Original Article

The position of eyespots and thickened segments influence their protective value to caterpillars

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It has long been believed that the paired circular markings ("eyespots") of Lepidoptera larvae, combined with their defensive postures (where the larvae swell their anterior body segments) protect them from potential predators. These traits could inhibit attacks by enhancing the similarity of the prey item to the predator's own natural enemies (notably snakes), but alternatively, they may simply exploit the predator's wariness of novel and/or conspicuous objects. To differentiate between these contrasting explanations, we evaluated the responses of naive chicks (*Gallus gallus domesticus*) to artificial caterpillar-like prey. In the first experiment, chicks were presented with model caterpillars either without eyespots or with eyespots positioned in 1 of 2 locations on their bodies: anteriorly or centrally. In the second experiment, chicks were presented with model caterpillars either lacking the thickening associated with the defensive posture, or with models in which a thickened section was placed centrally or anteriorly. In both cases, the chicks were significantly more wary of prey with anteriorly positioned defensive traits than either prey without these traits or prey with centrally positioned traits. Because prey with central eyespots and central thickening were equally novel and conspicuousness to those with these traits positioned in a more head-like anterior position, we infer that predator wariness was primarily influenced by the similarity of the prey to their potential natural enemies. These results support the idea that both caterpillar eyespots, and the defensive posture examined here, deter predators not simply because they are conspicuous, but because they enhance caterpillars' resemblance to potentially dangerous vertebrates.

Key words: **antipredator, caterpillar, eyespot, mimicry, morphology, protective coloration.**

INTRODUCTION

Most organisms are under strong selection to avoid being eaten by predators, and consequently, prey animals have evolved a vast array of morphological and behavioral defenses that allow them to avoid or deter predators (Ruxton et al. 2004). One common defensive strategy found in a wide variety of taxa is the use of "eyespots": paired circular markings that to a greater or lesser extent resemble the eyes of vertebrate predators (Janzen et al. 2010). These markings have repeatedly been demonstrated to serve an antipredator function (Blest 1957; Stevens 2005), and in some species may also play a role in sexual selection or species recognition (Oliver et al. 2009; Kodandaramaiah 2011; San Martin et al. 2011). However, despite a large body of empirical work dating back more than 50 years, the mechanism(s) through which eyespots provide protection from predators remain poorly understood, and recent attempts

to rectify this shortfall in understanding have produced equivocal results (Stevens and Ruxton 2014).

Eyespots have been particularly well studied in Lepidoptera: in some species, eyespots appear to deflect predatory attacks towards less vital parts of the animal's body thereby facilitating escape (Ruxton et al. 2004; Olofsson et al. 2010; Vallin et al. 2011), whereas in other species, they are thought to intimidate and/or bluff predators into forgoing their attack (Vallin et al. 2005; Merilaita et al. 2011; Olofsson et al. 2013). However, why predators should direct their attacks towards eyespots, and/or be intimidated by them, has recently become a matter of controversy. Thus, although the majority of authors have long believed that eyespots exploit predators' innate fear of the eyes/faces of dangerous vertebrates (e.g., Weismann 1882; Pough 1988; Janzen et al. 2010), others have noted that we have yet to rule out the possibility that they simply exploit predators' wariness of novel and conspicuous objects (Stevens 2005; Kodandaramaiah 2011; Stevens and Ruxton 2014).

On the surface, the results from a number of early studies appear to suggest that eyespots deter predators because they resemble eyes

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(Scaife 1976a, 1976b; Jones 1980). For example, Scaife (1976a) demonstrated that eye-like shapes are more effective deterrents when placed in the context of a known threat (e.g., on a hawk) when compared with either eye-like shapes presented on their own or eye-like shapes in a novel context (e.g., on a kiwi). An eye mimicry function is also supported by a more recent study by Blut and colleagues (2012) who demonstrated that artificial prey with eyespots that had small conspicuous white sparkles at the top of the pupils (making them more eye-like by mimicking corneal light reflection) survived better than those with sparkles placed in unnatural positions (e.g., at the base or to the side of the pupils). Nevertheless, after conducting a series of field experiments with moth-like triangular targets, Stevens and colleagues argued that eyespots were most effective when they were highly conspicuous, but not necessarily when they were more eye-like (Stevens et al. 2007, 2008, 2009). Thus, although we know that conspicuousness influences the effectiveness of these markings, it remains unclear whether predators actually mistake eyespots for the eyes of dangerous vertebrates (Stevens and Ruxton 2014).

