



Defensive posture and eyespots deter avian predators from attacking caterpillar models



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ARTICLE INFO

Article history:

Received 31 January 2013

Initial acceptance 6 March 2013

Final acceptance 6 May 2013

Available online 18 June 2013

MS. number: A13-00102

Keywords:

antipredator

caterpillar

eyespot

mimicry

morphology

protective coloration

Many lepidopteran caterpillars appear to gain protection from insect-eating birds because they possess eyespots, a pair of conspicuous markings on the body generally thought to resemble the eyes of a predator. Similarly, many caterpillars widen their anterior body segments when threatened, and this defensive posture may also deter attack because it emphasizes the caterpillar's eyes and/or allows the caterpillar to resemble a snake more closely. Yet, given that this behaviour is not limited to caterpillars with eyespots, it is possible that the defensive posture may serve as a predator deterrent in itself. To disentangle the protective value of eyespots and widened anterior body segments, we created four types of artificial caterpillar in a 2×2 factorial design and deployed them on tree branches in the field where they were exposed to predation attempts by wild birds. The 'survivorship' of the artificial caterpillars was monitored over 90 h in six locations. We found a clear survival advantage for artificial prey with eyespots or a widened head shape. However, prey possessing both eyespots and a widened head did not survive longer than prey with either characteristic alone, suggesting that the traits did not act synergistically to deter attack. Interestingly though, only caterpillars in the eyespot-defensive posture treatment received more pecks directed towards the anterior 'head' region. Collectively, this study demonstrates clearly that caterpillars possessing either eyespots or widened anterior body segments are likely to experience reduced predation by wild birds, even at northern latitudes where there are relatively few arboreal snakes.

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The caterpillars of many lepidopteran species possess a pair of conspicuous eyespots. These eye-like markings are thought to protect caterpillars because they resemble the eyes of a predator and thereby deter the attacks of insect-eating birds (Janzen et al. 2010). To human eyes, eyespots do not always confer a close resemblance to any given model, and need not, since receivers of the signal need to respond quickly and the caterpillar is often partly concealed (Janzen et al. 2010). However, sometimes the extent of mimicry is compelling. Indeed, one of Darwin's contemporaries, Henry Walter Bates, described his encounter with one particular species of snake-mimicking caterpillar as 'The most extraordinary instance of imitation that I ever met...[the caterpillar] startled me by its resemblance to a small snake...' (Bates 1862, page 509).

Recently we demonstrated empirically that eyespots can provide caterpillars with protection from wild avian predators in the field (Hossie & Sherratt 2012). This study also showed that other aspects of prey body colour (i.e. countershading) affect how well

eyespots deter avian attack (Hossie & Sherratt 2012). Indeed, many eyespotted caterpillars possess additional traits or body patterns that resemble other features of a head or face that may help to place the spots in a more eye-like context to the receiver (Janzen et al. 2010). One such trait that may influence the protective value of eyespots is the characteristic defensive posture that many of these caterpillars adopt when threatened. This posture often involves widening the anterior body segments near the eyespots, which may function to deter an attack for a number of reasons, including emphasizing eyespots, threatening the potential predator and enhancing the subject's overall resemblance to snakes (Fig. 1a, c). Bates observed this first hand: 'The first three segments behind the head were dilatate at the will of the insect, and had on each side a large black pupillated spot, which resembled the eye of the reptile...' (Bates 1862, page 509). To our knowledge, the extent to which this defensive posture enhances the protection afforded by eyespots has never been examined experimentally.

Among large caterpillars the facultative widening of anterior body segments when threatened is relatively common. Many lepidopteran larvae possess both eyespots and the ability to inflate their anterior body segments (e.g. *Hemeroplanes ornatus*, *Madoryx oclus*, *Papilio troilus*), but it also occurs in species where eyespots

