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ORIGINAL PAPER

Deflective effect and the effect of prey detectability on anti-predator function of eyespots

Adrian Vallin · Marina Dimitrova · Ullasa Kodandaramaiah · Sami Merilaita

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Abstract Eyespots (patterns of roughly concentric rings) are often thought to have an anti-predator function. Previous experiments have lent support for the intimidation hypothesis by demonstrating a deterring effect of eyespots, but so far there is little evidence for the deflective effect (direction of attacks toward less vital body parts). We studied predators' responses towards large and small eyespots and towards prey with no, one, or a pair of eyespots and if this response is influenced by whether or not prey blend into background. In two experiments, we used artificial, triangular prey items and blue tits (*Cyanistes caeruleus*) as predators. In experiment 1, we found evidence for the deflective effect of small but not large eyespots, independent of whether the prey was presented on a concealing or exposing background. In experiment 2, we found that predators avoided the prey with a pair of small eyespots more than the prey without eyespots, but interestingly, we only found this deterring effect on the concealing background. There was no difference in attacks between the prey with one large and two small or one large and no eyespots. We conclude that deflective function may select for eyespots, and background may influence the deterring function of eyespots.

Keywords Predation · Prey coloration · Signaling · Deflection · Intimidation · Crypsis

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A. Vallin · S. Merilaita (✉)
Department of Biosciences, Åbo Akademi University, Turku, Finland
e-mail: sami.merilaita@abo.fi

M. Dimitrova · U. Kodandaramaiah
Department of Zoology, Stockholm University, Stockholm, Sweden

Introduction

Predation is a strong selective force and has led to spectacular morphological and behavioral adaptations in prey. Particularly, various types of protective coloration in prey have become classic examples of anti-predation adaptation (e.g., Wallace 1889). Protective coloration ranges from camouflage to highly conspicuous patterns that are used for startling predators or for warning predators of a defense (Ruxton et al. 2004; Stevens and Merilaita 2009). Regardless of its function, the optimal design of protective coloration is determined by predator perception and the signaling environment (Endler 1992; Rowe and Skelhorn 2004).

One example of a conspicuous prey color pattern are eyespots that are commonly found on the wings of Lepidoptera but also in other animals such as mollusks, fish, frogs, and birds (Poulton 1890; Blest 1957; Stevens 2005). Eyespots are patterns consisting of roughly concentric circles of contrasting colors. As the name implies, these patterns resemble, to a varying degree, the vertebrate eye.

Although the developmental aspects of eyespots particularly in Lepidoptera have been relatively well-investigated (Nijhout 1980, 1991; Brakefield et al. 1996), the adaptive function of eyespots is still far from fully understood. Among taxa, eyespots differ in their number, shape and placement, and also their function can vary from different ways of anti-predator defense to signaling in mate choice (Robertson and Monteiro 2005; Stevens 2005; Kodandaramaiah 2009). In insects, the functions of eyespots have mainly been investigated along the lines of two different anti-predation hypotheses, the deflection hypothesis and the intimidation hypothesis (Stevens 2005).

The deflection hypothesis typically concerns the small eyespots found at the wing margins of many butterflies. These markings are believed to capture the attention of an

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attacking predator and direct the attack away from vital body areas to wing margins. In butterflies, the wing margins tear easily and an attack in this area may offer an opportunity for the butterfly to break free and escape without being severely harmed. Although intuitively appealing, the deflection hypothesis has lacked firm empirical support. Almost all evidence so far has been indirect (Wourms and Wasserman 1985; Lyytinen et al. 2004; see also Meadows 1993 for a critique on earlier studies for their very low sample sizes), and also negative results exist (e.g., Lyytinen et al. 2003; Vlieger and Brakefield 2007). An exception is the very recent experiment by Olofsson et al. (2010), showing conditional evidence. They found that under artificial light conditions, the marginal eyespots of the woodland brown butterfly (*Lopinga achine*) had a deflective effect on birds when the ultraviolet intensity was high and longer wavelengths were low, but not when both or only longer wavelength intensity was high.

Evidence for the intimidation hypothesis and the deterring function of large conspicuous eyespots is more abundant, and currently firm evidence exists that naturally occurring conspicuous eyespots do deter visually oriented predators. This evidence comes from a series of experiments in which passerine birds were presented with butterflies having eyespots (Vallin et al. 2005, 2006, 2007; Kodandaramaiah et al. 2009). Also, experiments using artificial eyespots or prey have provided knowledge about the deterring qualities of eyespots (Scaife 1976; Jones 1980; Forsman and Herrström 2004; Stevens et al. 2007, 2008a, b). Evidence from earlier studies (Scaife 1976; Jones 1980) suggests that pairedness, horizontal orientation, and the presence of an “iris” and a “pupil” is important. Recent studies (Stevens et al. 2007, 2008a) found the most important deterring features to be larger size and high internal pattern contrast, whereas eyespots occurring in pairs were no better than three eyespots or a single eyespot with the same total area.