Despite the current lack of supporting evidence, it seems likely that, in some species at least, eyespots are mistaken for predatory eyes. This argument is particularly compelling for caterpillars that appear to mimic snakes. The larvae of a number of Lepidoptera species possess eyespots on either their anterior or posterior segments and inflate these segments when attacked by predators (Bates 1862; Poulton 1890; see Supplementary Figure S1 for an example). Hossie and Sherratt (2012, 2013) have recently shown empirically that both the eyespots and the defensive posture can protect caterpillar-like models from attack by wild birds in the field. Furthermore, by comparing the "head shape" and position of eyespots in putative snake-mimicking caterpillars to the head shape and position of real eyes in photographs of preserved snakes, Hossie and Sherratt (2014) demonstrated that assuming the defensive posture increases caterpillars' resemblance to snakes. However, although these results are consistent with the idea that predators mistake eyespots for snake eyes, the possibility remains that both the eyespots and defensive posture of caterpillars deter avian predators simply because these traits are novel and/or conspicuous.

To elucidate the mechanism behind the protective value of eyespots and the defensive posture in lepidopteran caterpillars, we designed experiments to disentangle the protective effects of both novelty and conspicuousness from the effects of eye- or face-like mimicry. Here, naive domestic chicks were presented with model caterpillars either without eyespots or with eyespots in 1 of 2 different positions on their bodies (Experiment 1). Likewise, we presented chicks with model caterpillars either without enlarged body segments or with enlarged segments in 1 of 2 different positions on their bodies (Experiment 2). We reason that if eye/snake mimicry rather than novelty/conspicuousness alone is responsible for the deterrent effect of both eyespots and defensive postures, then we should only see a benefit of these traits when they are present and positioned in a way that resembles a snake. Thus, we test the specific prediction that eyespots and defensive postures will deter predators more effectively when positioned anteriorly, than when positioned centrally.

MATERIALS AND METHODS
Subjects and housing

Fifty-nine newly hatched domestic chicks (*Gallus gallus domesticus*) of the "Ross" strain were purchased from a commercial hatchery in Berwickshire, UK. They were transported to the laboratory by car

(approximately 90-min drive), where they were housed in 3 cages measuring $120 \times 50 \times 50$ cm. The floor of cages was covered with wood chips, and each cage contained 2 food and 2 water hoppers. Birds were subject to a 14:10h light cycle under florescent lighting (Sylvania Luxline Plus FH049W/T5/840), and the temperature of the laboratory was maintained at 24–27 °C using 2 room heaters. Water was provided ad lib., as were chick starter crumbs, except during training and experimenting when brief periods of food (but not water) deprivation were necessary to ensure chicks were motivated to forage. All chicks were marked with nontoxic "child-friendly" colored marker pens to enable individual identification: this did not appear to have any adverse effect in chick behavior. Chicks were weighed and visually inspected by J.S. daily to ensure they remained in good health: all chicks gained weight as the experiment progressed. At the end of the experiments, chicks were euthanized by dislocation of the neck by a trained technician, as outlined in the Code of Practice for the Humane Killing of Animals under Schedule 1 to the Animals (Scientific Procedures) Act 1986. The experiments were conducted under local ethical approval from Newcastle University (ERC Project ID No. 340) and complied with both UK Home Office regulations and the Association for the Study of Animal Behaviour's Guidelines for the Treatment of Animals in Research and Teaching.

Artificial prey

Artificial caterpillars were designed to assess whether the position of either eyespots or the thickened body segments associated with the defensive posture, influenced their antipredatory benefit. We followed Hossie and Sherratt's (2013) method of producing countershaded pastry caterpillars based loosely on the appearance of the late instars of *Papilio canadensis* and *Papilio glaucus* caterpillars. We chose to do this for a number of reasons: because eyespots have been found to be more effective deterrents when placed on countershaded caterpillars (Hossie and Sherratt 2012); to maintain a degree of ecological relevance; and so that our findings could be more directly compared with those of Hossie and Sherratt (2013). We used the same pastry recipes as Hossie and Sherratt (40 mL of lard, 118 mL of flour, and either 3 drops of Leaf Green food dye in 20 mL of water to produce light green pastry or 6 drops of the same dye in 15 mL of water to produce dark green pastry), and identical ingredients, dyes, and paints (shipped to the United Kingdom from Canada). Countershaded prey with dark dorsal and light ventral sides (4 cm long with a diameter of ≈0.7 cm) was produced by pressing together dark and light pastry cylinders (4 cm long with a diameter of ≈0.5 cm).

