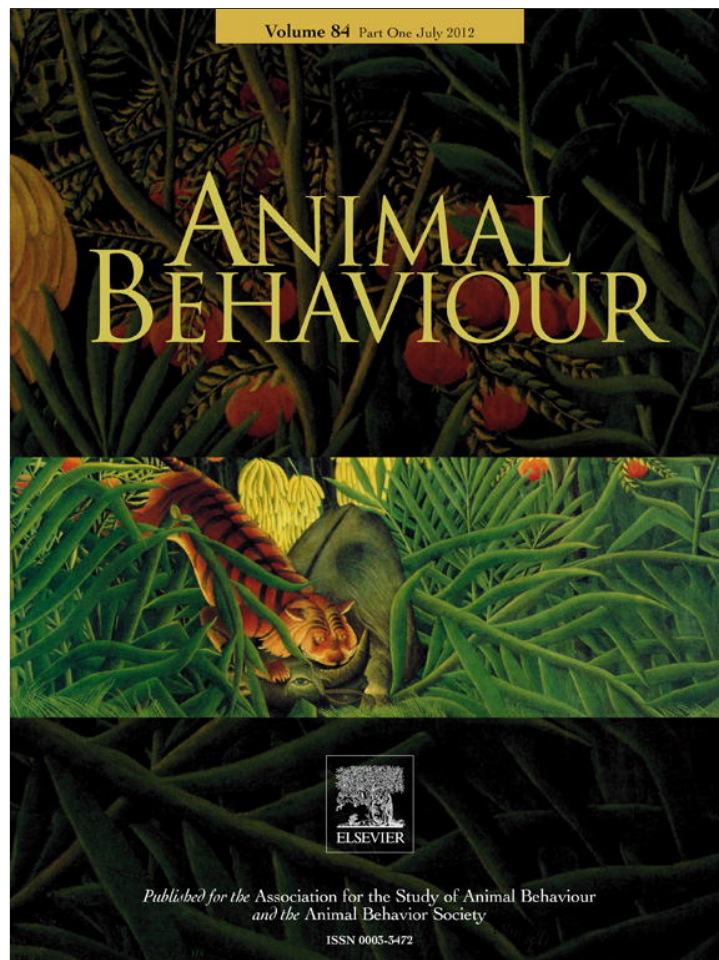


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# Animal Behaviour

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## Eyespots interact with body colour to protect caterpillar-like prey from avian predators

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A wide range of animals possess conspicuous eye-like markings on their bodies, commonly called 'eyespots'. These markings are generally thought to serve an antipredator function, particularly in lepidopteran caterpillars where eyespots may mimic the eyes of their enemies' predators. Despite this, the protective value of caterpillar eyespots remains unclear and has yet to receive empirical study. Moreover, most eyespot caterpillars are otherwise cryptically coloured, suggesting that the efficacy of eyespots may depend on additional aspects of prey coloration. We assessed the protective value of caterpillar eyespots with and without a form of countershading by pinning artificial caterpillars to tree branches in the field and monitoring their survival over 90 h. Eyespot and countershading treatments were arranged in a 2 × 2 factorial design, allowing us to quantify both main and interactive effects. We also photographed attacked prey to determine whether eyespots affected the area of the prey's body that was attacked (i.e. pecked by an avian predator). We observed no main effect of eyespots or countershading on survival; instead, countershading and eyespots acted synergistically to reduce predation, although whether this interactive effect reflects a countershading mechanism or different background matching when prey are viewed from above is unclear. Importantly, bird pecks were directed both towards eyespots and towards the body section furthest from the eyespots. This is the first field experiment to examine the protective value of eyespots on caterpillar-like prey and demonstrates that the extent of protection depends on additional factors, including body colour and possibly behaviour.