When studying eyespots as well as other types of prey coloration, it is important to bear in mind that the detectability of a given prey appearance or pattern depends on the background against which it is viewed. Lyytinen et al. (2004) showed in a laboratory experiment with dead *Bicyclus anynana* butterflies that on a simulated dry season background consisting of dry leaves, the wet season phenotype that has eyespots was more readily detected by great tits (*Parus major*) compared with the dry season phenotype that lacks eyespots. On a simulated wet season background with green plants, however, both phenotypes were equally hard to detect. Furthermore, it is possible that the function of eyespots is influenced by how well (or poorly) the rest of the prey body coloration blends into the background. In a recent field study, Stevens et al. (2008b) recorded attack rates on artificial prey that matched or mismatched the average perceived lightness of the habitat. Simple, circular spots on the “wings” of the prey increased the attack rate on the mismatching prey but not on the prey matching the average lightness of the backgrounds.

Here, we present a study on the anti-predation functions of eyespots, addressing two main topics, namely (a) deflective effect of eyespots and (b) the influence of prey detectability on deflective and deterring function of eyespots. We also hypothesized that the efficacy of eyespots should vary with context; eyespots could stand out more strongly and the response they elicit could be stronger when the rest of the prey body highly matches the background than when the rest of the prey body provides a highly visible target. This might also provide an explanation for why evidence particularly for the deflective function has been scarce in previous experiments, in which the prey has been easily detectable.

As mentioned above, the previous studies by Lyytinen et al. (2004) and Stevens et al. (2008b) have tested for some aspects of the influence of the visual background on eyespots. However, the anti-predator effects of eyespots have not previously been compared between highly background-matching prey and prey that mismatches the background. Therefore, in the present experiment, we wanted to be able to manipulate the actual degree of background matching of the prey when the predators encounter them. For this purpose, we used artificial backgrounds and prey items that made it possible for us to fully control for the degree of background matching of the prey.

Our study on the response elicited by small and large eyespots on predators and how the rest of the prey blending into the background influences this response consists of two experiments, in which we used blue tits (*Cyanistes caeruleus*) as predators. In our first experiment, we studied the deflective effect and used prey with a small or large eyespot on one side of the prey and investigated whether the birds directed their attacks to that side. We did this both on an exposing and on a concealing background. In the second experiment, we studied the influence of prey background-blending on the deterring effect of eyespots. In this experiment, we produced three prey types: prey without eyespots, prey with one eyespot and prey with two eyespots, and presented these in combinations of two. We tested if the predators’ avoidance response was dependent on background-blending of the prey.

Methods

Predators

We used wild caught blue tits, *C. caeruleus*, as predators, because they are insectivorous passerines and have been successfully used in previous predation experiments on butterflies and eyespots (e.g., Vallin et al. 2006, 2007). The birds were trained to search for prey items that were made of printed paper and covered a reward, a small piece ($2 \times 2 \times 2$ mm^3) of peanut sunk in the background. This set up allowed us to easily manipulate prey and background patterns. We performed this study from January to March in 2009 at Tovetorp Zoological Research Station (Stockholm University) situated in South Eastern Sweden. The experimental protocol and housing of birds was reviewed and approved by the regional ethical committee in Linköping (Dnr: 62–08). We caught blue tits at the research station using mist nets and housed them indoors in individual cages ($80 \times 60 \times 40$ cm). The cages were fitted with perches and birds always had access to water, suet, sunflower seeds, and peanuts. The temperature was kept at 16°C and a light regime that followed the local day length. Birds were always allowed at least 1 h of undisturbed foraging in the home cage at both the beginning and the end of a day. We kept the birds in captivity for a maximum of 14 days. Before the present study, the birds were first used in another experiment. In that experiment, they all were treated alike and had searched for cryptic, rectangular prey items. Because the color pattern of the prey items and the backgrounds were very different between these two studies, we have no reason to assume that this would have had any effect on the response of the birds towards eyespots. The birds were ringed and released at the site of their capture after use in the present study. All the birds maintained their condition and were healthy upon release.