For Experiment 1, we produced caterpillars without eyespots, with anterior eyespots (≈0.7 cm from the anterior tip of the caterpillars), and with central eyespots (see Figure 1). Prey with eyespots was produced by painting 2 yellow circles (≈0.45 cm diameter) dorsolaterally on the prey 0.4 cm apart. A black circle (≈0.3 cm diameter) was then painted inside each of these; and finally, a small white spot of pain (≈0.1 cm diameter) was added to the centre of the black circles.

For Experiment 2, we produced caterpillars with no thickening and caterpillars with either anterior or central thickening (see Figure 2). In order to produce thickened segments for use in Experiment 2, we pressed the 4-cm long caterpillars into plaster of Paris molds with either anterior or central thickening. In this way, we created prey with the same volume and color as unmolded caterpillars but just 3 cm in length. Molds used by Hossie and Sherratt
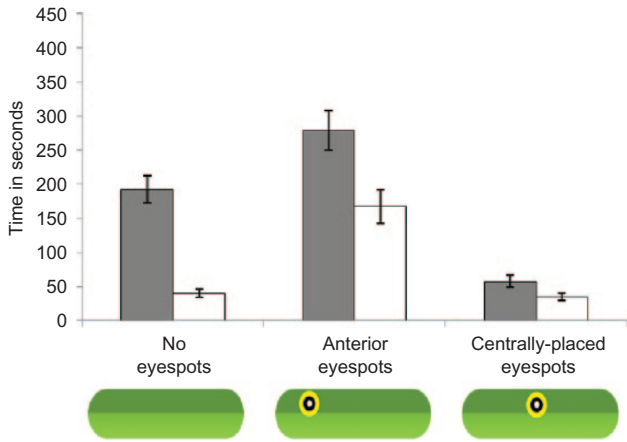
Figure 1

The mean time in seconds (±1 standard error of the mean) taken to attack the pastry caterpillar (filled bars), and between first inspecting and attacking the caterpillar (open bars), for birds in each of the 3 experimental groups (*n* = 9 per group) in Experiment 1.
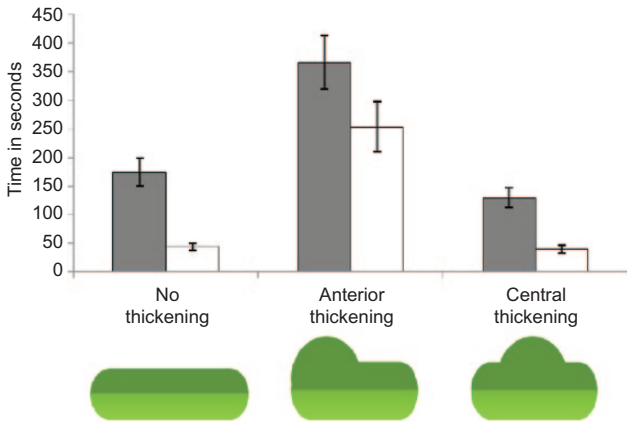

Figure 2

The mean time in seconds (±1 standard error of the mean) taken to attack the pastry caterpillar (filled bars), and between first inspecting and attacking the caterpillar (open bars), for birds in each of the 3 experimental groups (*n* = 8 per group) in Experiment 2.

(2013) were shipped to United Kingdom to ensure that our anterior-thickening treatment was identical to theirs.

Training

On Day 1 posthatch, chicks were left to acclimatize to the laboratory for 1 h before being individually color marked (see previously). On Day 2, 51 experimental chicks were trained to eat chick starter crumbs from the white paper-covered floor of an experimental arena. Two identical arenas were used, each consisting of a cage identical to the housing cages, with a section measuring $20 \times 50 \times 50$ cm partitioned off using wire mesh to create a separate "buddy arena." In all training and experimental trials, 2 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 8 individuals and were changed every 3 trials. These chicks were not given access to artificial prey at any point during the experiment.