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Many animals possess conspicuous circular markings that superficially resemble vertebrate eyes. These markings are commonly called 'eyespots' and while they may play a variety of roles (e.g. sexual signalling, San Martin et al. 2011), they are widely thought to have evolved as an antipredator defence (Blest 1957; Edmunds 1974; Stevens 2005; Kodandaramaiah 2011; see Supplementary Table S1 for a summary of possible antipredator functions of eyespots). Accordingly, the possession of eyespots may deflect a predator's attack away from vulnerable body parts and/or prevent attacks altogether through predator intimidation. Nevertheless, empirical tests of these proposed mechanisms have been partly contradictory (intimidation: Vallin et al. 2005; Stevens et al. 2007; Merilaita et al. 2011; deflection: Lyytinen et al. 2003, 2004; Olofsson et al. 2010), and it remains unclear the extent to which eyespots increase prey survival under field conditions. Moreover, when such tests have been conducted they have sometimes challenged popular belief. For example, recent work has suggested that

the intimidation effect of eyespots, at least in adult Lepidoptera, may be a consequence of predators avoiding prey with conspicuous markings, rather than eyespots resembling the eyes of a predator's own enemies (Stevens et al. 2008a; Stevens & Merilaita 2009).

Regardless of the specific mechanism, the fact that eyespots have arisen independently in several animal groups (Ruxton et al. 2004a), and in a range of life-history stages, indicates that their protective value may be considerable. Eyespots occur on caterpillars from numerous independent lineages within Lepidoptera and have long been believed to confer protection against insectivorous birds through enhancing their resemblance to predatory snakes (e.g. Poulton 1890; Edmunds 1974; Chittka & Osorio 2007). Indeed, in his classic monograph, Henry Walter Bates described a caterpillar with eyespots that resembled a snake as 'The most extraordinary instance of imitation I have ever met' (Bates 1862, page 509). Most recently, Janzen et al. (2010) detailed the natural history of many Neotropical caterpillars with eyespots and proposed that these markings represent an eye-mimicry complex generated by avian predators, which innately fear predator eyes. Yet while predator eye mimicry is entirely plausible, to date no experimental work has examined this function for caterpillar eyespots in the field. Specifically, we lack empirical evidence showing that (1)

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eyespot function in caterpillars is related to the mimicry of snake or other predators' eyes, as opposed to the more general effects of neophobia or dietary conservatism.

To date, only a few preliminary attempts have been made to understand the function of eyespots in caterpillars. For instance, Poulton (1890) described three simple trials (each using only a single caterpillar) that supported an intimidation function for caterpillar eyespots; however, this work was anecdotal, and the eyespotted caterpillar in question was eventually consumed in two of the three cases described. Work by Blest (1957) found that captive birds directed their pecks towards a single eyespot that had been painted on mealworm larvae. Shirota (1980) likewise presented caterpillars with artificial eyespots to six captive birds, but found that all caterpillars were promptly attacked, with delays to attack only occurring when eyespots were unrealistically large (i.e. diameter of 1–2 cm). Intriguingly, the same birds did not attack a live *Deilephila elpenor lewisii* caterpillar, which naturally possesses eyespots (Shirota 1980). Therefore, each of the above studies only provides anecdotal observations, and the protective value of eyespots within a natural setting remains to be demonstrated.

The caterpillars of many lepidopteran species are generally thought to mimic snakes as they possess a pair of eyespots on enlarged anterior body segments during late instars (e.g. *Papilio* sp., see Scriber et al. 1995; Allen et al. 2005; Wagner 2005 for photos). Several of these species are abundant in extratropical areas that lack the snake species that might pose a significant threat to birds. In these instances, eyespots could continue to be maintained by migratory birds that express either a learned or innate aversion to predatory snakes encountered in tropical wintering grounds, or a more general innate aversion to larger creatures with eyes (Janzen et al. 2010). As several species of caterpillars that have eyespots are apparently palatable, it seems unlikely that eyespots function as warning signals (Brower 1958; Leslie & Berenbaum 1990; Takagi et al. 1995). A deflection function for caterpillar eyespots has also been dismissed previously on the grounds that damage to any part of a caterpillar's body would be lethal (Janzen et al. 2010); however, in some cases, eyespot-targeted strikes would direct attacks towards specialized organs that release noxious chemicals when caterpillars are harassed. For example, Blest (1957) suggested that eyespots of *Papilio* caterpillars might direct attacks towards the osmeterium (Frankfater et al. 2009). In addition, deflecting pecks away from the true head might allow caterpillars to mount behaviours that heighten the intimidation effect of eyespots, thereby thwarting attacks before lethal damage is done. Experimental work in the field is clearly needed to clarify whether caterpillar eyespots function to intimidate predators from attacking and/or whether the presence of eyespots also serves to deflect or direct strikes.

