

CrossMark
click for updates

Research

Cite this article: De Bona S, Valkonen JK, López-Sepulcre A, Mappes J. 2015 Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots. *Proc. R. Soc. B* **282**: 20150202.

<http://dx.doi.org/10.1098/rspb.2015.0202>

Received: 29 January 2015

Accepted: 16 March 2015

Subject Areas:

behaviour, evolution, ecology

Keywords:

eyespots, animal coloration, predator–prey interactions, mimicry, IRTree GLMMS, deception

Author for correspondence:

Sebastiano De Bona

e-mail: sebastiano.debona@gmail.com

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2015.0202> or via <http://rspb.royalsocietypublishing.org>.

Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots

Sebastiano De Bona¹, Janne K. Valkonen¹, Andrés López-Sepulcre^{1,2} and Johanna Mappes¹

¹University of Jyväskylä, Centre of Excellence in Biological Interactions, Department of Biological and Environmental Science, Jyväskylä, Finland

²CNRS UMR 7618, Institute of Ecology and Environmental Sciences of Paris (iEES), Université Pierre et Marie Curie, Paris, France

Large conspicuous eyespots on butterfly wings have been shown to deter predators. This has been traditionally explained by mimicry of vertebrate eyes, but recently the classic eye-mimicry hypothesis has been challenged. It is proposed that the conspicuousness of the eyespot, not mimicry, is what causes aversion due to sensory biases, neophobia or sensory overloads. We conducted an experiment to directly test whether the eye-mimicry or the conspicuousness hypothesis better explain eyespot efficacy. We used great tits (*Parus major*) as model predator, and tested their reaction towards animated images on a computer display. Birds were tested against images of butterflies without eyespots, with natural-looking eyespots, and manipulated spots with the same contrast but reduced resemblance to an eye, as well as images of predators (owls) with and without eyes. We found that mimetic eyespots were as effective as true eyes of owls and more efficient in eliciting an aversive response than modified, less mimetic but equally contrasting eyespots. We conclude that the eye-mimicry hypothesis explains our results better than the conspicuousness hypothesis and is thus likely to be an important mechanism behind the evolution of butterfly eyespots.

1. Introduction

The animal kingdom exhibits a stupendous diversity of visual anti-predator signals that has fascinated biologists for decades and dominated the study of interspecific communication [1–3]. Among them, eyespots have been championed as the ultimate example of deceptive communication through predator mimicry [1]. These visual markings, consisting of concentric circles resembling a vertebrate eye, can be found in a variety of insects, fishes, molluscs, amphibians and birds [1,4–6]; yet, their ubiquitousness and diversity in lepidopterans are unmatched [6–8]. Some species have small, marginally located spots assumed to have a deflection function, driving the predator's attention to a non-vital part of the body [1,9–11]. Many others have larger central eyespots which are thought to intimidate potential predators [1–3]. The traditional explanation for this intimidation effect has been the eyespots' resemblance to vertebrate eyes and, therefore, to a potential enemy of the attacker [1,2]. Although intuitively appealing, this classical example of mimicry remained untested and was recently called into question [6].

Several studies have confirmed the efficacy of intimidating eyespots in inducing a startle response and improving prey survival [4,12–15]. However, Stevens [6] proposed that the intimidating effect of eyespots is merely due to their conspicuousness, and that the features shared with vertebrate eyes (e.g. circularity, being in pairs) represent common developmental constraints [6,16,17]. Predators are known to have strong aversions towards conspicuous colours, which can induce a neophobic reaction [18], initial or learnt avoidance [19], or a delay in attack due to the increased information processing time (sensory overload [6]). Mimicry, Stevens argues [6], need not be invoked to explain the startling effect of eyespots on predators. This criticism of the long assumed hypothesis triggered a series of studies aimed at contrasting them [20–23].

Stevens created artificial paper moths carrying different types of eyespots and tested for survival differences between models (e.g. [20,21]). In support of the

	OW	OE	BR	BM	BW
total	29	29	50	46	38
females	9	10	16	15	10
males	20	19	34	31	28
adults	11	16	22	23	18
juveniles	18	13	28	23	20

Figure 1. Treatments, sample sizes and the planned comparisons tested. OE, owl with eyes; OW, owl without eyes; BR, butterfly with real eyespots; BM, butterfly with modified eyespots; BW, butterfly without eyespots.

conspicuousness hypothesis, these studies showed that size and number matter for the efficacy of these markings, but not the shape nor the colour arrangement of the concentric circles. By contrast, Merilaita *et al.* [22] found that eyespot number (2 or 4) had no survival effect on manipulated real peacock butterflies (*Inachis io*) presented to blue tits (*Cyanistes caeruleus*) in a laboratory setting. This lack of effect of stimulus intensity is in contrast with the conspicuousness hypothesis, but does not support the eye-mimicry either, since two eyes should create a more convincing predator imitation than four. In an elegant study, Blot *et al.* [23] created artificial prey moths and manipulated the crescent-shaped sparkle mark within the pupil. Sparkle marks are present in many butterfly eyespots and increase the three-dimensional appearance of the eyespot, creating the illusion of light reflecting on an eyeball [23]. In this field experiment, they found that sparkles in a natural position increased the deterrent efficacy of the eyespot, giving some support for the eye-mimicry explanation.