Background and prey

The exposing backgrounds were made of A4-sized (27×21 cm) plain brown sheets of cardboard. The concealing backgrounds were produced by covering the plate with a sheet of printed paper. The patterning of the prey items and the concealing background were made using a purpose written program in Matlab R2008b (MathWorks Inc, Natick, MA). The concealing background was grey with two types of black pattern elements, dots and lines (Fig. 1a). One thousand dots and 300 lines were distributed randomly with respect to position and orientation on the background. The prey items were isosceles triangular ($b \times h$: 30×15 mm^2), and their grey ground color was identical to the grey of the concealing background (Fig. 1b, c). In addition, there were three lines and nine dots, identical to the lines and dots of the concealing background, randomly distributed on each prey item. Prey types differed both within and between the experiments with respect to number, placement, and size of artificial eyespots.

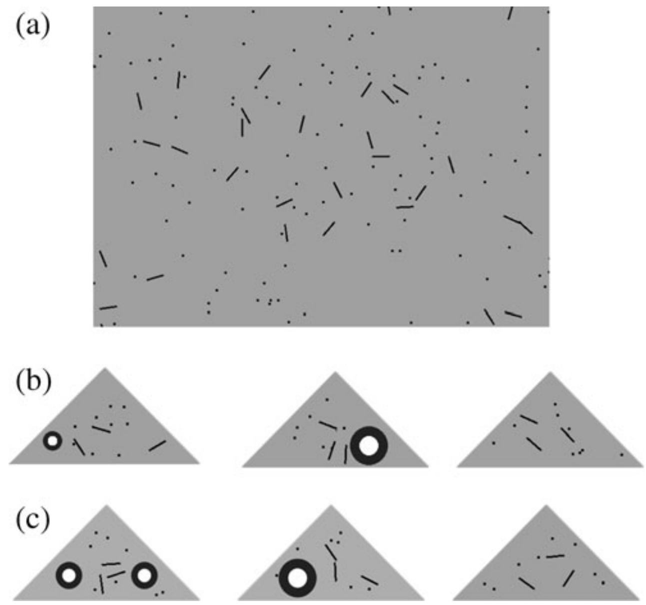

Fig. 1 A sample of the colour pattern of the concealing background (**a**). As the exposing background, we used typical non-tinted plain brown cardboard (not shown). Examples of the three prey types used in the first experiment (**b**) from left to right: small (3 mm) and large (6 mm) eyespot on the left or right, and no eyespot. Examples of the three prey types used in the second experiment (**c**): two small (4 mm), one large (6 mm) eyespot and no eyespots. Prey items were presented in pairs combining two different prey types. In both experiments, the *lines* and *dots* were distributed randomly, and the exact position of the eyespot at the wing tip varied randomly

Training

We trained the birds prior to the experiments to associate the triangular pieces of paper with the pieces of peanut under them, so that the birds would be willing to search for and attack them in the experiment. The training procedures were identical in the two experiments. The training was done in the experimental cage that was made of plywood ($W \times H \times D$: $55 \times 90 \times 70$ cm^3) and was lit by two high frequency fluorescent day light bulbs (15 W, BIOlight, Narva). One of the short side cage walls contained an observational window made from one-way see-through plastic (10×12 cm^2) that allowed observation without noticeably disturbing the bird. A perch was placed 20 cm below the ceiling on the long side wall to the left of the wall fitted with the observational window, and on the long side wall to the right, a hatch was situated at cage floor level where the background plates with prey could be inserted and removed. Additionally, a bowl of fresh water was present at all times in the cage.

Before every training and experiment session, each bird was allowed an acclimatization period of 45–60 min in the experimental cage without access to food. In the first training step, a bird received a plain cardboard (11×15 cm^2) with five prey items: (a) one white prey item with a piece of peanut glued on top of it, (b) two uniform grey prey items, lacking any patterning, that were glued to the cardboard at one point and had a piece of peanut glued to its underside (the peanut was visible from the side), and (c) two uniform grey prey items, lacking any patterning, glued from all corners and covering a peanut piece in a hole in the cardboard (the peanut piece was completely invisible). The prey items were placed randomly, but with the restriction that no prey was placed less than 2 cm from the edge or, in the second experiment, from another prey. A bird was allowed to search for prey items during 60 min. Birds that found and consumed all five of the peanuts within this time advanced to the second training step that took place the following day. Birds that failed were given two more chances on consecutive days before unsuccessful individuals were excluded from the experiment. We chose to use only prey without eyespots in the training (a) to have uniform training protocol for all birds, (b) to not familiarize the birds with the eyespots, and (c) to exclude the possibility that the birds would form a search image for the eyespot, giving an additional benefit for the spotless prey.