The efficacy of eyespots in defending against predators may be affected by additional features of the prey's coloration. For instance, in a field experiment using moth-like targets, Stevens et al. (2008b) showed that eyespots reduced predation on conspicuous, but not camouflaged, prey. Yet, most eyespot caterpillars are cryptically coloured (Poulton 1890; Edmunds 1974; Janzen et al. 2010), and many 'snake mimic' caterpillars are countershaded (e.g. *Papilio canadensis*, *Papilio glaucus*; Wagner 2005). Other potential examples of caterpillars with eyespots that appear countershaded include *Hebomoia glaucippe* (Pieridae), *Daphnis nerii* (Sphingidae), *Xylophanes ceratomioides* (Sphingidae) and *Hemeroplanes triptolemus* (Sphingidae). As countershading (i.e. darker pigmentation on surfaces is generally illuminated) has been suggested to be an effective means of crypsis in caterpillars (Rowland et al. 2007, 2008), then it is possible that eyespots and countershading have an interactive effect on caterpillar survival.

To determine whether constantly displayed eyespots were effective in deterring avian predation and/or deflecting strikes, we deployed artificial caterpillars in the field and monitored their 'survival'. Specifically, caterpillar treatments followed a 2×2 factorial design: with or without 'eyespots' and with or without countershading. This design allowed us to compare directly the survival of prey with eyespots against two alternate prey with greater levels of crypsis (i.e. solid green-no eyespots, and countershaded-no eyespots). Furthermore, our design permitted us to test for an eyespot–countershading interaction. Overall, we predicted that eyespots would protect prey by intimidating predators from attacking, and if eyespots serve a deflection function, we predicted that pecks would be directed towards the caterpillar's eyespots.

## METHODS

### Site Description

Field trials were conducted near Kemptville (Site 1: 45°03'00"N, 75°39'44"W; Site 2: 44°59'48"N, 75°38'22"W) and Oxford Mills (Site 3: 44°56'27"N, 75°38'56"W), Ontario, Canada. Experiments were run between 25 May and 27 August 2010, which corresponds roughly to the dates when Canadian tiger swallowtail, *Papilio canadensis*, caterpillars could be found in the wild in our study area (Hall et al. 1996; T. J. Hossie, personal observation). All three sites were composed primarily of secondary growth of mixed deciduous forest. Each site was no closer than 5.5 km from another site. Preliminary scouting of each site confirmed the presence of *Pa. canadensis* adults, snakes, as well as several species of insectivorous birds. Insectivorous bird species observed in each of the field locations are detailed in Supplementary Table S2.