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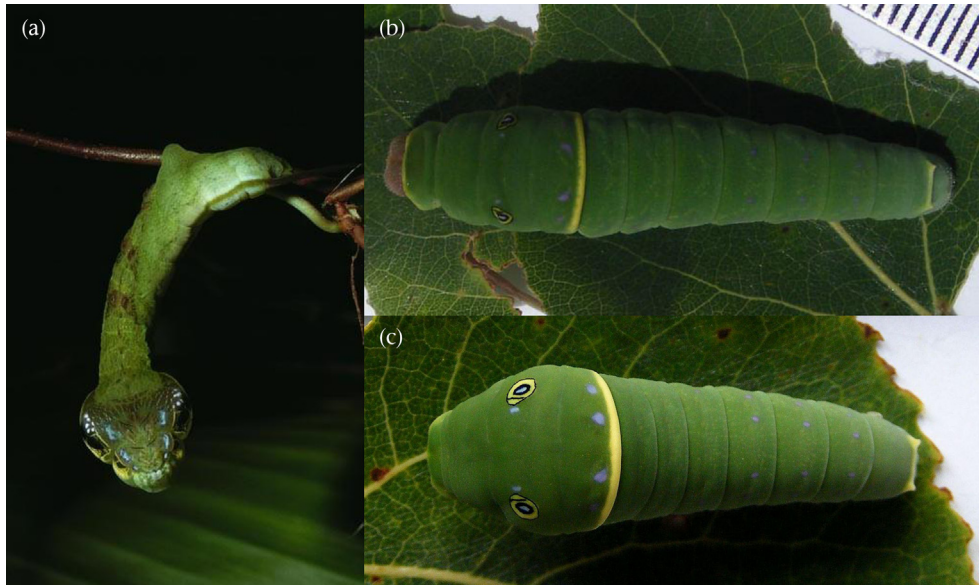


Figure 1. (a) Penultimate instar of a Costa Rican *Hemeroplanes triptolemus* (Sphingidae) caterpillar displaying its defensive posture; what appears to be the dorsal side of the snake head is in fact the ventral side of the caterpillar thorax. Photo is from Janzen & Hallwachs (2012), specimen number 03-SRNP-11366 (used with permission). Ultimate instar *Papilio canadensis* caterpillar in (b) resting posture and (c) defensive posture (photos by T. J. Hossie).

are poorly developed or absent altogether (e.g. *Eumorpha phorbis*, *Papilio cresphontes*). In the several cases we have reviewed, expanding the anterior body segments does tend to enhance the 'head-like' appearance (to humans) of the caterpillar's dorsal segments, particularly in those caterpillars that do have eyespots (Fig. 1b, c). Interestingly, several 'nonvenomous' colubrid snakes behaviourally modify their head shape when threatened, a process known as head triangulation, which is generally thought to increase mimetic resemblance to vipers (Werner & Frankenberg 1982; Werner 1985, 1986). Recent work has revealed that some bird species are less likely to attack artificial snakes with triangular 'viper-shaped' heads, compared to prey with a narrower 'colubrid-shaped' head (Valkonen et al. 2011). Of course, vipers are not the only snakes that pose a threat to insect-eating birds: many non-viperine snakes pose an equivalent threat to these birds (some of which also have a triangular-shaped head). We emphasize that the inflated anterior body segments of these caterpillars need not resemble the triangular head of a viper specifically to gain protection; a better approximation to any predator of an insect-eating bird could be sufficient to favour the evolution or maintenance of these traits.

Although Janzen et al. (2010) provided strong indirect evidence that caterpillars deter avian predators by combining eyespots and behaviour, to date direct tests have been poorly replicated and/or lacking an appropriate ecological context (but, for examples with adult Lepidoptera, see: Vallin et al. 2006, 2007; Kodandaramaiah et al. 2009; Merilaita et al. 2011). Specifically, Poulton (1890) described the defensive postures of elephant hawkmoth (*Deilephila elpenor* and *Deilephila porcellus*) caterpillars, which have eyespots, as well as the results from staged presentations of these caterpillars to birds (Poulton 1890). Briefly, these tests indicated no protection from a tame jay, weak avoidance of the caterpillar by domestic fowl, and the deterrence of small birds (e.g. 'sparrows') from a feeding tray containing seed or bread crumbs (Poulton 1890). Additionally, Shiota (1980) observed that six captive starlings (*Sturnus cineraceus*) feared, and did not attack, live final-instar *Deilephila elpenor lewisii* caterpillars within 3 min, although this work suffered from various weaknesses in experimental design. Together these results suggest that eyespots and the defensive

posture may deter bird attacks, yet well-replicated field studies are clearly better suited to measure their realized protective value.