The plate used in the second training step was A4-sized, and half of the plate was plain cardboard and the other half was covered with the concealing background patterning. There were two grey prey items on each half of the plates. The prey lacked any patterning, and they all covered a piece of peanut that was in a hole, below the level of the surface of the plate. One prey item on each half was attached to the plate at only one of three tips and its detectability was increased by slightly bending one tip up. After successfully completing the second step (according to the same criteria as for step 1), a bird advanced to either of the two experiments the following day.

Experiment 1

In the first experiment, which tested for the deflective function of eyespots on the two backgrounds, the birds were presented prey without any eyespots (hereafter "spotless") and prey with one eyespot either at the right or the left tip of the "wing" (Fig. 1b). This allowed us to observe whether an eyespot had any effect on where the birds directed their attacks. To be able to assess the effect of eyespot size on the birds' responses, we used two different sizes. Prior to the experiment, 30 birds were randomly assigned to either the large (6 mm diameter) or small (3 mm) eyespot treatment. Every bird was successively offered 18 experimental plates, each having a single prey of the three types, (1) eyespot on the right tip and (2) eyespot on the left tip, or (3) spotless (Fig. 1b). Half of the presentations were made on the exposing and the other half on the concealing background. Each bird received all the combinations of background and prey type three times. The presentation order was randomized for each bird. For every presentation, we recorded both at which side of the midline, left or right, the bird attacked the prey item, and the effective time to attack. Effective time to attack was defined as the total time a bird was actively searching for prey on the plate. Timing started when a bird landed on the plate, was paused if the bird left the plate, and finally stopped when the bird ripped the prey from the background and consumed the peanut. The plate was then removed from the cage and the next plate in the sequence presented to the bird.

Experiment 2

In the second experiment, which tested for the deterring effect of eyespots on the two backgrounds, we used three different prey types (Fig. 1c): (1) spotless, (2) one large eyespot (diameter=6 mm), and (3) two smaller eyespots (diameter=4 mm). Because previous experiments suggest that the deterring effect increases with the total area of eyespots (Stevens et al. 2008a), we kept the total areas of black and white of the two small eyespots equal to those of the large eyespot. This allowed us to test if a pair of eyespots is more deterring than a single eyespot (when controlled for area), and if prey concealment influences this effect. On each plate, which either had the exposing or the concealing background, we presented one of the three possible combinations of prey: (1) spotless and one eyespot, (2) spotless and two eyespots, and (3) one eyespot and two eyespots. All 15 birds were presented with all three prey combinations on both backgrounds and this protocol was repeated three times in a randomized order, again giving a total of 18 presentations for each bird. The two prey items were randomly placed on the background but never closer than 10 cm from each other and never closer than 2 cm from the edge of the plate. We recorded which prey was attacked, and the effective time to attack. Birds were only allowed to attack one of the two prey items, after which the plate was removed and a new plate was inserted into the cage.

Statistical analyses

We used R 2.9.2 for Windows (R Development Core Team 2009) for analyzing the data. We used logistic regression to analyze the frequencies of attacks towards the side of the prey with the eyespot in the first experiment and frequencies of first attacks towards each prey type in the second experiment. This was done using the lme4 package, which allowed us to include background as a repeated measurement variable in the analysis. Thus, the logistic regression models consisted only of the term background as an explanatory variable. Its significance was tested with Wald

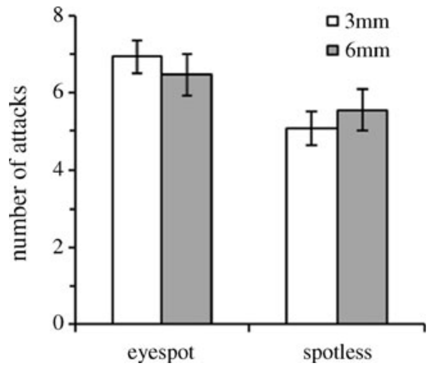

Fig. 2 The mean frequencies (± SE) of attacks on the two sides of the prey items, the side that had an eyespot and the spotless side. All birds were presented the prey with one eyespot either on the left or on the right "wing" for 12 times. Half of the birds (n=15) received prey with 3-mm eyespots (*white bars*) and the other half (n=15) received prey with 6-mm eyespots (*grey bars*)

statistics. Further, if the test was non-significant, we confirmed the result by deleting the term from the model and by checking that this decreased the Akaike information criteria (AIC) value, indicating improved fit of the model to the data (e.g., Crawley 2007). In both experiments, the effective times to attack met the requirements for parametric analysis after power transformations suggested by the Box-Cox analysis. We analyzed attack times using repeated measurements ANOVA.