Chicks underwent 6 training trials at regular intervals: 3 on Day 2 posthatch and 3 on Day 3 posthatch. In all trials, chick crumbs were scattered over the floor of the experimental arena, and chicks were then placed in the arena for approximately 2 min. In the first 2 trials, chicks were placed in the arena in groups of 3; in the following 2 trials, chicks were placed in the arena in groups of 2; and in the final 2 trials chicks were placed in the arena individually. In the final trial, a 50-cm long Sycamore (*Acer pseudoplatanus*) branch was placed in the centre of the arena. This was to familiarize chicks with branches in order that they did not show a neophobic response when presented with a branch in the subsequent test trial (see following section). Chicks were food deprived for 30 min before each of the training trials on Day 2 to ensure they were motivated to forage in the experimental arena. During all training trials, the 2 arenas were used simultaneously. However, during the single test trial, only 1 subject performed the experiment at any 1 time, in order to ensure that measurements were taken accurately. By the end of the training trials, all chicks had pecked the branch and were eating crumbs in the experimental arena. At the end of training, birds were split into 2 groups: 27 chicks went on to take part in Experiment 1, whereas the remaining 24 went on to take part in Experiment 2. Both experiments were run concurrently, and the same 8 buddy chicks were used in both experiments.

Experiment 1: does the position of eyespots influence their antipredator efficacy?

On Day 4, the 27 birds that were to be the subjects of Experiment 1 were randomly assigned to 1 of 3 experimental groups (*n* = 9 per group). Birds in all 3 groups received a single test trial: they were food deprived for 30 min before being placed in the experimental arena individually. Here, each chick encountered a Sycamore branch (50 cm in length) supporting a single pastry caterpillar. Caterpillars were presented on a branch for a number of reasons: to reflect (to a limited degree) the natural background against which many caterpillars with eyespots are viewed; to increase comparability with previous studies (Hossie and Sherratt 2012, 2013); and to ensure chicks did not stand on prey (which could potentially have caused prey to become misshapen or displaced in a manner that obscured the eyespots). The branch was located in the centre of the arena, with 1 end 25 cm from the buddy arena and the other 25 cm from the rear wall of the experimental arena. Chicks were placed in a central position immediately in front of the rear wall of the experimental arena. The pastry caterpillars were attached to the branches using pins pushed into the branches in random positions. The tops of the pins were cut off leaving approximately 3 mm protruding from the branches, onto which pastry caterpillars were impaled.

The type of caterpillar that birds encountered differed among our 3 experimental groups: 1 group received a caterpillar without eyespots; 1 group received a caterpillar with anterior eyespots; and 1 group received a caterpillar with centrally placed eyespots. Birds remained in the arena until they attacked the caterpillar (maximum time = 417 s). The latency to first inspect the caterpillar (an unambiguous behavior in which chicks tilt their head dramatically to 1 side and view the caterpillar monocularly), the latency to attack the caterpillar, and the presence/absence of approach-retreat behavior (repeatedly approaching and retreating from the caterpillar) were recorded contemporaneously by the experimenter. Latency to first inspection is not an accurate measure of when the prey items were first detected, but instead is a measure of when birds first showed interest in prey. We, therefore, subtracted this value from

the latency to attack prey in order to obtain a measure of predator wariness. These measures were chosen because they were unambiguous, which was particularly important given that they were recorded by an observer that could also see the prey item. Finally, prey was examined and the position of the beak mark (produced by the chick attack) was classified as being in either one of the end thirds or in the middle third of the prey item. In 2 cases, the beak mark was on the boundary between 2 different sections, and so was classified as being in the section in which the majority of the beak mark occurred.

Experiment 2: does the position of thickened body segments influence their antipredator efficacy?

On Day 4, the 24 birds that were to be the subjects of Experiment 2 were randomly assigned to 1 of 3 experimental groups ($n = 8$ per group). Birds in all 3 groups received a single test trial identical to that described in Experiment 1. However, the type of pastry caterpillar that birds encountered in the experimental arena differed: 1 group received a caterpillar with no thickening; 1 group received a caterpillar with anterior thickening; and 1 group received a caterpillar with thickening in the centre of the body. Again, birds remained in the arena until they attacked the caterpillar (maximum time = 600 s), and the latency to first inspect the caterpillar, the latency to attack the caterpillar, the presence/absence of approach-retreat behavior, and the position of beak marks on prey were recorded.