Predation experiments conducted in the field often lack information on predator identity and behaviour during the attack. Therefore, the proximate effect of the eyespot can be misinterpreted or unobserved. Moreover, the simplicity of the achromatic paper eyespots used in many experiments may be insufficient to mimic a vertebrate eye and elicit a startle response. The higher survival of non-eye-mimic models in Stevens' studies [20,21] could be attributed to a lack of familiarity with the conspicuous shapes presented to the predator, rather than a startle effect. However, such system should not be evolutionarily stable, because lack of familiarity can only be a transitional state when the phenotype is rare (if it spread and became common, predators would become familiar). It is therefore essential to test the eye-mimicry and conspicuousness hypotheses in settings where direct observation of predator behaviour is possible.

In this study, we recreated an encounter between predator and prey in controlled conditions. We simulated a prey defence against the predator attack triggering an image animation on a computer monitor, and recorded the predator's behavioural reaction. As a model for an avian predator, we used wild caught great tits (*Parus major*). To avoid any bias due to prior learning, it was crucial to choose a novel eyespot pattern that birds could not have experienced before. We opted for testing a real butterfly image as the stimulus to maximize the realism of the eyespot pattern while manipulating only the feature at test (mimicry). Therefore, as a model, we chose a tropical

butterfly (*Caligo martia*) whose single pair of eyespots has a strong resemblance to owl eyes.

We manipulated the image of the butterfly by reversing the colour arrangement of the internal features of the wing eyespots (figure 1) in order to reduce the eye similarity while keeping contrast the same (see Material and methods). We compared the reactions of great tits (*P. major*) to manipulated eyespots, unmodified (eye-resembling) eyespots and to a butterfly with the eyespots digitally removed. Our experiment was designed to test both competing hypotheses against each other. It is important to note that we did not intend to investigate the specific adaptation of *Caligo martia* against its natural predators, but rather to test the properties that make eyespots generally aversive to a naive predator's eye. As a positive control, we tested whether our potential predators were indeed intimidated by real owl eyes, rather than their entire face. We did so by testing images of a pygmy owl face (*Glaucidium passerinum*) with and without eyes. If the eye-mimicry hypothesis is true, eyespots that to a human observer resemble the eyes of an owl should elicit similar aversive reactions to the face of an owl with open eyes, while non-mimetic but equally contrasting wing spots should elicit a weaker response. On the other hand, if the conspicuousness theory is true, eyespots differing in mimicry but with the same internal contrast should elicit a similar response, which should be stronger than the reaction to the butterfly without the eyespots.

2. Material and methods

(a) Predator handling

The study was conducted at Konnevesi Research Station, in Central Finland (62.6° N, 26.3° E) during January–April 2014. Wild great tits (*P. major*) were caught in the vicinity of the research station using trap boxes and mist-nets. We weighed birds upon capture, recorded sex and age, and housed them in artificially illuminated plywood cages (64 × 64 × 77 cm) with one or two perches that allowed them to rest. The light was set to be on between 8.30 and 20.00 EET. Birds were fed *ad libitum* with sunflower seeds, peanuts, crushed suet-balls and fresh water. Between 72 and 24 h before the experiment, two dead mealworms (*Tenebrio molitor*) were placed in the cage to habituate birds to the mealworm taste and encourage attack during the experiment. All birds were released after the experiment at the site of capture. Before release birds were weighed and ringed.