Results

Experiment 1: the deflection hypothesis and the effect of background

Concealment of the rest of the prey did not influence the deflective effect of the eyespots. For the smaller (3 mm) eyespot, the frequency of attacks on the eyespot side of the prey was not dependent on background type (Wald test: z=0, df=1, p=1.0). Similarly, the side of attack for the prey with the larger (6 mm) eyespot did not depend on background type (Wald test: z=0.46, df=1, p=0.64). In accordance with this, eliminating the term background from the logistic regression models decreased their AIC values, indicating improved fit to the data.

We proceeded by pooling the data for the two backgrounds and testing if the location of the eyespot influenced the side the birds directed their attacks. Interestingly, we found a significant deflective effect. The birds more frequently directed their attacks at the side where the spot was located in the 3-mm eyespot trials (Wilcoxon matched pairs test: W=74, n=15, p=0.047; Fig. 2). However, the effect was not significant in the 6-mm eyespot trials (Wilcoxon matched pairs test: W=67, n=15, p=0.37), although the direction of the effect was the same as in the 3-mm eyespot trials (Fig. 2).

We also tested if background or prey type (i.e., spot to the left, to the right, or no spot) influenced the measured attack times (Fig. 3). Birds attacked prey on the concealing background after a longer time compared with the exposing background (ANOVA: $F_{1,28}$=257, p<0.001). There was no effect of spot size (ANOVA: $F_{1,28}$=0.02, p=0.90) or prey type (ANOVA: $F_{2,56}$=1.44, p=0.24). Neither were any of the interactions significant (all $p \geq 0.19$).

Experiment 2: the intimidation hypothesis and the effect of background

We tested for the effect of background separately for the three prey combinations. Background did not affect which prey was attacked in the combination of no eyespot and large eyespot (Wald test: z=1.10, df=1, p=0.27; Fig. 4), and in the combination of one large and two small eyespots (Wald test: z=0.21, df=1, p=0.83). In contrast, attacks depended on background type in the combination of no eyespot and two eyespots (Wald test: z=2.10, df=1, p=0.036). The prey with the two eyespots was avoided more often when it was presented on the background that concealed the rest of its surface (Fig. 4).

Next, we tested if the number of eyespots influenced which prey type the birds attacked first. For those presentation pairs for which background had no effect (0 vs. 1 eyespot and 1 vs. 2 eyespots), we pooled the data from the two backgrounds. When the prey had the combination of no eyespot and one large eyespot, there was no difference in the number of attacks between the prey types (Wilcoxon matched pairs test: W=37, n=15, p=0.32; Fig. 4). When the prey had the combination

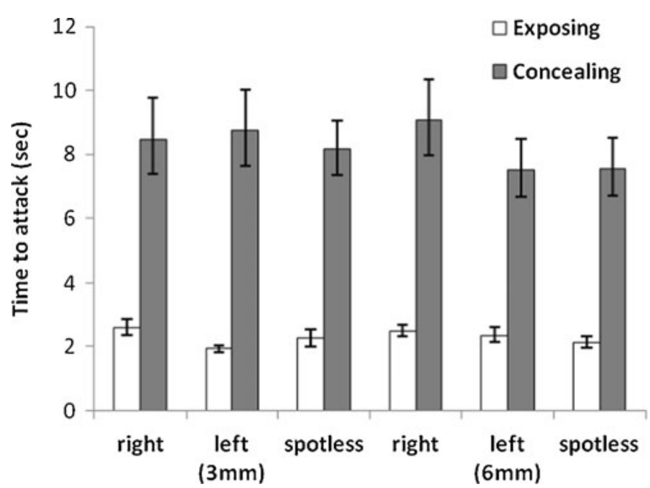

Fig. 3 Mean (± back-transformed SE) time to attack for the three prey types (eyespot on the right side, on the left side, or no eyespot) on the exposing (*white bars*) and concealing (*grey bars*) backgrounds with the 3-mm and the 6-mm eyespots

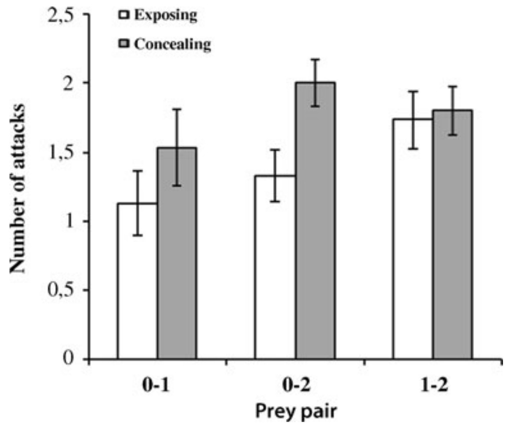

Fig. 4 The frequency of first attacks (mean±SE) by blue tits on the prey item having the smaller number of eyespots of a pair of prey items making up the two-choice test. The pairs consisted of prey with 0 and 1, 1 and 2, and 0 and 2 eyespots, respectively. All birds (n=15) were presented three times all three prey combinations on both the exposing (*white bars*) and on the concealing (*grey bars*) background