In general, the soft bodies of caterpillars are quite vulnerable to peck damage, so it seems unlikely that eyespots of caterpillars deflect attacks to assist their escape (Janzen et al. 2010). Yet, it seems possible that eyespots could direct the strikes of an attacking bird to areas of the body more resistant to damage, or facilitate the function of chemical defences. For example, Blest (1957) proposed that the eyespots in *Papilio* caterpillars might direct strikes towards the osmeterium, a specialized defensive structure that releases volatile chemicals (Frankfater et al. 2009). Indeed we have shown previously that eyespots influence the distribution of pecks on artificial caterpillar prey (Hossie & Sherratt 2012); however, it remains unclear whether a caterpillar's defensive posture augments such an effect. To examine the protective value of eyespots and inflated anterior body segments (i.e. modified 'head' shape) in caterpillars we deployed caterpillar models in the field and monitored their 'survival'. Treatments were arranged in a 2×2 factorial design, which allowed us to determine (1) whether eyespots or inflating anterior body segments effectively protect caterpillars from avian predators, and (2) whether combining these defences further increases their ability to deter attack.

METHODS

Artificial Prey

Artificial caterpillars were designed to assess whether eyespots and adopting a defensive posture (i.e. thickening of the anterior body segments) protect caterpillars from attack by wild birds such as those species listed in Supplementary Table S1. Manufacture of artificial caterpillars followed methodology similar to previous studies (e.g. Church et al. 1997; Rowland et al. 2008), and for ecological relevance our models were based loosely on the late instars of *Papilio canadensis* and *Papilio glaucus* caterpillars. A number of species in *Papilio* (Papilionidae) have caterpillars with conspicuous eyespots and adopt a presumably defensive posture when approached. Specifically, these caterpillars withdraw their true head into their thorax, or curl it under their body, while

inflating their anterior body segments (Fig. 1b, c; T. J. Hossie, personal observation).

Caterpillar models comprised four treatments: (1) no eyespots-resting posture; (2) no eyespots-defensive posture; (3) eyespots-resting posture; (4) eyespots-defensive posture. We have shown previously that eyespots effectively protect these artificial caterpillars from bird attacks only when the caterpillars are also countershaded (Hossie & Sherratt 2012), as commonly observed (de Ruiter 1956; Lederhouse 1990). We thus decided to make all treatments similarly countershaded. Briefly, pastry caterpillars were manufactured with a 3:1 mixture of white flour and lard. From this, light green pastry was produced by adding three drops of Leaf Green AmeriColor™ Soft Gel Paste™ (AmeriColor Corp., Placencia, CA, U.S.A.) and 20 ml of water to 40 ml of lard and 118 ml flour, whereas six drops of gel paste and 15 ml of water were added to the same volumes of flour and lard to produce the dark pastry. Dark and light pastry was rolled into 0.5 cm diameter strips, which were then cut into 4 cm lengths. Two-toned (i.e. countershaded) prey were then created by pressing together one piece of dark and light pastry such that the final artificial prey were cylinders 4 cm long with a diameter of ~0.7 cm. For our purposes the dark side was considered dorsal as prey were presented on the upper side of branches. To create prey with a modified 'head' shape, representing the caterpillar's defensive posture, half of these two-toned models were pressed into a mould made of plaster of Paris. This process created models that were equivalent to the unmoulded prey in volume and colour, but were 3 cm in length and asymmetrically widened on one end (Fig. 2b, d). The eyespot design was modified from Hossie & Sherratt (2012) such that here the spots were slightly smaller and had a central bright iris, so as to be more representative of the eyespots on *Papilio* caterpillars. We used Elmer's™ Non-toxic Tempera Paint (Elmer's Products, Inc., Westerville, OH, U.S.A.) to make eyespot treatments. First, two yellow circles (~0.45 cm in diameter, 1:1 mix of yellow and white paint) were painted dorso-laterally on the prey, 0.4 cm apart. Resting- and defensive-posture

treatments were different lengths (i.e. 4 cm and 3 cm, respectively); thus, we painted eyespots ~0.7 cm from the anterior tip for 'resting-posture' models, but only ~0.5 cm from the anterior tip of 'defensive-posture' models to maintain roughly equivalent proportional distance to the anterior end. Excess yellow paint was removed from the centre of the spots using a thin paintbrush and a 0.3 cm black circle was painted inside each. Finally, a small white spot of paint ~0.1 cm in diameter was added to the centre of the black circle. A dorsal view of an eyespot caterpillar is provided in Supplementary Fig. S1. Finished pastry caterpillars (e.g. Fig. 2) were checked against a standard model prey for each treatment to ensure uniformity in size and shape of the body, as well as the size and position of eyespots.