(b) Prey treatments

We designed five different treatments (figure 1) corresponding to the following images: an owl with open eyes (OE), an owl with eyes closed (OW), a butterfly with mimetic (real) eyespots (BR), a butterfly with modified (reversed) eyespots (BM) and a butterfly without eyespots (BW). The owl treatments (OW and OE) featured the face of a Eurasian pygmy owl (*Glaucidium passerinum*). This is a common and important predator [24,25] known to elicit strong aversion in great tits [26] and is commonly detected in the area where the study birds were captured (J. Mappes, personal observations since 1996). The OE and OW pictures featured the same owl face, with eyes being digitally removed in OW. For the butterfly images, we used a ventral picture of an owl butterfly (*Caligo martia*). The eyespots of the genus *Caligo* show a remarkable resemblance to owl eyes, at least to the human observer, hence their common name. While these butterflies rest with the wings folded, most probably showing one eyespot at the time, we decided to use a full ventral image, which maintain the same symmetry as the owl face and is more representative of what is found among a variety of butterfly species. It is important to note that our experiment does not aim to recreate *Caligo's* natural behaviour, but rather use the image of the species to test if its high resemblance to vertebrate eyes to a human observer was matched by a perception of danger in birds. BR consisted of an unmodified picture featuring natural eyespots (black pupil, yellow iris). For BM, we manipulated the same picture so that the eyespot colours were reversed: the pupil and contour were replaced with natural yellow, and the iris and crescent-shaped sparkle mark within the pupil with black (figure 1). We added a brown ring between the black iris and the yellow outer ring, as well as two yellow veins crossing the spot to keep the number of passages between contrasting colours and the surface of each feature equal (figure 1). Given that we kept all other features of the eyespot but the position of the colours equal, thus the contrast and number of transitions between adjacent colours in BR and BM are similar [27]. The manipulated eyespot (BM) showed slightly higher root mean square contrast [28] than the natural eyespot (BR), therefore making our estimate of the mimicry effect conservative (RMS contrast: BR = 68.90, BM = 74.51). The total surface of the owl eyes and butterfly eyespots was very similar (61 098 pixels and 61 732 pixels, respectively), as were the dimensions of the two figures when displayed on the computer monitor (owl 7.6×5.2 cm; butterfly: 6.7×6.5 cm). The overall tan coloration of the owl image was adjusted to be closer to the butterfly wing colour, yet still within the natural range, in order to standardize the features' backgrounds as much as possible. All image manipulations and adjustments were performed in Adobe Photoshop Cs4. The images were displayed to the birds on a computer monitor (Dell Professional™ P2311H 23"), against a solid white background to remove any effect of crypsis. In the display settings, the brightness and contrast of the monitor were set, respectively, to 30 and 75. While the lack of UV reflectance of a computer screen can cause colours to appear unnatural for birds, this effect was present in all treatments and thus controlled. Moreover, birds' scare reactions to the displayed pictures appeared real (see electronic supplementary material, video).

(c) Experimental set-up

The experimental trials were performed in a cage similar to the housing cages ($50 \times 49 \times 68$ cm), except for the presence of a one-way glass replacing one of the walls, through which the bird could be seen and filmed. The cage contained a light bulb, a perch and a water cup where fresh water was always available. The cage floor was modified to fit the computer monitor horizontally, covered by a transparent Plexiglas and a sheet of brown paper with a circular hole (10.5 cm diameter) cut out where the image was displayed. A 5 cm-high piece of grey plastic pipe (10.5 cm diameter) was placed around the hole so that the image display could be seen inside it (henceforth referred to as

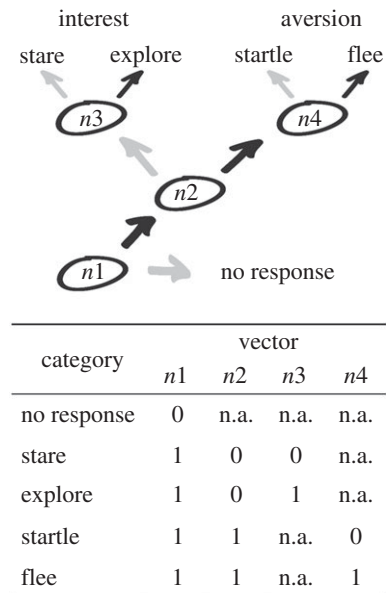


Figure 2. Structure of the binomial tree leading to the four behavioural response categories recorded. Circles represent binomial nodes with the two alternative outcomes coded as 1 (black arrows) or 0 (grey arrows). Table represents the binomial node values necessary to code each categorical outcome.