of one large and two small eyespots, there was an almost significant tendency for the birds to attack more often the prey with one eyespot (W=45, n=15, p=0.059; Fig. 4). Because background influenced the birds' preference in the prey combination of no eyespot and two eyespots, these data were analyzed separately for both the backgrounds. On the concealing background, the birds attacked the spotless prey significantly more often (Wilcoxon matched pairs test: W= 100.5, n=15, p=0.016). On the exposing background, however, no difference was found (W=49, n=15, p=0.52).

When analyzing the attack times, we again found that it took a longer time on the concealing than on the exposing background before the birds attacked the prey (ANOVA: $F_{1,14}$=118, p<0.001; Fig. 5). However, attack time was not influenced by prey type ($F_{2,28}$=0.36, p=0.70), and the interaction between background and prey type was not significant ($F_{2,28}$=0.57, p=0.57).

Discussion

In our first experiment, in which the eyespot was either on the left or on the right "wing" of the triangular prey, we found that the birds more often attacked the side with the eyespot, when the eyespot was small (3 mm). This is an important result because it lends support to the idea that some eyespots can be used to manipulate predators to direct their attack towards the eyespot and away from other body areas. It also implies that such deflective effect can select for eyespots and where they are placed.

Although the idea of deflective effect of eyespots is old (Poulton 1890), experimental studies investigating this effect have either produced negative results (Lyytinen et al. 2003, 2004; Vlieger and Brakefield 2007) or have not been statistically sound (Blest 1957) or have lacked a proper control (Wourms and Wasserman 1985). Lyytinen et al. (2003, 2004) found that naive pied flycatchers (*Ficedula hypoleuca*) caught fewer butterflies with marginal eyespots than did adult birds, but there was no difference in success of attacks directed towards butterflies with eyespots and without eyespots. Hence, the studies by Lyytinen and others provided some circumstantial support for higher survival of butterflies with marginal eyespots but no strong direct evidence for the hypothesis that eyespots could actually direct attacks to less critical body parts. An exception among previous studies is the recent study by Olofsson et al. (2010), which found a deflective effect but only under artificial light conditions when UV intensity was high and the intensity of longer wavelengths was low. They speculated that this finding could be related to the relatively higher proportion of UV in the dawn, when also the intensity of bird predation is high. However, in our experiment (using daylight imitating lights), an exceptionally high UV intensity was not required to induce a deflective effect. In conclusion, our study shows that small eyespots can be used by prey to manipulate where birds direct their attacks, which is a necessary condition for any survival benefit from the deflective effect. It also suggests that this effect can take place under broader, less specific light conditions than proposed by Olofsson et al. (2010).

Interestingly, we did not find a similar deflective effect with the larger (6 mm) eyespot. In real butterflies, eyespots as big as these are usually associated with deterring function (cf. Vallin et al. 2007). We therefore speculate that our larger eyespot may have had a weak deterring effect that has counteracted the deflective effect.

We hypothesized that if the rest of the prey body would be well-concealed, then the eyespots would attract more attention and that should strengthen the deflective effect. Contrary to our hypothesis, deflection of attacks did not depend on how well the rest of the prey body blended in the

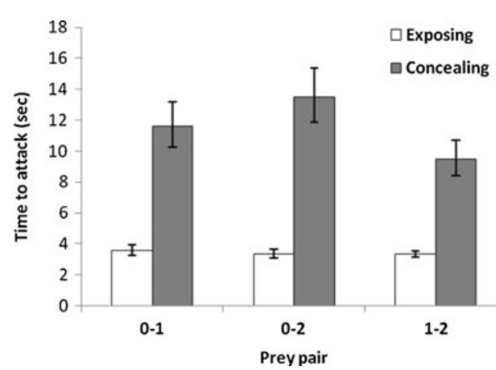

Fig. 5 Mean (± back-transformed SE) time to attack in the two-choice experiment. Pairs of prey with no and one (0–1), no and two (0–2), and one and two (1–2) eyespots were presented on the exposing (*white bars*) and concealing (*grey bars*) background

background. Importantly, this suggests that the birds did not direct their attacks more towards the eyespots simply because the eyespots would have been the only conspicuous part of the prey.