Site Description

Field trials were conducted at three sites in Ontario, Canada (site 1: near the Ferguson Forest Centre, Kemptville, 45°03'00"N, 75°39'44"W; site 2: the University of Guelph, Kemptville Campus, 44°59'48"N, 75°38'22"W; site 3: Oxford Mills, 44°56'27"N, 75°38'56"W). Experiments were run between 14 June and 31 August 2012, which overlaps with the dates when eyespotted *P. canadensis* (Fig. 1b, c) and *P. glaucus* caterpillars can be found in the wild in our study area (Hall et al. 1996; T. J. Hossie, personal observation). All three sites were composed of secondary growth mixed-deciduous forest. Each site was no closer than 5.5 km from another site. Preliminary scouting of each site confirmed the presence of *P. canadensis* adults and several species of insectivorous birds. Insectivorous bird species observed in each of the field locations are detailed in Supplementary Table S1.

Prey Presentation

Pastry caterpillars were deployed between 1600 and 1900 hours on the first day of a replicate site. Specifically, 24 trees were

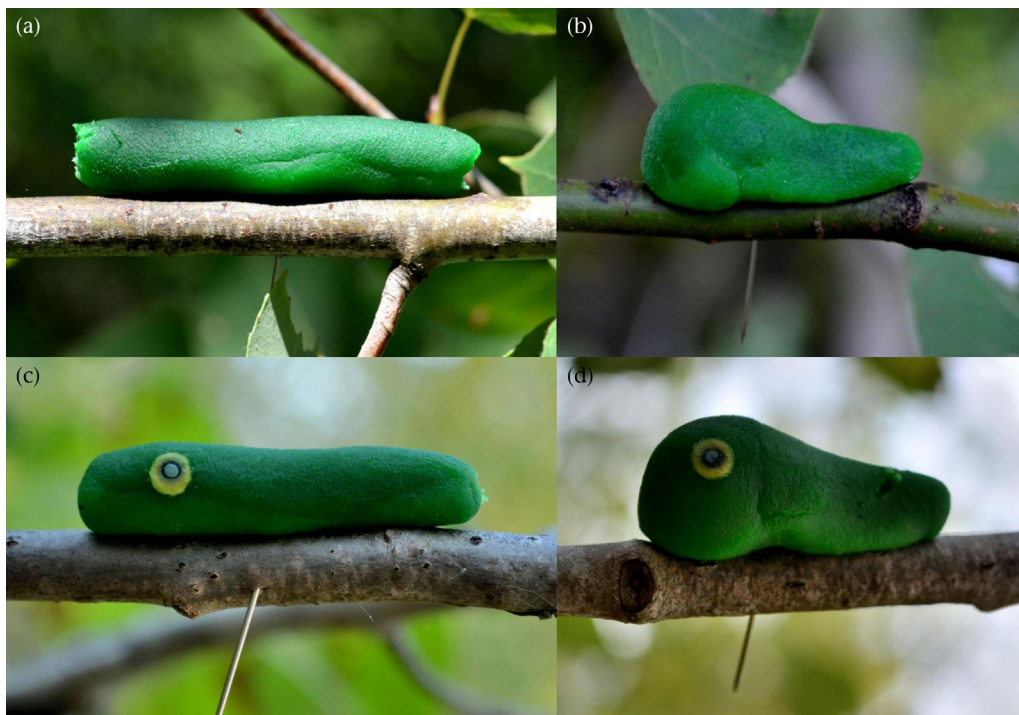


Figure 2. Examples of pecked pastry caterpillars from each of the four treatments used in this experiment: (a) no eyespots-resting posture; (b) no eyespots-defensive posture; (c) eyespots-resting posture; (d) eyespots-defensive posture.

selected along a linear transect (~10 m apart), and to each tree a single artificial prey was pinned on the upper side of a live branch in the north, south, east and west zone of a tree. Trees were restricted to one of four species (*Populus tremuloides*, *Populus deltoides*, *Betula papyrifera*, *Acer rubrum*; Table S2), which have all been identified as a host species of 'snake mimic' *Papilio* caterpillars (Scott 1986; Scriber et al. 1995; Allen et al. 2005). Each tree received a single pastry caterpillar from each treatment and branch zone (i.e. north, south, east, west), allocated randomly. Attaching four prey to each tree has the benefit of reduced variation in microhabitat and equal likelihood of encounter among treatments. Selected branches were 1–2 m from the ground and were roughly 0.5–1 cm in diameter. Pastry caterpillars were considered killed if they were no longer present or had been attacked by a bird (peck marks present; e.g. Fig. 2, Fig. S1). 'Survival' was checked at 14, 19, 24, 38, 43, 48, 62, 67, 74 and 90 h from deployment. In all cases of missing baits, the pin (invariably still stuck in the tree branch) was found and the ground underneath was searched for the bait or any remaining pieces of an attacked bait. All attacked prey were photographed, then removed. Artificial prey damaged by rain, slugs or ants were removed ($N = 111$) and considered censored (i.e. considered to have survived only to that time period, but not considered killed) in the final analysis. After 90 h, remaining prey were removed.