'ring'). The monitor was connected to a laptop (Sony Vaio™ SVF1421C5E) to control the image display. Approximately, 1 h 30 min before starting the experiment the bird was deprived of food to increase foraging motivation during the experiment. The bird was placed in the experimental cage 30 min prior to the experiment and left to habituate. During this time, the screen was on and displayed the same solid white background against which the images would appear later. We started the experiment placing a dead mealworm (*T. molitor*) on the Plexiglas inside the ring, exposing a harmless prey to the bird. As soon as the bird landed on the ring to attack the mealworm, we displayed the image using a split animation opening outwards along the width axis (using Microsoft Office 2013 PowerPoint®). This was done in order to standardize the encounter between the predator and the stimulus image. In this way, we also simulated the behaviour of a butterfly resting its wings closed and spreading them open, a situation often encountered by our predator model (great tits). The mealworm appeared to lay longitudinally along the butterfly body (treatments BR, BM, BW), or the owl beak (OE, OW). We removed the image after the bird caught the worm, or 10 min after the first attack. If the bird did not pick the mealworm after 10 min from the first attack, we removed it manually. After the worm was consumed or removed, we waited approximately 1 min, offered a new mealworm and ran a second trial with the same image treatment. If a bird's second trial was unsuccessful (the bird was too nervous or did not attack the prey, hence not facing the image again), we considered only the first trial. The experiment was filmed with a digital camera (Canon Legria™ HFR36). Each bird was tested in a single treatment, except in two cases in which the birds were recaptured after release and tested a second time. Given the time lapse between the two experiments and the apparent absence of learning, we did not consider it necessary to exclude those birds. We also included bird ID as a random effect in the analysis to account for pseudoreplication (see below). Eight birds that did not try to attack the mealworm (and hence did not face a picture) during the whole experiment were excluded from the analysis and released to the place of capture. We successfully tested 97 birds in 99 experiments, for a total of 192 successful trials (figure 1). We observed the bird's reaction to the displayed images, classifying it according to five categories: 'no response', 'stare', 'explore', 'startle' and 'flee'. We assigned

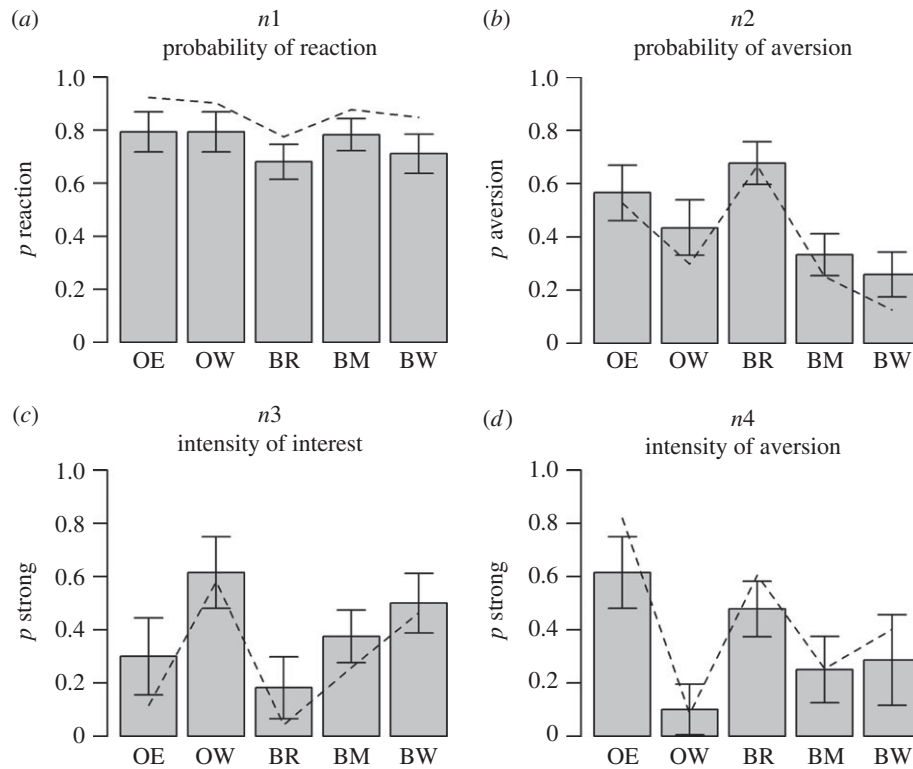


Figure 3. Observed proportions (grey bars) and fitted values (dashed lines) for the response tree GLMMs at all four tree nodes. Bar errors correspond to standard errors of the proportion.

the ‘no response’ category when birds did not show recordable behaviour in response to the image, while taking the mealworm. The ‘stare’ category was assigned when the bird stopped before picking up the prey, staring at the displayed image; if the bird moved or hopped around the ring edge to more carefully examine the prey we classified it as ‘explore’. If a bird startled, hopping or flying away from the ring we classified it as ‘startle’. If the bird showed a clear attempt to escape through the one-way glass, and/or emitted a warning call, we classified it as ‘flee’ (for a representative example of the behaviours see the electronic supplementary material, video). We also measured the predator recovery time after the display, considered as the time lapsed between the bird first landing on the ring and the bird capturing the mealworm. This lag was assumed to be a good estimate for a prey’s escape window, i.e. the period of time in which a real prey would have attempted to escape. If a bird did not catch the mealworm within the first 10 min after we triggered the image display, we coded the escape window as a missing value. In four cases, owing to a small delay in triggering the display, the bird reacted after seizing the mealworm and thus escape window was considered invalid (missing value) too. We collected a total of 179 valid observations for escape window.

