2010*b*), and demonstrates that birds misclassify masquerading caterpillars as twigs. However, birds given only twigs (Br-1T and BR-2T groups) took significantly longer to attack the stimuli than birds given only caterpillars (Br-1C and Br-2C groups; Kruskal–Wallis test: $\chi^2 = 5.29$, $p = 0.021$, d.f. = 1). Therefore, although the caterpillars gained some protection from predation by being misclassified as twigs, the visual resemblance between twigs and caterpillars was not perfect.

Birds trained on unmanipulated branches took significantly longer to attack caterpillars when twigs were absent (Br-1C and Br-2C groups) than

when twigs were present (BR-C+T group) (Kruskal–Wallis test: $\chi^2 = 9.57$, $p = 0.002$, d.f. = 1), demonstrating that in our experiment caterpillars benefitted less from masquerade when found alongside their models than when found in isolation from their models. In addition, birds in the Br-T+C group showed a significant tendency to attack the caterpillar before the twig (binomial test; $p = 0.0078$, $n = 8$): all birds in this group attacked the caterpillar first, demonstrating that they could discriminate between twigs and masquerading caterpillars when they were presented simultaneously.

By contrast, birds trained on manipulated branches took a similar amount of time to attack twigs and caterpillars: birds in the ManBr-1C and ManBr-2C groups did not differ significantly from birds in the ManBr-1T and ManBR-2T groups in the time taken to attack the stimuli (Kruskal–Wallis test: $\chi^2 = 0.103$, $p = 0.75$, d.f. = 1). Furthermore, they took a similar amount of time to attack caterpillars presented in the absence of twigs and caterpillars presented alongside twigs: birds in the ManBr-1C and ManBr-2C groups did not differ significantly from birds in the ManBR-C+T group in the time taken to attack either the first stimuli (Kruskal–Wallis test: $\chi^2 = 0.06$, $p = 0.806$, d.f. = 1), or the caterpillar (Kruskal–Wallis test: $\chi^2 = 0.737$, $p = 0.391$, d.f. = 1). In addition, birds in the ManBR-C+T group showed no significant tendency to attack caterpillars before twigs (binomial test; $p = 0.762$, $n = 8$). This demonstrates that in the absence of masquerade, the presence of other stimuli in the chicks' fields of view did not inhibit their willingness to attack caterpillars.

4. DISCUSSION

In our experiment, birds learned that branches were non-rewarding (see the electronic supplementary material, figure S2). Those trained with unmanipulated branches generalized their learned avoidance/indifference to both twigs and caterpillars (irrespective of how they were presented), and those trained with manipulated branches did not. Caterpillars therefore, benefitted from masquerade both when presented in the absence of models and when presented alongside models, but only if birds had experienced unmanipulated branches. As we predicted, caterpillars benefitted more from masquerade in the absence of models. We suggest that the challenge of correctly classifying the masquerader as something different from the model is easier when an example of the model can be viewed simultaneously with the masquerader. In this case the viewer has an actual frame of reference for what a twig should look like, rather than just a 'cognitive representation' against which to compare the twig-mimicking caterpillar. However, whilst predator cognition is clearly important in the evolution of masquerade (in order for masquerade to be effective predators have to learn that models are non-rewarding), it is unclear whether birds used cognitive or sensory processes to discriminate between twigs and caterpillars.

Our results suggest that the strength of resemblance exhibited by different species of masqueraders might be driven at least in part by the likelihood of model and masquerader being viewed simultaneously. All
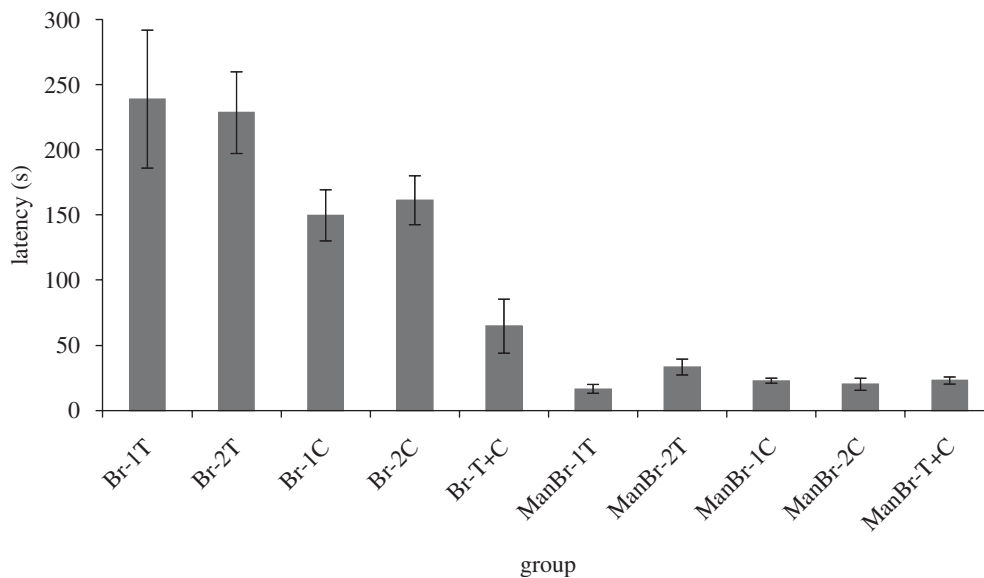
Figure 1. Latency in seconds (mean ± s.e.) to attack the test stimulus for each group ($n = 8$ for each group).

else being equal, we would predict that element imitators (those viewed alongside models, e.g. twig-mimicking caterpillars) may have to be closer mimics than object imitators (those viewed in isolation from models, e.g. caterpillars that resemble bird-droppings). We would also expect masqueraders to select, or evolve in, microhabitats that minimize their likelihood of being simultaneously viewed alongside examples of the model. However, such selection may be limited by the need to avoid allowing predators to use micro-habitat as a cue for differentiating models and masqueraders (Skelhorn *et al.* 2010a). In addition, our experiment only considers the behaviour of naive predators in a simple environment, and exploring how experience with caterpillars, or increased environmental complexity influences how useful a frame of reference is to a predator will provide intriguing areas for further study.

We also found that the birds in this experiment could discriminate between caterpillars and twigs. There are a number of potential reasons why Early Thorn caterpillars are not perfect masqueraders. This species may be under selection to resemble the specific host plant, and resemblance will increase over time; caterpillar appearance may be a compromise allowing exploitation of a range of host plants with slightly different looking twigs; there may be physiological constraints preventing perfection of resemblance; selection for perfect resemblances may not be strong because, although discrimination is possible, it is sufficiently time-consuming that predators can more profitably turn their attention to other prey; and/or discrimination may be more difficult in natural situations than in our laboratory because natural visual environments are more complex.

We have shown that predators are more likely to misclassify masquerading prey as the models that they resemble when they are viewed in isolation from the model than when viewed alongside the model, and have suggested a number of lines of enquiry to further understanding of the evolutionary ecology of masquerading species. Our results may also be relevant to the study of Batesian mimicry, where profitable prey species visually resemble toxic or otherwise unprofitable species (Cott 1940; Endler 1981). We would predict that innocuous Batesian mimics may have to resemble the defended model species more closely in situations where the two live in very close spatial proximity, such that examples of the two species may often be simultaneously viewed by the predators.

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