### Artificial Prey

Artificial caterpillars were designed to assess the protective value of eyespots and countershading on caterpillar prey in general; however, for ecological relevance, our prey were modelled loosely on *Papilio canadensis*. We created artificial prey following similar protocols to previous studies (e.g. Church et al. 1997; Rowland et al. 2008). Specifically, pastry caterpillars were manufactured with a 3:1 mixture of white flour (White Rose™, White Rose Flour Mills, Byford, WA, U.S.A.) and lard (Tenderflake™, Maple Leaf Foods, Toronto, ON, Canada). From this, we made light green pastry by adding three drops of Leaf Green AmeriColor Soft Gel Paste™ (AmeriColor Corp., Placentia, CA, U.S.A.) and 20 ml of water, and we made dark green pastry by adding six drops of gel paste and 5 ml of water. Strips of dark and light green pastry were produced by pressing the dough through a circular opening 0.5 cm in diameter using a modified Play-Doh™ Mini Fun Factory™ (Hasbro Canada Corp., Longueuil, QC, Canada); these strips were then cut into 4 cm lengths. To make uniform, light green prey, two 4 cm strips of light pastry were pressed together and rolled such that the final artificial prey were cylinders 4 cm long with a diameter of about 7 mm. Countershaded prey were produced using the same methods, but combining one light and one dark strip of pastry. This created a two-toned prey similar to Rowland et al. (2008); for our purposes the dark side was considered dorsal, as prey were presented on the upper side of branches. We used Elmer's™ Non-toxic Tempera Paint (Elmer's Products, Inc., Westerville, OH, U.S.A.) to create eyespot treatments, by painting two 0.6 cm black circles dorsolaterally on the prey, 0.3 cm apart and 0.5 cm from one end. Excess black paint was removed from the centre of the black spots using a thin paintbrush and a 0.4 cm white circle was painted inside each. Relatively large eyespots were used in an attempt to maximize the



antipredator effect of the markings, as larger eyespot size greatly influences avoidance behaviour of predators (Stevens et al. 2008a). A dorsal view of an eyespot caterpillar is provided in Supplementary Fig. S1. Tempera paint is an appropriate medium for eyespots as it dries in a smooth matte finish and the colours do not change over time (Mayer 1985). Finished pastry caterpillars (e.g. Fig. 1) 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. In this experiment we did not attempt to mimic the thickened head area that many eyespot caterpillars (e.g. *Pa. canadensis*) exhibit in their defensive posture. Although of interest, this thickening is expressed facultatively upon harassment, and here we focused on continuously displayed signals. Similarly, the caterpillars of a number of species (e.g. *Deilephila elpenor*, *Xylophanes tersa*) possess several pairs of eyespots. Whether additional pairs of eyespots serve a specific function or affect caterpillar survival remains untested; however, here we focused only on a single pair of eyespots.

#### Prey Presentation

Pastry caterpillars were deployed between 1700 and 1900 hours on the first day of a replicate site. Specifically, 24 trees were 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 tree 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*; Supplementary Table S3), 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 and west) was allocated randomly. Attaching four prey to each tree has the benefit of reduced variation in microhabitat among treatments. Selected branches were 1–2 m from the ground and were roughly 5–9 mm 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. 1). 'Survival' was checked at 14, 19, 24, 38, 43, 48, 62, 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 ( $N = 88$ ) 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.

The entire process was replicated nine times (three times per site, albeit at a different location within site) for a total of 864 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, we always conducted consecutive replicates 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.). We conducted additional checks for pecked pastry caterpillars in the early replicates, but time constraints precluded us from checking for pecked pastry caterpillars in the remaining replicates. Specifically, in one replicate trial, a check for pecked/missing pastry caterpillars was conducted at 67 h from the start of the trial, and in two replicate trials, a 72 h check was conducted. Pastry caterpillars were designed to be fully palatable to birds, and our experimental design was approved by the Carleton University Research Ethics Committee (Protocol B10-14).

#### Analyses

We analysed the survival of artificial prey using Cox proportional hazards regression (Cox 1972), 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 the



**Figure 1.** Examples of pecked pastry caterpillars from each of the four treatments used in this experiment. (a) Solid-no eyespots; (b) Countershaded-no eyespots; (c) Solid-eyespots; (d) Countershaded-eyespots.

possible lack of independence between baits on the same tree by clustering the baits by tree in our analysis. Overall significance of the model was assessed using the Wald test, which does not assume independence of observations within a cluster. Factors in our model included colour (i.e. solid versus countershaded), eyespot (i.e. eyespots versus no eyespots), and the colour  $\times$  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 also [Supplementary Figs S2, S3](#)). We therefore stratified the analysis to permit baseline hazard rates specific to location and to 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 (i.e. with or without countershading), 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. Similarly, for caterpillars without eyespots, 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 or treatments, we used a generalized mixed effects model with a Poisson error distribution and logit 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 log likelihood ratio test. We also compared the fit of our models using Akaike's Information Criterion (AIC). This analysis was conducted in R ([R Development Core Team 2008](#)) using the `lmer4` package ([Bates et al. 2011](#)).