(d) Statistical analysis

All analyses were performed using R Program v. 3.0.2. Reaction and escape window responses contained replicates of the same individual, so all models incorporate individual as a random effect to account for pseudoreplication.

In order to allow quantitative analysis of the categorical responses to the treatments, we analysed them using item response tree GLMMs [29]. This technique, borrowed from sociometrics, can be adapted and used as a way to handle categorical data of behaviour [30]. In essence, the method involves the conceptualization of the categorical responses as resulting from a decision tree composed of several nodes with two alternative outcomes. In other words, nodes correspond to sequential binary choices leading to each of the behavioural categories. The nodes should ideally be chosen to represent biologically meaningful alternatives.

Once coded this way, the data can be analysed as a multinomial response variable within a generalized linear mixed model (GLMM) framework, where the correlation among node probabilities are conditioned by the structure of the tree. Figure 2 depicts the decision tree we used to model our data. Node $n1$ determines the presence (1) or absence (0) of an observed reaction of any type (essentially distinguishing between ‘no response’ and the rest of categories). If a bird reacts ($n1 = 1$), node $n2$ further determines the type of reaction. This groups conceptually the four remaining responses into two groups: interest (0; ‘stare’ and ‘explore’), or aversion (1; ‘startle’ and ‘flee’). Nodes $n3$ and $n4$ model the strength of reaction as a probability of the strongest reaction occurring. $n3$ divides weak interest (‘stare’, 0) from strong (‘explore’, 1), while $n4$ separates weak aversion (‘startle’, 0) from strong (‘flee’, 1) (figure 3).

In practice, each observation was re-coded as a series of zeroes and ones that represent the sequence of node outcomes required to obtain the recorded response (figure 2) [30]. We then fit a GLMM with a binary response and logit link using `glmmPQL` function (MASS package). Node and the interaction between node and treatment contrast were included as fixed effects in order to account for different probabilities and treatment effects for different nodes. The planned contrasts specified are shown in figure 1. The repeated measures on single individuals were accounted for by adding node-specific random effects for individual birds.

Escape window scores were analysed with a mixed effects Cox model using the `coxme` function (package `coxme`). We only included data on individuals that caught the worm (i.e. there was no censored data), since the birds were given enough time and an absence of prey consumption was therefore considered a failed attempt, rather than a lag. We specified the same planned contrasts as the analysis of the categorical responses (figure 1).

3. Results

Table 1 summarizes the results of the response tree GLMM of bird behavioural responses (figure 3). We found that the

colour arrangement of the butterfly eyespot (mimicry effect, BR versus BM) affected the type of reaction observed (node *n2*). Real mimetic eyespots (BR) were more effective in inducing an aversive reaction than the equally contrasting modified ones (BM). There was also a non-significant trend for a mimicry effect on nodes *n3* and *n4*. The effect size suggests that, in contrast to modified eyespots (BM) the real eyespots (BR) tended to induce a stronger aversion ('fleeing', node *n4*), and weaker interest ('stare', node *n3*). The presence of eyes in the owl image (OE versus OW) increased the probability of fleeing when the reaction was aversive (node *n4*). We found also a non-significant trend of the owl eye presence on node *n3*, suggesting that the owl without eyes elicited stronger interest (exploration). We did not find any significant difference between owl eyes and their butterfly mimics (OE versus BR) for any node, indicating similar responses and suggesting strong efficacy of the mimicry. Neither did we find an effect of the conspicuousness *per se* for any node (BM versus BW).

For the escape window, no significant differences were found for any of the contrasts (all $p > 0.6$; electronic supplementary material, table S1 and figure S1). In addition, we ran the same analysis replacing one of the contrasts (model-mimic, OE versus BR) with the comparison between the two treatments we expected to produce the highest and the lowest mean escape window time (OE–BW) and yet we did not find a significant difference ($p = 0.42$; electronic supplementary material, table S1). This suggests that the bird's own tendency to hesitation might be more important than the treatment for determining the attack delay.

4. Discussion

In this study, we tested whether the intimidating effect of butterfly eyespots is caused by the mere conspicuousness of the spot or by its mimicry of a predator eye. Our results provide clear support for the eye-mimicry hypothesis but not the conspicuousness hypothesis. Mimetic eyespots were more effective in inducing an aversive response in a passerine predator (*P. major*) than the less mimetic but equally contrasting eyespots. Furthermore, owl faces with eyes elicited a stronger aversive response than faces without eyes, demonstrating the importance of this feature in recognizing potential predators [31,32]. We did not find a difference in the reaction of birds whether they were presented images of butterflies with non-mimetic spots or no spots. We therefore conclude that it is unlikely that the adaptive value of eyespots is due to sensory or information overload caused by the conspicuousness of colour pattern [16].