We repeated the entire process six times (two times per site, albeit at a different location within site) for a total of 576 artificial prey deployed. Each consecutive replicate was conducted in an entirely new location within one of the three larger sites and was at least 200 m from any previous location within that site. To avoid temporal confounds among sites, consecutive replicates were always conducted at a different site in a systematic order (i.e. site 1, location 1; site 2, location 1; site 3, location 1; site 1, location 2, etc.).

Analyses

We analysed the survival of artificial prey using Cox (1972) proportional hazards regression, which can handle both censored data and nonuniform changes in predation risk. Since pastry caterpillars were pinned singly on branches, individual caterpillars may well have been encountered independently on the trees (e.g. Rowland et al. 2008); however, we controlled for the possible lack of independence between baits on the same tree by clustering the baits by tree in our analysis. We assessed overall significance of the model using the Wald test, which does not assume independence of observations within a cluster. Factors in our model included head shape (i.e. resting versus defensive posture), eyespot (i.e. eyespots versus no eyespots) and the head shape \times eyespot interaction. The effects of location and tree species were not of direct interest, and we did not have a priori hypotheses regarding their effect on survival. However, preliminary analysis indicated that the hazard rate differed among tree species and locations (see Figs S2, S3). We therefore stratified the analysis to permit separate baseline hazard rates for location and tree species. This analysis was conducted in R (R Development Core Team 2008) using the survival library (Therneau & Lumley 2008), and the assumption of proportionality was tested using the `cox.zph` function.

We quantified the number and location of bird pecks on pastry caterpillars by analysing digital photographs of all retrieved baits that showed evidence of being attacked. For each pastry caterpillar with eyespots, we counted the number of pecks in each of six evenly sized continuous sections: anterior tip, body section with eyespots, mid-anterior body, mid-posterior body, hind mid-posterior body, and posterior tip. For the no eyespots-resting posture treatment, we counted the number of pecks in each body section, with the anterior–posterior designation assigned randomly by a coin toss. To determine whether the number of

pecks differed between body sections and between treatments, we used a generalized mixed effects model with a Poisson error distribution and log link function. We included section as a fixed factor and set individual as a random factor to control for the lack of independence of multiple pecks on a given caterpillar. The significance of a factor was examined by comparing models with and without the parameter of interest using a likelihood ratio test. We also compared the fit of our models using Akaike's Information Criterion (AIC). These analyses were conducted in R (R Development Core Team 2008) using the `lmer4` package (Bates et al. 2011).

RESULTS

The 90 h mortality rate ranged from 28% to 89% per location (mean \pm SE: $63.6 \pm 9.12\%$). Hazard rate was significantly reduced by the main effects of both head shape ($z = -2.65$, $P = 0.008$) and eyespots ($z = -1.98$, $P = 0.048$), but there was no significant interaction between head shape \times eyespot ($z = 1.72$, $P = 0.086$). The modified head shape reduced hazard by $23.6 \pm 10.1\%$, whereas possessing eyespots reduced hazard by $18.5 \pm 10.4\%$ (Table 1). To assess the relative survival rates of the four treatments, we conducted pairwise comparisons of the four treatments (Fig. 3). This analysis revealed that the 'no eyespots-resting position' treatment suffered the highest predation rate, with comparatively lower hazards arising in the 'no eyespot-defensive posture' treatment ($22.1 \pm 9.6\%$ reduction; Wald = 6.84, $P = 0.009$) and the 'eyespot-defensive posture' treatment ($21.1 \pm 10.9\%$ reduction; Wald = 4.75, $P = 0.029$; Fig. 3). The reduction in hazard between the 'eyespot-resting posture' treatment and 'no eyespots-resting position' treatment approached significance ($17 \pm 10.4\%$ reduction; Wald = 3.63, $P = 0.057$; Fig. 3). Classifying only prey recovered with peck marks as killed (i.e. considering all missing prey as censored) produced qualitatively similar results (Table S3).