## RESULTS

The total 90 h mortality rate for a location ranged from 32% to 91% (mean  $\pm$  SE:  $68 \pm 6.32\%$ ). The main effects of colour and of eyespots did not explain a significant amount of the variation in survival (Wald = 0.73,  $P = 0.47$ ; Wald = 1.58,  $P = 0.11$ , respectively; [Table 1](#)), but a significant eyespot  $\times$  colour interaction was detected (Wald =  $-2.36$ ,  $P = 0.019$ ; [Table 1](#)). To interpret the significant interaction, we conducted pairwise Cox proportional hazard regressions on experimental treatments. We found a strong reduction in the hazard of countershaded prey with eyespots relative to solid prey with eyespots ( $\sim 15.4\%$  reduction; Wald = 5.05,  $df = 1$ ,  $P = 0.025$ ). Remaining pairwise comparisons were not significant (results from all pairwise Cox proportional hazard models are

presented in [Fig. 2a](#)). Therefore, the significant interaction indicated that eyespots increased hazard on solid prey but decreased hazard on countershaded prey. Note that qualitatively equivalent results were obtained when missing prey were censored instead of classified as prey killed ([Supplementary Table S4](#)).

Given that the baseline hazard rate differed between tree species ([Supplementary Fig. S3](#)), we compared treatment-related survival by conducting further Cox regression models separately for each tree species. With the exception of *Populus tremuloides*, there was no significant difference in the survival rate of caterpillars between the four eyespot/colour treatments (*P. tremuloides*: overall Wald = 14.67,  $df = 3$ ,  $N = 320$ ,  $P = 0.002$ ; remaining  $P > 0.38$ , see [Supplementary Material](#) for details). For those pastry caterpillars on *P. tremuloides*, a significant eyespot  $\times$  colour interaction contributed to the overall significance (Wald =  $-3.77$ ,  $P < 0.001$ ). Pairwise treatment comparisons revealed the same directionality in survivorship as the full analysis but they showed greater strength ([Fig. 2b](#)). Notably, the artificial caterpillars pinned to *P. tremuloides* experienced a relatively high baseline hazard rate ([Supplementary Fig. S3](#)).

Although many prey items presumed attacked had been fully removed by predators (52%, 334/642), the remaining attacked baits were recovered from the field (48% recovered, 308/642). Overall, a similar proportion of caterpillars disappeared upon attack in all four treatments (mean proportion  $\pm$  SE =  $0.50 \pm 0.05$ ;  $F_{3,24} = 1.27$ ,  $P = 0.31$ ). There was no significant effect of treatment on the number of pecks received by attacked pastry caterpillars recovered from the field ( $F_{3,307} = 1.12$ ,  $P = 0.295$ , overall mean  $\pm$  SE =  $3.78 \pm 0.18$ ). Pecks were not evenly allocated along all sections of the pastry caterpillars ( $\chi^2_5 = 459.34$ ,  $P < 0.001$ ), and the distribution of pecks was affected by the presence of eyespots ( $\chi^2_6 = 49.858$ ,  $P < 0.001$ ), but not by countershading ( $\chi^2_6 = 9.904$ ,  $P = 0.13$ ). Results from model comparison using AIC confirmed these results ([Supplementary Table S5](#)). Specifically, prey with eyespots were pecked less in the section anterior of the eyespot ( $z = -3.699$ ,  $P < 0.001$ ) and pecked more frequently within the eyespot section ( $z = 4.406$ ,  $P < 0.001$ ) and on the posterior tip furthest from the eyespots ( $z = 5.091$ ,  $P < 0.001$ ) than prey without eyespots ([Fig. 3](#), [Supplementary Table S6](#)). The middle sections of prey with and without eyespots rarely received pecks ([Fig. 3](#)). Therefore, pecks were not distributed evenly along the bodies of any caterpillar type, but eyespots affected where birds pecked the prey.