The majority of vertebrate predators of birds (e.g. raptors, felids and snakes) have eyes with an inner dark pupil surrounded by a light iris. Recent field experiments using artificial paper prey models with eyespots always visible showed that colour arrangement did not affect survival [20,21]. By contrast, we found that the colour arrangement appeared to strongly influence the efficacy of inducing aversion in a passerine predator (table 1). It is possible that the differences in our results are related to the differences in the set up. Eyespots tested on artificial paper prey are usually simple black and white concentric circles [4,20,21,33]. Their resemblance to a predator eye is far from the one achieved by the real eyespots of many lepidopteran species. For instance, the three-dimensional illusion achieved by the sparkle mark has proved to be

Table 1. Response tree GLMM of great tit categorical reactions. Eye effect OE—OW; model-mimic OE—BR; contrast effect BM—BW; mimicry effect BR—BM. Significant results are presented in bold.

	<i>n1</i> probability of reaction			<i>n2</i> type of reaction (prob. aversion)			<i>n3</i> intensity of interest			<i>n4</i> intensity of aversion						
	estimate	s.e.	<i>T</i>	estimate	s.e.	<i>T</i>	estimate	s.e.	<i>T</i>	estimate	s.e.	<i>T</i>	<i>p</i>			
intercept	1.92	0.34	5.59	0.00	0.00	–2.03	0.04	0.41	–1.21	0.41	–2.93	0.00	–0.39	0.48	–0.81	0.41
eye effect	0.26	1.24	0.21	0.83	1.05	0.91	0.36	–2.37	1.41	–1.68	0.09	0.09	3.91	1.68	2.33	0.02
model-mimic	1.25	1.08	1.16	0.25	0.97	–0.60	0.55	1.09	1.55	0.70	0.48	0.48	1.09	1.24	0.88	0.38
contrast effect	0.25	0.98	0.26	0.80	0.91	0.92	0.36	–0.92	1.01	–0.91	0.37	0.37	–0.68	1.51	–0.45	0.65
mimicry effect	–0.73	0.89	–0.82	0.42	0.84	2.13	0.03	–2.07	1.30	–1.60	0.11	0.11	1.51	1.17	1.29	0.20

important [23], and this feature lacks in most studies that use artificial preys. Moreover, the static nature of paper model prey used in some studies (e.g. [20,21]) may limit the interpretability of the results. Predator–prey interactions in nature often involve dynamic encounters between the partners, and eyespots are more likely to appear suddenly in front of the predator. Their intimidating effect has been shown to increase when they are revealed abruptly [12]. This may occur in different ways, through a deimatic display performed by the attacked prey [3,19,34], or simply due to its natural movements (wing tilting, change of position, flight). Our set up successfully simulates the nature of a sudden encounter with the stimulus, hence adding more realism to the interaction.

The presence of eyes on the owl's face increased the intensity of the aversive reaction, confirming their important role as recognizable features of a potential threat [31,35]. We did not find a clear difference in the type of response elicited (interest versus aversion). It is possible that other features of the owl's face (such as its shape or bright yellow beak) were sufficient to cause a scare reaction in great tits [32]. Interestingly, when compared with an owl with eyes, the owl face without eyes caused a stronger tendency towards exploration in the individuals that showed interest. This is suggestive of eyes being important as a quick clue to recognize danger (i.e. their presence requires no further exploration).

In this study, the presumed model (owl eyes) and the mimic eyespots appeared to elicit similar reactions both in terms of response type (interest versus aversion) and its intensity (startle versus flee), adding support to the eye-mimicry hypothesis. Nevertheless, we should note that the owl with eyes was the only treatment that elicited warning calls from the tested birds. This, however, happened in only three out of the 192 trials and we therefore cannot draw statistical conclusions. As already suggested by Janzen *et al.* [7], it is likely that eyespots do not mimic only one specific model; the suggestion of any predator species is sufficient to elicit the scare reaction.

We were surprised not to find differences between butterflies with reversed eyespots and spotless butterflies. Many studies have proved that conspicuous markings are effective in causing an avoidance reaction in passerine birds [4,20,21,33], but in our system, conspicuousness itself does not seem to provide a detectable advantage. It is possible that the sudden appearance of a large butterfly masked the feeble effect provided by the presence of reversed eyespots. Moreover, the eyespots of *Caligo* butterflies used as model in our experiment bear an astonishing resemblance (to the human observer) to owl eyes. It would be interesting to compare the efficacy of eyespots characterized by different levels of resemblance to predator eyes, and testing whether conspicuousness plays a larger role when lower degrees of mimicry are achieved. This hypothesis would also explain the results found in many field studies [20,21].