Statistical analyses

We used the time between first inspection and attack as a measure of how wary birds were of the caterpillar; and the overall latency to attack as a measure of the net antipredator benefit of the caterpillars' visual appearance. In both experiments, the within-group variances in these measures exhibited heteroscedasticity, so data were log$_{10}$ transformed. The resulting data met the assumptions of parametric tests: normality (Kolmogorov–Smirnov tests, $P > 0.497$ for all groups) and homoscedasticity (Levine's tests $P > 0.663$ for all measures). We then used analysis of variance (Anova) to determine whether the resulting measures differed among our experimental groups; and Bonferroni corrected t-tests to determine between which groups differences lay. Finally, we used Fisher's Exact tests to determine whether the number of birds showing approach-retreat behavior differed among experimental groups and Binomial tests to determine whether birds had a tendency to direct attacks toward particular areas of prey.

RESULTS

Experiment 1: eyespot presence and position

Our measure of wariness (the time between first inspections and attack) differed significantly among the 3 experimental groups (Anova, $F_{2,24} = 30.356$, $P < 0.001$; see Figure 1). We could not reject the null hypothesis that birds given caterpillars without eyespots were equally wary as birds given caterpillars with centrally placed eyespots (Bonferroni corrected t-tests with modified α of 0.0167; $t_{16} = 0.870$, $P = 0.397$). However, birds given caterpillars with anterior eyespots were significantly more wary than birds given either artificial caterpillars lacking eyespots or caterpillars with centrally placed eyespots ($t_{16} = 7.073$, $P < 0.001$; $t_{16} = 6.743$, $P < 0.001$, respectively).

Similarly, the number of birds showing approach-retreat behavior did not differ between the group given caterpillars without eyespots and the group given caterpillars with centrally placed eyespots (Fisher's Exact test, $P = 1$). However, significantly more birds showed approach-retreat behavior in the group given caterpillars with anterior eyespots than in the group given caterpillars without eyespots ($P = 0.0152$) or in the group given caterpillars with centrally placed eyespots ($P = 0.0023$).

The latency to attack the pastry caterpillars also differed significantly among our experimental groups (Anova, $F_{2,24} = 47.191$, $P < 0.001$; see Figure 1). Although birds given caterpillars without eyespots and birds given caterpillars with anterior eyespots did not differ significantly in the time taken to attack the caterpillars (Bonferroni corrected t-test with modified α of 0.0167; $t_{16} = 2.403$, $P = 0.029$), birds in both of these groups took significantly longer to attack the caterpillar than birds given caterpillars with centrally placed eyespots ($t_{16} = 6.751$, $P < 0.001$; $t_{16} = 9.072$, $P < 0.001$, respectively).

Finally, more chicks than expected by chance (33%) attacked prey in the central third of their bodies when prey had no eyespots (6 of the 9 chicks pecked the centre; Binomial test, $P = 0.04$, $n = 9$) and when prey had central eyespots (8 of the 9 chicks; $P = 0.001$, $n = 9$). However, when prey had anterior eyespots, more chicks than expected by chance attacked the end of the prey that contained the eyespots (7 of the 9 chicks; $P = 0.008$, $n = 9$). Thus, in the absence of eyespots, chicks tended to attack the centre of prey, but when eyespots were present, they tended to direct attacks towards the eyespots.

Experiment 2: head presence and position

As in the previous experiment, our measure of wariness differed significantly among experimental groups (Anova, $F_{2,21} = 32.447$, $P < 0.001$; see Figure 2). We could not reject the null hypothesis that birds given caterpillars without any thickening and birds given caterpillars with central thickening were equally wary of caterpillars (Bonferroni corrected t-tests with modified alpha of 0.0167; $t_{14} = 0.498$, $P = 0.626$). In contrast, the birds given caterpillars with anterior thickening were significantly more wary than both the birds given caterpillars without any thickening and the birds given caterpillars with central thickening ($t_{14} = 6.648$, $P < 0.001$; $t_{14} = 7.045$, $P < 0.001$, respectively).