## DISCUSSION

Our experiment examined the protective value of eyespots and countershading in caterpillar-like prey subject to predation by wild avian predators. Surprisingly, we did not observe a strong main effect of eyespots or countershading on survival. However, a significant interaction indicated that countershading and eyespots act synergistically to reduce predation. Specifically, we found that eyespots increased the survival of countershaded prey but reduced survival of solid prey. These patterns were more apparent when analyses were restricted to include only prey pinned on trembling aspen (*Populus tremuloides*), the primary host of *Pa. canadensis* ([Scriber et al. 1995](#)). Finally, pecks were not randomly or symmetrically distributed on eyespot caterpillars, indicating that eyespots may also affect where predators strike the prey. To our knowledge this is the first experiment to demonstrate that eyespots can protect caterpillar prey from predation by wild birds in the field, albeit to a limited extent.

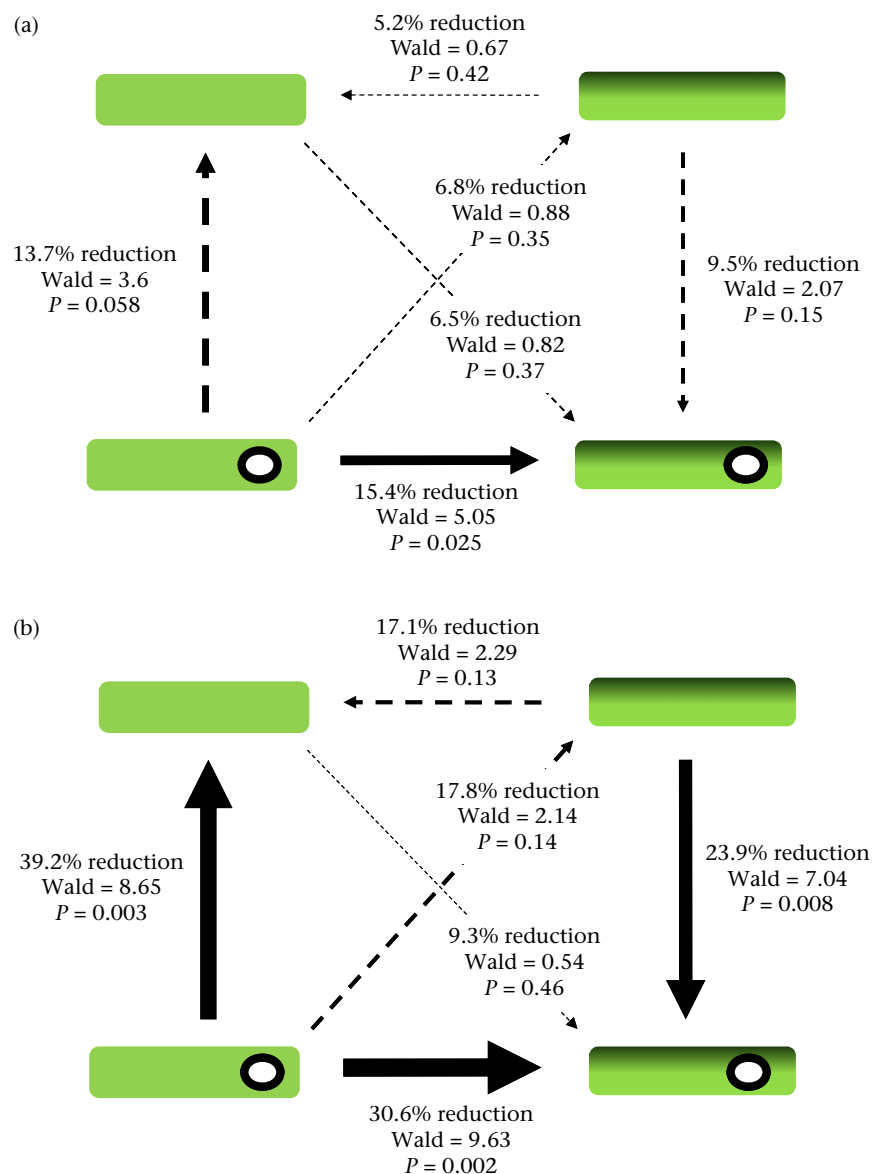
If eyespots do protect caterpillars through predator eye mimicry, then the weak avoidance of eyespot caterpillars may be a result of our design necessarily excluding relevant caterpillar behaviours.