The difference between the concealing and the exposing background in attack times indicates that our manipulation of prey concealment through the backgrounds was successful. Interestingly, there was no effect of prey type on search time although the eyespots appeared relatively distinct. Because the prey used in training had no eyespots, the predators may have learned to attend to other cues for detecting the prey on the concealing background. This implies that the detectability cost of eyespots for a cryptic prey, at least when eyespots are a novel trait, may be surprisingly low. This is interesting when considering the evolution of eyespots (see also Lyytinen et al. 2003).

In the second experiment, we focused on the intimidation hypothesis, and presented the predators with paired combinations of prey with one large (6 mm) eyespot or two symmetrically placed, smaller (4 mm) eyespots with equal total area as the large eyespot or with no eyespot at all. As in the first experiment, attack times were longer on the concealing background, suggesting that our manipulation of the background had been successful. We found an effect of background only in the choice between the prey with two eyespots and no eyespots. The birds were more prone to attack the prey without eyespots when the prey items were presented on the concealing background, whereas there was no difference when they were presented on the exposing background. Because the prey with no eyespots was the more concealed one of the two, we can be confident that the longer time to attack the prey with two eyespots on the concealing background was caused by increased hesitation. These results suggest that if a prey has two eyespots, the deterring effect is strengthened compared to a prey with no eyespots, provided the rest of the prey is relatively well concealed. Interestingly, Stevens et al. (2008b) found in a field experiment using artificial prey the opposite effect, such that a pair of simple spots decreased predation risk more in a more conspicuous prey. There are, however, several methodological differences between these two studies. For example, in our study, the ground coloration of the prey highly matched the concealing background and the eyespots consisted of two concentric, contrasting circles, whereas in Stevens et al. (2008b) the more concealed prey only matched the average perceived lightness of the tree trunks on which they were presented, and the spots were simple circles. Furthermore, in our experiment, the preys were presented at a relatively close range and to a single predator species, whereas in the field study by Stevens et al. (2008b), the detection range and predator species were not limited. Nevertheless, these differing results suggest that more research on how background influences the deterring properties of eyespots is needed.

When there was a difference of one in the number of eyespots between the two prey (0 vs. 1 and 1 vs. 2), the background did not influence which prey was attacked. When the data for these presentations were pooled for the two backgrounds, we found that one large eyespot was not more deterring than no eyespots, and increasing the number of eyespots from one to two, but keeping their total area constant, caused an almost significant increase in the deterring effect. In a field experiment with artificial prey, there was no difference in predation rate on prey either with one, two, or three equally sized eyespots or when prey with one large eyespot was compared with prey with two eyespots with half the area of the large (Stevens et al. 2008a).

Although firm evidence exists for the idea that conspicuous eyespots can thwart attacks of birds (Vallin et al. 2005, 2006, 2007; Kodandaramaiah et al. 2009), our understanding of which features make them effective is still very basic. Much of the present knowledge of the deterring features comes from experiments using simple representations of eyespots and suggests that for example high internal pattern contrast (Jones 1980; Stevens et al. 2007), area (Stevens et al. 2008a), shape (Scaife 1976; Stevens et al. 2008a) and number (Scaife 1976; but see Stevens et al. 2008a) are important for eliciting aversive responses in birds. Although the prey items in our experiment featured several of these qualities, they did not elicit any strong aversive response in the blue tits. One reason may be the size of the eyespots used. In order to achieve the same total area of black and white in the one spotted prey and the two spotted prey, the diameter of spots of the two-spotted prey were 4 mm each. Vallin et al. (2007) measured the eyespots of seven peacock butterflies (*Inachis io*) which elicited a deterring effect on blue tits, and found their diameter to be 6.4±0.5 mm. This suggests that the blue tits in the present experiment might have found a larger pair of eyespots more deterring. Another property in *I. io* that strengthens the deterring effect is that the display is seldom static, but is often combined with behaviors, such as sudden exposure of the eyespots that is accompanied with noise, and the display is usually directed towards the predator so that the apparent size of the display will be maximized (Blest 1957; Vallin et al. 2007). However, little is known about how widespread such behaviors are among butterflies that have deterring eyespots. In any case, the deterring effect elicited by the prey with two eyespots in our two-choice experiment confirms that even artificial and static eyespots can deter a predator from attacking and are therefore useful in research on natural selection on eyespots.

To summarize our main results, we found a deflective effect caused by small eyespot but, interestingly, not by large eyespots, and this effect was not dependent on prey concealment. This result is important because it provides evidence for the hypothesis that prey can use small eyespots to manipulate passerine birds so that attack probabilities will increase towards some body areas and consequently decrease towards other areas. Our results also imply that the deflective effect can select for the occurrence, placement, and size of some eyespots. In addition, we found some evidence for the effect of prey detectability on the efficacy of deterring eyespots.