We found no treatment effect on the escape window, which measured the delay from prey detection to successful attack. We expected that the more intimidating treatments would delay the attack more than the less intimidating ones. The lack of an effect could be attributed to several reasons. First, the relatively small dimensions of the experimental cage forced the bird to stay near the prey even after a startle event. Because of this, birds often found themselves examining the image from a further point and re-evaluating the risk. In the analysis, we did not include those cases in which the bird gave up in trying to capture the prey after a startle. This happened mostly with the owls treatments (six for OE and OW pooled, two for BR, one for BM and none for BW), but including them as censored data (though theoretically incorrect) did not allow the detection of a significant difference. Moreover, the escape window may be an inappropriate measure of the eyespots efficacy, since the first seconds of predator hesitation may be enough to allow for escape [3]. The immediate reaction of the predator may therefore be much more relevant than its prolongation. Furthermore, in natural conditions a bird would likely flee in the presence of a perceived predator, rather than be forced to re-evaluate the situation as in our confined setting [4].

To summarize, we provide a direct experimental demonstration that butterfly eyespots do indeed deter predators by mimicking the eyes of their predators, rather than being a developmental by-product providing contrast and conspicuousness [6,16]. While highly intuitive, without rigorous testing the eye-mimicry hypothesis remains a *just so story* [36]. However, as evolutionary psychologist Robert Kurzban put it, our 'goal should not be to expel stories from science, but rather to identify the stories that are also good explanations' [37], and the one about *the butterfly who copied the owl's eyes* seems to be a good one after all.

Ethics statement. The experiment was carried out with permission from the Central Finland Centre for Economic Development, Transport and Environment (KESELY/1017/07.01/2010), and licence from the National Animal Experiment Board (ESAVI-2010-087517Ym-23).

Data accessibility. Datasets supporting this study are available in the Dryad Repository, at the link: doi:10.5061/dryad.5dk4p.

Acknowledgements. We would like to thank Helinä Nisu and Diana Abondano for the invaluable work in the aviary, and Konnevesi Research Station for facilities and logistics. Emily Burdfield-Steel, Franziska Dickel, Dalial Freitak, Juan Galarza, Swanne Gordon, Bibiana Rojas, Katja Rönkä and all members of the Darwin-club commented on the manuscript.

Author contributions. J.M., J.V. and S.D.B. conceived and designed and S.D.B. conducted the experiment. S.D.B. and A.L.S. analysed the data. S.D.B., J.V., A.L.S. and J.M. interpreted the data and drafted the manuscript. All the authors gave final approval for publication.

Funding statement. The Academy of Finland (project no. 252411) and an Erasmus-Socrates scholarship to Sebastiano De Bona provided funding.

Competing interest. We have no competing interests.

References

1. Poulton EB. 1890 *The colours of animals: their meaning and use especially considered in the case of insects. The international scientific series.* New York, NY: D. Appleton and Co.
2. Cott HB. 1940 *Adaptive coloration in animals.* London, UK: Methuen & Co. Ltd.
3. Edmunds M. 1974 *Defence in animals: a survey on anti-predator defences.* New York, NY: Longman.
4. Blest AD. 1957 The function of eyespot patterns in the Lepidoptera. *Behaviour* **11**, 209–255. (doi:10.1163/156853956X00048)
5. Duellman WE, Trueb L. 1994 *Biology of amphibians.* New York, NY: McGraw Hill Publ. Co.
6. Stevens M. 2005 The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biol. Rev.* **80**, 573–588. (doi:10.1017/S1464793105006810)