**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	Wald	P
Colour	0.04576	1.04682	0.11343	0.06303	0.726	0.4678
Eyespot	0.10715	1.1131	0.11316	0.06766	1.583	0.1133
Colour $\times$ eyespot	-0.22295	0.80016	0.16243	0.09466	-2.355	0.0185

Parameter estimates and associated significance tests depict how eyespots and countershading 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 and used the robust standard error to calculate the Wald statistic.



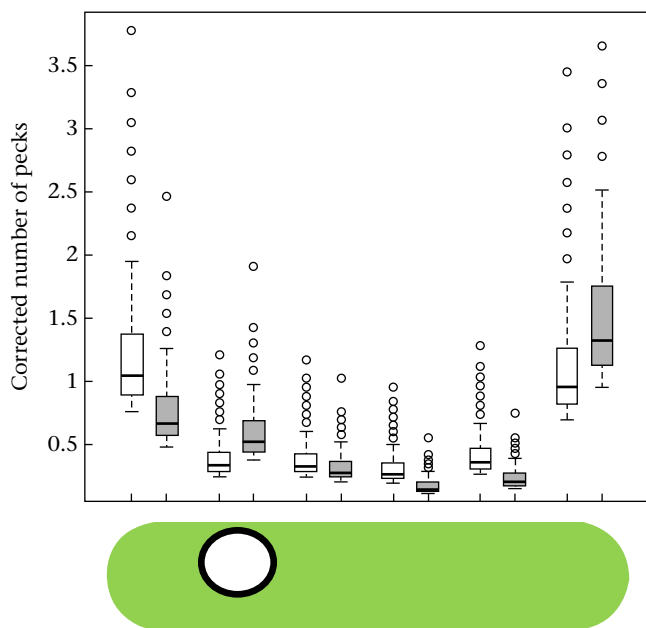
**Figure 2.** (a) Results from pairwise Cox proportional hazard regression comparisons of pastry caterpillar survival stratified by tree species and location, and clustered by the tree to which pastry caterpillars were pinned. Eyespot (no eyespots versus eyespots present) and colour (solid green versus countershaded) treatments were arranged in a 2x2 factorial design. (b) Results from the same analyses conducted using only those pastry caterpillars pinned to *Populus tremuloides* (primary host of *Papilio canadensis*; Scriber et al. 1995). In both panels, arrow width is proportional to the relevant Wald statistic and points towards the treatment with lower hazard rate. Solid arrows are significant at  $P < 0.05$ .

Such behaviours have long been recognized in caterpillars with eyespots (Bates 1862; Poulton 1890) and also occur in *Papilio* sp. caterpillars (T. J. Hossie, personal observation). The extent to which variation in behaviour and morphology influence predation of eyespot caterpillars by avian predators is currently being evaluated. The diversity of eyespots observed in the natural world, many of which only vaguely resemble eyes, suggests that eyespots need not closely resemble a predator's eyes; however, it is conceivable that the reduced efficacy of eyespots that we observed may also have been the result of our eyespot design.