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References

Blest AD (1957) The function of eyespot patterns in Lepidoptera. Behaviour 11:209–256

Brakefield PM, Gates J, Keys D, Kesbeke F, Wijngaarden PJ, Monteiro A, French V, Carroll SB (1996) Development, plasticity and evolution of butterfly eyespot patterns. Nature 384:236–242

Crawley MJ (2007) The R book. Wiley, Chichester

Endler JA (1992) Signals, signal conditions, and the direction of evolution. Am Nat 139:S125–S153

Forsman A, Herrström J (2004) Asymmetry in size, shape, and colour impairs the protective value of conspicuous color patterns. Behav Ecol 15:141–147

Jones RB (1980) Reactions of male domestic chicks to two-dimensional eye-like shapes. Anim Behav 28:212–218

Kodandaramaiah U (2009) Eyespot evolution: phylogenetic insights from Junonia and related butterfly genera (Nymphalidae: Junoniini). Evol Dev 11:489–497

Kodandaramaiah U, Vallin A, Wiklund C (2009) Fixed eyespot display in a butterfly thwarts attacking birds. Anim Behav 77:1415–1419

Lyytinen A, Brakefield PM, Mappes J (2003) Significance of butterfly eyespots as an anti-predator device in ground-based and aerial attacks. Oikos 100:373–379

Lyytinen A, Brakefield PM, Lindström L, Mappes J (2004) Does predation maintain eyespot plasticity in *Bicyclus anynana*? Proc R Soc Lond B 271:279–283

Meadows DW (1993) Morphological variation in eyespots of the foureye butterflyfish (*Chaetodon capistratus*): implications for eyespot function. Copeia 1993:235–240

Nijhout HF (1980) Pattern formation on lepidopteran wings: determination of an eyespot. Developm Biol 80:267–274

Nijhout HF (1991) The development and evolution of butterfly wing patterns. Smithsonian Institution, Washington

Olofsson M, Vallin A, Jakobsson S, Wiklund C (2010) Marginal eyespots on butterfly wings deflect bird attacks under low light intensities with UV wavelengths. PLoS ONE 5:e10798

Poulton EB (1890) The colours of animals. Paul, Trench. Trübner & Co, London

Robertson KA, Monteiro A (2005) Female *Bicyclus anynana* butterflies choose males on the basis of their dorsal UV-reflective eyespot pupils. Proc R Soc B 272:1541–1546

Rowe C, Skelhorn J (2004) Avian psychology and communication. Proc R Soc Lond B 271:1435–1442

Ruxton GD, Sherratt TM, Speed MP (2004) Avoiding attack: the evolutionary of crypsis, warning signals and mimicry. Oxford University Press, Oxford

Scaife M (1976) The response to eye-like shapes by birds. II. The importance of staring, pairedness and shape. Anim Behav 24:200–206

Stevens M (2005) The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. Biol Rev 80:573–588

Stevens M, Merilaita S (2009) Animal camouflage: current issues and a new perspective. Phil Trans R Soc Lond B 364:423–427

Stevens M, Hopkins E, Hinde W, Adcock A, Connelly Y, Troscianko T, Cuthill IC (2007) Field experiments on the effectiveness of "eyespots" as predator deterrents. Anim Behav 74:1215–1227

Stevens M, Hardman CJ, Stubbins CL (2008a) Conspicuousness, not eye mimicry, makes "eyespots" effective antipredator signals. Behav Ecol 19:525–531

Stevens M, Stubbins CL, Hardman CJ (2008b) The anti-predator function of 'eyespots' on camouflaged and conspicuous prey. Behav Ecol Sociobiol 62:1787–1793. doi:10.1007/s00265-008-0607-3

Vallin A, Jakobsson S, Lind J, Wiklund C (2005) Prey survival by predator intimidation: an experimental study of peacock butterfly defence against blue tits. Proc R Soc Lond B 272:1203–1207

Vallin A, Jakobsson S, Lind J, Wiklund C (2006) Crypsis versus intimidation—anti-predation defence in three closely related butterflies. Behav Ecol Sociobiol 59:455–459

Vallin A, Jakobsson S, Wiklund C (2007) "An eye for an eye"—on the generality of the intimidating quality of eyespots in a butterfly and a hawkmoth. Behav Ecol Sociobiol 61:1419–1424

Vlieger L, Brakefield PM (2007) The deflection hypothesis: eyespots on the margins of butterfly wings does not influence predation by lizards. Biol J Linn Soc 92:661–667

Wallace AR (1889) Darwinism. Macmillan, London

Wourms MK, Wasserman FE (1985) Butterfly wing markings are more advantageous during handling than during the initial strike of an avian predator. Evolution 39:845–851