

The function of animal ‘ eyespots ’ : Conspicuousness but not eye mimicry is key

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Abstract Many animals are marked with conspicuous circular features often called ‘ eyespots ’ , which intimidate predators , preventing or halting an attack . It has long been assumed that eyespots work by mimicking the eyes of larger animals , but recent experiments have indicated that conspicuousness and contrast is important in eyespot function , and not eye mimicry . We undertake two further experiments to distinguish between the conspicuousness and mimicry hypotheses , by using artificial prey presented to wild avian predators in the field . In experiment 1 , we test if eyespot effectiveness depends on the marking shape (bar or circle) and arrangement (eye-like and non-eye-like positions) . We find no difference between shapes or arrangement ; all spots were equally effective in scaring birds . In experiment 2 , we test if the often yellow and black colors of eyespots mimic the eyes of birds of prey . We find no effect of shape , and no advantage to yellow and black spots over non-eye-like but equally conspicuous colors . The consistent finding is that eyespot function lies in being a conspicuous signal to predators , and not necessarily due to eye mimicry [*Current Zoology* 55 (5) : 319 – 326 , 2009] .

Key words Eyespots , Conspicuousness , Mimicry , Predation , Protective coloration

Various markings on prey animals function as anti-predator signals (Ruxton et al. , 2004). These include high contrast approximately circular features , generally termed ‘ eyespots ’ , found on a range of animals from insects to fish . Such eyespots are thought to have various functions , including deflecting predatory attacks , but in particular , startling and intimidating predators , preventing or halting an attack altogether (reviewed by Stevens , 2005). Indeed , there is strong evidence that some eyespots are highly effective in startling birds , for example those of peacock butterflies *Inachis io* (Blest , 1957 ; Vallin et al. , 2005 , 2007) and eyed hawk moths *Smerinthus ocellatus* (Vallin et al. , 2007). Although eyespots are often used in startle displays (such as those of the peacock butterfly) , other eyespots are continuously displayed (e. g. owl butterflies *Caligo* , emperor moths *Saturnia pavonia*) , and such stimuli can be highly effective in preventing attacks by birds (Stevens et al. , 2007 , 2008a ; Kodandaramaiah et al. , 2009).

Although the experiments of Vallin et al. (2005 , 2007) show that eyespots work in intimidating predators , they do not illustrate why , and understanding the mechanistic basis for eyespot function is crucial to understand their form and evolution (Stevens , 2007). For over 100 years it has been assumed that eyespots mimic the eyes of larger animals , thus scaring away potential predators , because to humans they often subjectively resemble a pair of eyes (e. g. Blest , 1957). However , eyespots may work simply because they are highly contrasting conspicuous features , which

promote avoidance behavior in foraging predators , such as neophobia and dietary conservatism (Marples and Kelly , 1999) ; the ‘ conspicuous signal ’ hypothesis (Stevens , 2005). Recent work , presenting artificial prey to wild avian predators has provided support for this conspicuousness theory , in that eyespots were most effective when comprising large size , high contrast , and a range of shapes , including bars and squares (Stevens et al. , 2007 , 2008a). In both studies , more eye-like spots had no effect on survival provided conspicuousness remained constant . Thus , we use the term ‘ wingspot ’ (for butterflies and moths) instead of ‘ eyespot ’ in describing these features henceforth (Stevens et al. , 2008a). See below for a fuller discussion of the conspicuousness hypothesis , including with respect to the current findings . Although there is no objective experimental evidence supporting the eye-mimicry hypothesis , it is still widely assumed to be correct (e. g. Rota and Wagner , 2006 ; Merilaita , 2007 ; Vallin et al. , 2007).