Of the 364 prey assumed killed, we recovered 185 (50.8%) that had been pecked by birds in the field. Overall, a similar proportion of caterpillars disappeared, and were presumably attacked, in all four treatments (mean proportion \pm SE = 0.48 ± 0.05 , $F_{3,20} = 0.486$, $P = 0.70$). However, the total number of pecks on each caterpillar model was affected by both eyespots (mean number of pecks \pm SE: no eyespots: 4.39 ± 0.38 ; eyespots: 6.13 ± 0.57 ; $F_{1,181} = 8.26$, $P = 0.005$) and head shape (rest: 4.31 ± 0.34 ; defensive: 6.25 ± 0.60 ; $F_{1,181} = 6.51$, $P = 0.012$), but there was no significant interaction between eyespot and head shape ($F_{1,181} = 0.02$, $P = 0.88$). More pecks were directed towards the section of the prey with eyespots in the defensive posture when compared to an equidistant position at the other end (i.e. section 5: $z = 3.119$, $P = 0.002$) and when compared to the same position (i.e. section 2) on defensive-posture prey lacking eyespots ($z = 3.194$, $P = 0.0011$;

Table 1

Results from fitting Cox proportional hazards regression model of pastry caterpillar survival stratified by location and tree species

	Coef	Exp(coef)	SE(coef)	Robust SE	z	P
Eyespots	-0.2049	0.8147	0.1499	0.1037	-1.976	0.0481
Head shape	-0.2687	0.7644	0.1469	0.1014	-2.65	0.00805
Eyespots \times head shape	0.2274	1.2553	0.2129	0.1326	1.715	0.0864

Parameter estimates and associated significance tests depict how eyespots and posture affect survival of pastry caterpillars. Since fates of pastry caterpillars that were pinned to the same tree were not necessarily entirely independent, we clustered caterpillars that were pinned to the same tree in the analysis. In our analysis we used a binary coding system (i.e. 0/1) for our factors indicating the absence/presence of eyespots or a widened head shape.

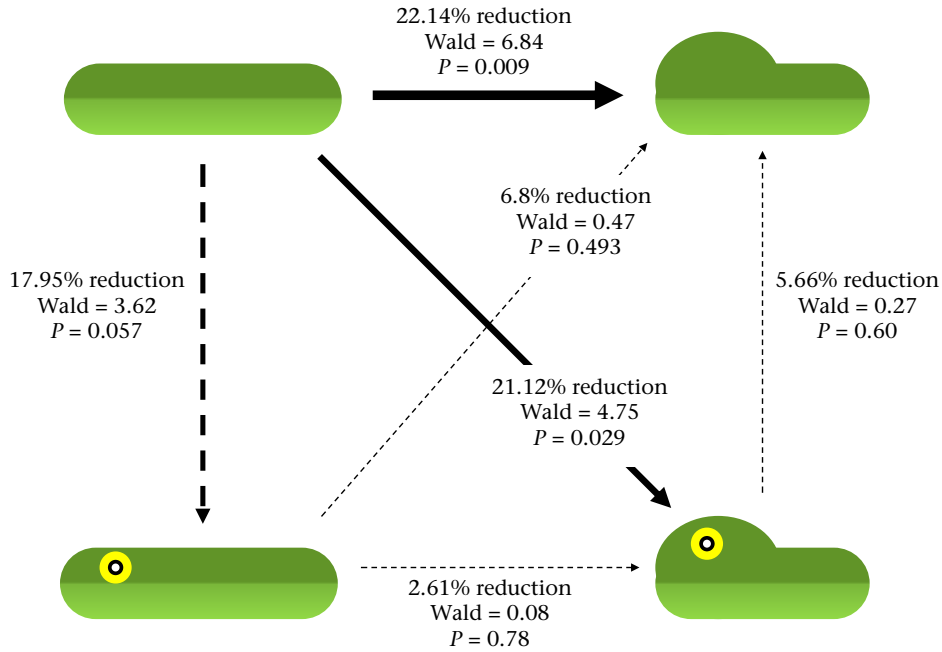


Figure 3. Results from pairwise Cox proportional hazard regression comparisons of pastry caterpillar survival stratified by tree species and location, and clustered by the tree to which pastry caterpillars were pinned. Eyespot (i.e. no eyespots versus eyespots present) and head shape (i.e. resting posture versus defensive posture) treatments were arranged in a 2×2 factorial design. Arrow width is proportional to the relevant Wald statistic and points towards the treatment with lower hazard rate. Solid arrows are significant at $P < 0.05$.