7. Janzen DH, Hallwachs W, Burns JM. 2010 A tropical horde of counterfeit predator eyes. *Proc. Natl Acad. Sci. USA* **107**, 11 659–11 665. (doi:10.1073/pnas.0912122107)
8. Kodandaramaiah U. 2011 The evolutionary significance of butterfly eyespots. *Behav. Ecol.* **22**, 1264–1271. (doi:10.1093/beheco/arr123)
9. 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. (doi:10.2307/2408684)
10. 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. (doi:10.1098/rspb.2003.2571)
11. 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. (doi:10.1371/journal.pone.0010798)
12. 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. B* **272**, 1203–1207. (doi:10.1098/rspb.2004.3034)
13. 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. (doi:10.1007/s00265-005-0069-9)
14. 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. (doi:10.1007/s00265-007-0374-6)
15. Olofsson M, Løvlie H, Tibblin J, Jakobsson S, Wiklund C. 2013 Eyespot display in the peacock butterfly triggers antipredator behaviours in the naïve adult fowl. *Behav. Ecol.* **24**, 305–310. (doi:10.1093/beheco/ars167)
16. Stevens M, Ruxton GD. 2014 Do animal eyespots really mimic eyes? *Curr Zool.* **60**, 26–36.
17. Brakefield PM. 1998 The evolution–development interface and advances with the eyespot patterns of *Bicyclus* butterflies. *Heredity* **80**, 265–272. (doi:10.1046/j.1365-2540.1998.00366.x)
18. Marples NM, Kelly DJ. 1999 Neophobia and dietary conservatism: two distinct processes? *Evol. Ecol.* **13**, 641–653. (doi:10.1023/A%3A1011077731153)
19. Sargent TD. 1990 Startle as an anti-predator mechanism, with special reference to the underwing moths, (*Catocala*). In *Insect defences: adaptive mechanisms and strategies of prey and predators* (eds DL Evans, JO Schmitd), pp. 229–249. Albany, NY: State University of New York Press.
20. Stevens M, Hopkins E, Hinde W, Adcock A, Connolly Y, Troscianko T, Cuthill IC. 2007 Field experiments on the effectiveness of 'eyespot' as predator deterrents. *Anim. Behav.* **74**, 1215–1227. (doi:10.1016/j.anbehav.2007.01.031)
21. Stevens M, Hardman J, Stubbins CL. 2008 Conspicuousness, not eye mimicry, makes 'eyespot' effective antipredator signals. *Behav. Ecol.* **19**, 525–531. (doi:10.1093/beheco/arm162)
22. Merilaita S, Vallin A, Kodandaramaiah U, Dimitrova M, Ruuskanen S, Laaksonen T. 2011 Number of eyespots and their intimidating effect on naïve predators in the peacock butterfly. *Behav. Ecol.* **22**, 1326–1331. (doi:10.1093/beheco/arr135)
23. Blut C, Wilbrandt J, Fels D, Girgel El, Lunau K. 2012 The 'sparkle' in fake eyes—the protective effect of mimic eyespots in Lepidoptera. *Entomol. Exp. Appl.* **143**, 231–244. (doi:10.1111/j.1570-7458.2012.01260.x)
24. Jędrzejewska B, Jędrzejewski W. 1993 Summer food of the pygmy owl *Glaucidium passerinum* in Białowieża National Park, Poland. *Ornis Fenn.* **70**, 196–201.
25. Suhonen J, Halonen M, Mappes K. 1993 Predation risk and the organization of the *Parus* guild. *OIKOS* **66**, 94–100.
26. Curio E, Klump G, Regelman K. 1983 An anti-predator response in the great tit (*Parus major*): is it tuned to predator risk? *Oecologia* **60**, 83–88. (doi:10.1007/BF00379324)
27. Endler JA. 2012 A framework for analysing colour pattern geometry: adjacent colours. *Biol. J. Linn. Soc.* **107**, 233–253. (doi:10.1111/j.1095-8312.2012.01937.x)
28. Peli E. 1990 Contrast in complex images. *J. Opt. Soc. Am. A* **7**, 2032–2040. (doi:10.1364/JOSAA.7.002032)
29. De Boeck P, Partchev I. 2012 IRTrees: tree-based item response models of the GLMM family. *J. Stat. Softw.* **48**, 1–28.
30. Lopéz-Sepulcre A, De Bona S, Valkonen JK, Umbers KDL, Mappes J. Submitted. Item response trees: a recommended method for analysing categorical data in behavioural studies. *Behav. Ecol.*
31. Scaife M. 1976 The response to eye-like shapes by birds. I. The effect of context: a predator and a strange bird. *Anim. Behav.* **24**, 195–199. (doi:10.1016/S0003-3472(76)80115-7)
32. Beránková J, Veselý P, Sýkorová J, Fuchs R. 2014 The role of key features in predator recognition by untrained birds. *Anim. Cogn.* **17**, 963–971. (doi:10.1007/s10071-014-0728-1)
33. Stevens M, Cantor A, Graham J, Winney IS. 2009 The function of animal 'eyespot': conspicuousness but not eye mimicry is key. *Curr. Zool.* **55**, 319–326.
34. Umbers KD, Lehtonen J, Mappes J. 2015 Deimatic displays. *Curr. Biol.* **25**, R58–R59. (doi:10.1016/j.cub.2014.11.011)
35. Curio E. 1975 The functional organization of anti-predator behaviour in the pied flycatcher: a study of avian visual perception. *Anim. Behav.* **23**, 1–115. (doi:10.1016/0003-3472(75)90056-1)
36. Gould SJ, Lewontin RC. 1979 The Spandrels of San Marco and the Panglossian Paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B* **205**, 581–598. (doi:10.1098/rspb.1979.0086)
37. Kurzban R. 2012 Just so stories are (bad) explanations. Functions are much better explanations. *Evol. Psychol.* (www.epjournal.net/2504).