Despite previous experiments to distinguish between eye-mimicry and conspicuousness , several issues remain . First , previous work (Stevens et al. , 2007 , 2008a) comprised prey with spots all generally arranged in eye-like orientations (generally a pair of markings arranged either side of the prey midline) , and so the lack of an advantage to more eye-like shapes could simply be if all treatments looked sufficiently eye-like to work . That is , provided the arrangement of the stimuli is approximately eye-like (a pair of markings on either side of the body) ,

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this may be sufficient to present an apparent pair of eyes. Second, previous work used achromatic prey lacking color information, whereas many wingspots have colors thought to be similar to those found in vertebrate eyes. For example, many spots have a dark centre and a light yellow surrounding ring, which may mimic the dark pupil and yellow iris of many predatory birds (e.g. owl butterfly spots are thought to mimic the eyes of owls; Cott 1940). In our study site, sparrow hawks *Accipiter nisus* are the most common avian predator of small birds, and have yellow and black eyes. Overall, the intimidatory role of wingspots is a valuable system to explore what features are important in the evolution and efficacy of protective signals.

We undertake two experiments to distinguish between the eye-mimicry and conspicuousness hypotheses, by presenting artificial 'moth' prey to wild avian predators in the field, marked with specific wingspot patterns. We use a powerful 'field psychophysics' methodology, previously employed to investigate both camouflage (e.g. Cuthill et al., 2005) and wingspot (e.g. Stevens et al., 2007) function, with prey not intended to mimic any real species, but rather to determine what features underlie wingspot function. In experiment 1, we test the survival of prey with markings either resembling eyes in shape (circles) or not resembling eyes (bars) (as in Stevens et al., 2008a), placed in eye-like or non-eye-like positions and orientations on the targets. In experiment 2, we created targets with color information, calibrated to correspond to avian color and luminance (perceived lightness) vision, with colors that do or do not mimic eyes.

1 Materials and Methods

1.1 General Methods

The general protocol followed that of Stevens et al. (2008a). We created artificial prey, 55 mm wide by 30 mm high, from triangular pieces of waterproof paper (HP LaserJet Tough Paper; Hewlett Packard, Palo Alto, USA), printed on a Hewlett Packard Color LaserJet 2605 dn printer at 300 dpi. Targets comprised a grey 'wing' background, with non-control targets marked with a pair of stimuli with a black centre and either a white (experiment 1) or colored (experiment 2) surround. Stimuli were created in Photoshop Elements 5.0 (Adobe Systems Inc., San Jose, USA). For the calibrations we measured the reflectance of the relevant stimuli using an Ocean Optics (Dunedin, FL, USA) USB4000 spectrometer held at 45° to normal, with illumination by a PX-2 pulsed xenon lamp, followed by modeling the photon catches of a blue tit's *Cyanistes caeruleus* single and double cones (Hart et al. 2000) with irradiance spectra collected in the study site with the cosine corrected spectrometer (as Maddocks et al., 2001). The background target grey was calibrated to be lighter than

the trees (in terms of the double cone values) to which the targets were pinned, as wingspots can switch from being protective to increasing predation when placed on prey that match the background luminance (Stevens et al., 2008b). Although targets were pinned to various tree species, the targets were much lighter than the bark of all species, which differ relatively little in comparison to each other.

Targets were pinned to trees of various species at a height of 1–3 m in the University of Cambridge Madingley Woods, Cambridgeshire, UK (0°3.2' E, 52° 12.9' N), a mixed deciduous woodland with various avian predators (see Stevens et al., 2008a). Attached underneath the targets, partially projecting out, was a dead mealworm (*Tenebrio molitor* larvae) providing an edible component for birds. Targets were checked at approximately 4, 24, 48, and 72 hours, with predation revealed by disappearance of all or most of the mealworm from the target. Non-avian predation was identified, as slugs left slime trails, ants were seen swarming on the target, and spiders left hollow exoskeletons. Non-avian predation, target disappearance, or the 'survival' to 72 hours, were incorporated as censored values in the survival analysis (Cuthill et al., 2005).