Fig. 4). Similarly, the adjacent section (i.e. section 3) in defensive-posture prey also received more pecks if the prey had eyespots ($z = 2.603$, $P = 0.009$). The pecks were distributed symmetrically around the midpoint in the remaining three treatments (all pairwise tests: $P > 0.06$; Table S4), with most pecks directed towards the tips and relatively few pecks directed towards the middle sections (Fig. 4). Therefore, eyespots affected where birds pecked the artificial caterpillars only when the caterpillars were in a defensive posture and not when they were in a resting posture.

DISCUSSION

Our experiment examined the protective value of eyespots and a widened head shape in caterpillar models subject to the possibility

of predation by wild avian predators. We found a clear survival advantage for caterpillar models that possessed either, or both, eyespots and a widened head shape. Both eyespots and head shape were significant main effects in explaining variation in caterpillar survival. Moreover, only caterpillars with both the eyespot and widened head received more pecks directed towards the anterior ‘head’ region (Fig. 4). Treatment-related effects on crypsis are insufficient to explain these observed protective effects, as eyespots and modified body shape would both be expected to reduce, rather than enhance, relative crypsis. We had expected to observe a significant interactive effect between eyespots and head shape mimicry, however, the interaction only approached significance. Also, we found that artificial prey possessing both eyespots and the widened head shape did not survive longer than those with either

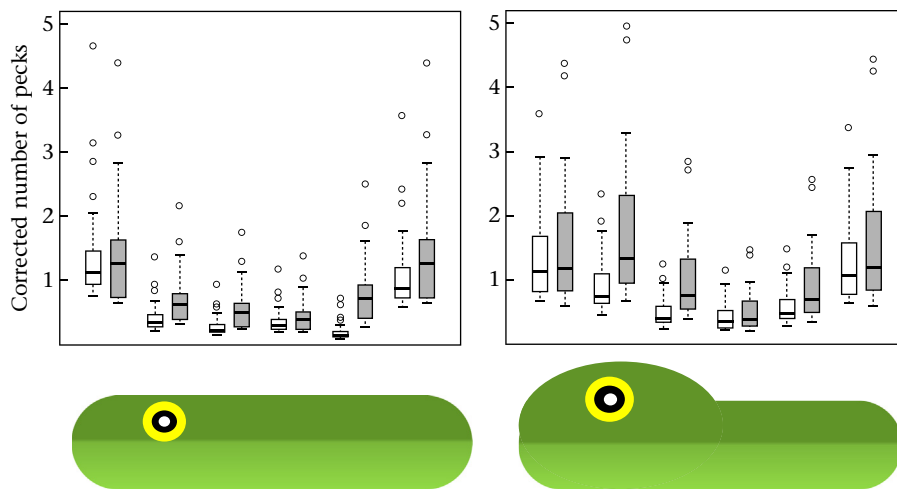


Figure 4. Box plot depicting the median number and location of peck attacks on pastry caterpillars deployed in the field after controlling for the lack of independence of multiple pecks on a given individual prey item. White boxes: no-eyespot treatment; grey boxes: eyespot treatment. The corrected median number of pecks is depicted by the bar inside the box, hinges indicate the 25% and 75% quartiles, and whiskers extend to 1.5 times the interquartile range, with values outside of this range depicted as open circles.

defence alone. Therefore, we found no strong evidence that eyespots and modified head shape act synergistically to enhance protection. To our knowledge this is the first simultaneous examination of the roles of eyespots and head shape in reducing attack on caterpillar prey. While we have not demonstrated a synergy, our results clearly highlight the protective advantage of these traits for caterpillars at risk of predation by birds.

The protective value of eyespots observed here is much greater than what we had observed previously (Hossie & Sherratt 2012). However, in our previous study the eyespots were purposefully designed to be large and conspicuous in an attempt to augment their possible effect on survival (Hossie & Sherratt 2012), whereas here we modified the design to be a closer approximation of the eyespots possessed by *Papilio* caterpillars. Our revised eyespot design also incorporated a white 'sparkle' marking in the centre of the black pupil (Fig. 2). Such 'sparkle' features have recently been shown to enhance the protective effect of eyespots in adult butterflies (Blut et al. 2012) and may operate similarly in caterpillars. Therefore, it is possible that we observed a greater deterrent effect of eyespots in this study because we used a more realistic (and hence effective) design. While possessing eyespots clearly confers a protective advantage, we can infer that aspects of eyespots, including their size and sparkle, critically influence their protective value to caterpillars that possess them.