Similarly, the number of birds showing approach-retreat behavior did not differ significantly between the group given caterpillars without thickening and the group given caterpillars with central thickening (Fisher's Exact test, $P = 1$). However, significantly more birds showed approach-retreat behavior in the group given caterpillars with anterior thickening than in either the group given caterpillars without thickening ($P = 0.0014$) or the group given caterpillars with central thickening ($P = 0.007$).

The latency to attack the pastry caterpillars also differed significantly among our experimental groups (Anova, $F_{2,21} = 15.689$, $P < 0.001$; see Figure 2). Birds given caterpillars without any thickening and birds given caterpillars with central thickening took a similar amount of time to attack the caterpillar (Bonferroni corrected t-tests with modified α of 0.0167; $t_{14} = 1.553$, $P = 0.143$), whereas birds in both these groups attacked caterpillars significantly sooner than birds given caterpillars with anterior thickening ($t_{14} = 3.903$, $P = 0.002$; $t_{14} = 5.436$, $P < 0.001$, respectively).

Finally, more chicks than expected by chance (33%) attacked prey in the centre of their bodies when prey had no thickening (7 of the 8 chicks; Binomial test, $P = 0.002$, $n = 8$). However, when

prey had either central or anterior thickening, all of the chicks directed their attacks towards the area of the prey where the thickened and nonthickened sections of the prey met.

DISCUSSION

Both the eyespots and the defensive postures of many Lepidoptera larvae could deter predators because they mimic the eyes/head of predators' own predators (mimicry hypothesis), or because their conspicuousness induces wariness, sensory overload, and/or biases (conspicuous signal hypothesis). In a recent thoughtful review, Stevens and Ruxton (2014) argued that the majority of previous work was not appropriately designed to discriminate between these competing hypotheses and highlighted the need for further research in this area. Our experiments clearly demonstrate that predators are more wary of prey (i.e., they inspected them for longer and performed more approach-retreat behaviors) with eyespots and with thickened sections, but only when these traits are placed in an anterior position consistent with the position of the head in dangerous vertebrates. In fact, predators were no more wary of prey with centrally positioned eyespots or thickened sections than prey that did not possess these features. It is difficult to conceive any way in which this positioning effect can be explained by a change in conspicuousness of the stimuli. In the absence of eyespots/thickening, chicks showed a significant tendency to direct their attacks towards the centre of prey, so the deterrent effect of anterior eyespots cannot be explained by assuming that conspicuous markings are a more effective deterrent when located in the position in which predators usually attack prey. Conversely, anterior eyespots are unlikely to function by drawing attention away from preferred attack sites. If this were the case, we would expect centrally positioned eyespots to reduce prey wariness by drawing attention towards preferred attack sites, and this was not observed. However, the anterior positioning of the eyespots and the thickened section inevitably have the effect of making the artificial caterpillars appear more akin to a hostile vertebrate (e.g., a snake). Combined with the demonstration that the "head" shape and the position of the eyespots of many caterpillars in their defensive posture align with the head shape and location of the real eyes of snakes (Hossie and Sherratt 2014), we now have strong empirical support for a mimetic function of these traits in caterpillars. Furthermore, because the predators used in our experiment were naive to both their own potential predators and caterpillar-like prey, we can also conclude that these traits function by exploiting predators' *unlearned* aversions to stimuli that resemble the eyes and heads of vertebrate predators.

Although our results clearly demonstrate that anterior eyespots induced wariness in naive predators, we did not find compelling evidence that they delayed predatory attacks relative to the no-eyespot control group. It may be that although prey with anterior eyespots benefit from inducing wariness in predators, this effect is counteracted by concurrent reductions in time to detection relative to prey without eyespots. Consistent with this explanation, predators were quicker to attack prey with centrally positioned eyespots than prey without eyespots, suggesting that the former prey are more conspicuous but fail to elicit additional predator wariness. In isolation, these results suggest that the anterior eyespots and no-eyespots (i.e., less conspicuous) phenotypes represent distinct, but approximately equivalent defenses. However, it is worth noting that our experimental paradigm likely provides a conservative estimate of the deterrent effect of eyespots because chicks were unable to flee the experimental arena and trials continued until chicks attacked the

caterpillar model they were presented with. In contrast, wild birds react to eye-like features through a "startle" response coupled with distancing and are then unlikely to risk being attacked themselves by returning for additional investigation (Janzen et al. 2010).