Countershaded prey without eyespots in our study did not have greater survival than solid green prey. This contrasts with previous work (Edmunds & Dewhurst 1994; Rowland et al. 2007, 2008), although a protective effect of countershading has not always been observed (Ruxton et al. 2004b; Speed et al. 2004). It is possible that microhabitat choices of prey strongly influence the efficacy of countershading, or that the bird community in our study areas may

have been composed of species where countershading does not affect prey detection (e.g. Speed et al. 2004). Countershading might have been more effective if caterpillars had been deployed on leaves (see also: Speed et al. 2004), yet Rowland et al. (2008) observed a protective effect of countershading for green prey similarly deployed on branches. Instead, we suspect that countershading may be insufficient for our larger-bodied prey positioned on branches as the prey used in our experiment were also larger than those used in previous studies (e.g. Rowland et al. 2008). A limitation of our design is that our solid treatment was the same colour as the ventral, rather than dorsal, side of the countershaded prey, meaning that treatments would have been seen as different colours when viewed from above. As we observed no significant main effect of prey body colour, this constraint most likely had little effect on our results; nevertheless, the design of our body colour treatment may not adequately distinguish between the effect of background matching and countershading.





**Figure 3.** Box plot depicting the 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.

A key result of our study is that eyespots reduced the hazard to countershaded prey but not solid prey. Thus, the protective value of conspicuous spots most likely depends on other aspects of prey coloration (see also, Stevens et al. 2008b). One possible reason for this interactive effect is that combining eyespots with countershading makes the caterpillar a more convincing snake mimic, perhaps by acting additively as a cue signalling danger, as many arboreal snakes are also countershaded. Indeed, birds may use patterns of dangerous snakes that function in crypsis (e.g. the zigzag pattern of *Vipera* sp.) as avoidance cues (Wüster et al. 2004; Valkonen et al. 2011). Whether the observed strong effects on *Po. tremuloides* are explained by the difference in sample size relative to other tree species, or by a specific biological factor remains unclear, and future work should investigate whether eyespots interact with countershading specifically or body colour more generally. Regardless of the mechanism, our results indicate that the effect of eyespots is not independent of habitat or additional aspects of prey coloration, emphasizing the epistatic effects of different traits on fitness.

Eyespots also affected the area of the pastry caterpillar's body that was pecked by birds, which may represent an additional advantage to the caterpillars. When eyespots were lacking, pecks were symmetrically directed towards the terminal sections; however, when eyespots were present, the terminal section anterior of the eyespot received relatively few pecks. In addition, both the section with eyespots and the terminal section furthest from the eyespots were pecked more than the comparable positions on caterpillars without eyespots (Fig. 3). Thus, while some avian predators may target the eyespots upon attack, others may be intimidated from attacking the end of the caterpillar with eyespots. Still other birds probably do not fear eyespots as eyespot prey were occasionally removed completely. Blest (1957) found that birds were more likely to peck towards a single spot painted on mealworm prey, and our finding that pecks were directed towards

eyespots is consistent with his suggestion that eyespots may direct strikes towards the osmeterium in papilionid larvae. Whether such change in the distribution of pecks increases caterpillar survival remains unknown; however, fewer pecks anterior of the eyespots should minimize the change of damage to a caterpillar's true head, located anterior to the eyespots in many eyespot caterpillars including *Pa. canadensis*.

Overall, our work is consistent with the proposed intimidation function for caterpillar eyespots, but many questions remain to be addressed empirically, namely: (1) do caterpillar eyespots represent a form of mimicry, (2) do tropical migratory birds carry either learned or innate aversions towards eye-like features to temperate areas, and (3) what selection pressures favour eyespots over other defences? Although challenging, addressing these questions will help us understand the widespread phenomenon of eyespots and may provide new insights into the evolution of mimicry.

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### Supplementary Material

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