Pastry caterpillars with a widened 'head' region (i.e. the defensive posture) survived significantly longer than prey in the 'rest posture', but from pairwise tests we note that this protective effect was not augmented by the presence of eyespots (neither was it lowered). That is, having either eyespots or a widened 'head' protected prey as well as both traits together. This result is consistent with Valkonen et al. (2011), who found that although both viper-like patterning and head shape reduced predation on clay snakes, models with both traits did not survive better. Interestingly, there are several examples of caterpillars that, despite lacking eyespots, possess a behavioural 'head-widening response' equivalent or superior to closely related species that possess both traits. For example, *Eumorpha phorbis* has much less conspicuous eyespots than the closely related *Eumorpha labruscae* (to human eyes), but still produces a convincingly snake-like head shape when threatened (see specimens 11-SRNP-2980 and 78-SRNP-35, Janzen & Hallwachs 2012). Similarly, *Papilio cresphontes* and *Papilio thoas* have a thickened 'head' area despite lacking the eyespots possessed by more apparent 'snake mimics' (e.g. *P. glaucus*, *Papilio troilus*). This is perhaps additionally indicative that these defensive strategies may function in isolation. Ongoing research is currently examining the evolutionary history of these defences in lepidopteran caterpillars in part to determine whether acquiring or losing eyespots best explains the specific trait combinations observed in modern species.

Peck marks were distributed symmetrically in the resting-posture treatments regardless of eyespot presence, whereas more 'head'-directed pecks occurred on prey in the defensive posture particularly when eyespots were also present. Specifically, prey with both a thickened head area and eyespots received significantly more pecks directed at the body segments containing eyespots and the adjacent segment (i.e. sections 2–3), compared to either the equidistant sections at the other end (i.e. sections 4–5) or sections 2–3 on defensive-posture prey lacking eyespots (Fig. 4). It remains unclear whether birds direct attacks towards the 'head' in an attempt to immobilize a prey item, or whether they attack the eyes or head of a threatening object like a snake, or perhaps simply out of curiosity. In our previous work we found that birds that were not deterred from attack targeted the eyespots even though prey were not in the defensive posture (Hossie & Sherratt 2012). We suspect that this difference results from changes in the eyespot design

between experiments (see above), and it would be interesting to investigate whether such differences in features (e.g. size, composition) determine how an eyespot functions (i.e. deflection versus intimidation).

Although the distribution of pecks provides important insight into the reaction of predators to static prey, there are important constraints to interpreting attack distributions using a design similar to ours. Specifically, in our study, it was difficult or impossible to know (1) whether multiple peck marks on a model involved attacks by multiple birds or a repeated attack by one bird, (2) which mark represented a bird's first attack after it repeatedly attacked the model, (3) the distribution of points of attack on prey that were completely consumed or removed, and (4) why attacks on recovered prey did not result in the removal of the prey. Importantly, any inferences drawn from the distribution of pecks on artificial prey deployed in the field must be tempered by these limitations.

This work demonstrates the protective effect of eyespots in caterpillar-like prey. In addition, our experiment demonstrates that a thickened head region also provides a clear protective advantage against attacking birds. The slightly weaker protective effect of eyespots compared to head shape mimicry ($18.5 \pm 10.4\%$ versus $23.6 \pm 10.1\%$, respectively) might indicate that a widened head shape more reliably indicates a true threat than eyespots. Somewhat surprisingly, our pairwise comparisons indicated that simultaneous possession of both eyespots and the thickened head area did not confer more protection than either trait alone, despite prey with both defences drawing more 'head'-directed attacks. Earlier work has also failed to observe a synergistic effect of eyespots and sound production in the protection of peacock butterflies from avian predators (Vallin et al. 2005). Thus, future research should investigate the learning process of avian predators to develop our understanding of how predators decide which cues reliably indicate danger, and why they sometimes fail to incorporate information from multiple cues when deciding to attack a given prey item. For example, are there adaptive limits to learning the reliability of multiple cues? Such research is likely to help us better understand the evolution of mimetic traits and may help explain why many mimics only imperfectly resemble their model.

Acknowledgments

We thank H. Kienzle for help during the implementation of this work. We also thank B. and E. Hossie, the University of Guelph Kemptville Campus and the Ferguson Forest Centre for allowing use of their property. L. Touzin helped facilitate work on crown land. We thank D. H. Janzen, E. Fernández-Juricic and two anonymous referees for critical comments on our manuscript. D. H. Janzen and W. Hallwachs kindly provided the image of *H. triptolemus* and the opportunity to study *E. phorbis* in the field. This work was funded through a Natural Sciences and Engineering Research Council of Canada Discovery grant awarded to T.N.S.

Supplementary Material

Supplementary material for this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2013.05.029>.

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