Our results also help us to understand the evolution of the defensive postures seen in many Lepidoptera larvae. We found that anterior thickening both enhanced predator wariness and delayed predatory attacks. This finding is in line with a previous field study that demonstrated that artificial caterpillars with anterior thickening survived better than those without (Hossie and Sherratt 2013) and suggests that selection will favor defensive postures that result in anterior thickening. In contrast, predators' latencies to attack prey with central thickening were similar to their latencies to attack prey without thickening. One explanation for this is that like the defensive postures of caterpillars (Hossie and Sherratt 2014), anterior (but not central) thickening may have increased the resemblance of our models to snakes by creating a distinct "head" shape. Intriguingly, these results also suggest that, unlike eyespots, there do not seem to be significant costs associated with increased detectability resulting from swollen body segments, anterior or otherwise. Thus, although there is likely to be strong selection against prey with eyespots in "inappropriate" (i.e., in central segments) positions, thickening in inappropriate positions simply fails to provide any adaptive value.

Because we did not manipulate the position of thickened segments and the position of eyespots simultaneously, we are unable to say how these factors may interact to influence the efficacy of snake mimicry. In a recent field experiment using pastry caterpillars, Hossie and Sherratt (2013) found that both the presence of eyespots and anterior thickening reduced predatory attacks, but there was no synergistic effect of these 2 traits. Stevens and Ruxton (2014) claimed that such a synergistic effect would be expected if the presence of eyespots and the defensive posture contributed to enhancing snake mimicry in caterpillars. However, we do not consider the presence or absence of interactions between traits as a critical prediction of the mimicry hypothesis. Failure to observe a subsequent increase in protection when both traits are present could result simply from prey reaching maximal protection from either trait alone, distinct subsets of the predator guild utilizing distinct features as predator cues, or overshadowing effects where predators use a few salient features to classify prey and ignoring other possibly informative features (Aronsson and Gamberale-Stille 2012; Gamberale-Stille et al. 2012; see also Valkonen et al. 2011). Understanding how multiple mimetic traits interact to influence the efficacy of mimicry, therefore, remains an interesting avenue for future research, and if paired with phylogenetic analyses could shed light on the order in which these traits likely evolve.

Overall, we hope we have risen to the challenge laid down by Stevens and Ruxton (2014), but there is more that could be done. In particular, our experiments used wariness and attack latency as measures of the efficacy of prey defenses. Consequently, all of the prey items in our experiment were "killed." Studies monitoring the survival of caterpillars in the field (e.g., Hossie and Sherratt 2012, 2013) are therefore needed, in order to determine how differences in wariness/attack latency in the laboratory translate to differences in survival (and thus selective pressures) in natural settings where predators are free to disperse and find alternative prey. Furthermore, our data suggest that birds direct their attacks towards 1) eyespots and 2) the areas where "heads" meet "bodies." This may be because chicks tend to direct attacks towards small contrasting spots (Osorio et al. 1999); or alternatively, this could be

an adaptive strategy for dealing with potentially dangerous prey, and it would be worth investigating whether birds direct attacks toward comparable body areas in snakes and caterpillar mimics. Finally, our artificial caterpillars were immobile, and yet, many caterpillars react behaviorally when approached by predators. Some caterpillars have elaborate displays that seem more like behavioral mimicry than simply a startle response. For example, *Hemeroplanes* sp. (Sphingidae) caterpillars are among the most convincing snake mimics morphologically, and when threatened, they adopt a rigid curve to their body and proceed to sway from side-to-side until the threat diminishes (Moss 1920; Hossie TJ, personal observation). Moreover, many caterpillars only display their eyespots when threatened with attack, either because the eyespots are hidden within folds of their cuticle or because the entire caterpillar is hidden within a leaf shelter (Janzen et al. 2010). It is possible that through using static artificial prey, we have effectively overlooked key behaviors, startle or otherwise, that have a strong influence on the adaptive value of eyespots, and ongoing work is evaluating the role of behavior in eliciting the intimidation effect.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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