Both experiments were randomized block designs, consisting of 7 blocks each with a sample of 8 replicates per treatment (total sample size = 56 per treatment, 392 in total, in both experiments), randomly pinned to trees in June and July 2008. Blocks were non-linear transects ca 1 km long and 20 m wide, using less than 5% of the available trees, each in different woodland regions on different dates, with targets randomly allocated to suitable trees. The low target density and use of different woodland areas minimized the chance of the same bird encountering multiple targets. Whilst some previous work has been done in the same woodland using artificial prey with wingspot features, the most recent study (Stevens et al., 2009) finished approximately five months prior to the current experiments. Also, the low density of targets in both the previous and current experiments, and that various new individual birds will have entered and left the resident population (due to births, deaths, and migration), makes it unlikely that the birds in the current study will have encountered similar targets before.

Survival analysis for experiment 1 was by Cox proportional hazards regression (Cox, 1972; Klein and Moeschberger, 2003; Cuthill et al., 2005), a semi-parametric technique with significance tested with the Wald statistic (abbreviated *W*). Cox regression assumes the hazard ratios between treatments should be similar across time (the proportional hazards assumption), an assumption not met for experiment 2, and so here we used non-parametric survival analysis, with Kaplan-Meier estimates for survival functions and a log rank test statistic to compare survival curves (see Stevens et al., 2009).

Survival statistics can incorporate censored values , which , rather than treated as missing data , still provide information on those replicates up until the time at which a non-avian predation event occurred. Planned pairwise contrasts (Ruxton and Beauchamp , 2008) compared specific treatments , and we used no more comparisons than remaining degrees of freedom , meaning *P*-value correction was not required (Rosenthal et al. , 2000 ; see Stevens et al. , 2008b). Effect sizes are odds ratios (OR), with a value of 1.00 indicating two treatments have identical survival.

1.2 Experiment 1

In experiment 1 , targets were marked with either a pair of bars or circles , with markings comprising a black central component and white surround. The areas of the central and surrounding components were the same for both bars and circles. Treatments were marked with ; a pair of circles arranged horizontally about the midline (CH); bars of the same arrangement in a horizontal orientation (BH); circles arranged vertically along the

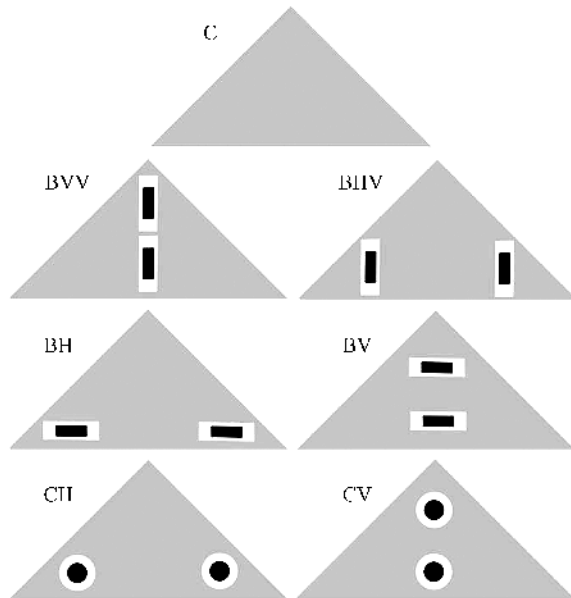


Fig.1 Stimuli used in experiment 1

CH : Circles arranged horizontally about the midline. CV : Circles arranged vertically along the midline. BH : Bars arranged horizontally about the midline and orientated horizontally. BV : Bars arranged vertically along the midline and orientated vertically. BVV : Bars arranged vertically along the midline and orientated vertically. BHV : Bars arranged horizontally about the midline and orientated vertically. C : Uniform unmarked control.

1.3 Experiment 2

In experiment 2 , we tested for a difference between targets marked with bars or circles with different colors , of the same area (see experiment 1 above). We had seven treatments ; targets with a pair of circles with a black centre and either a red (CR), yellow (CY), or blue (CB) surround ; targets marked with a pair of bars with a black centre and either a red (BR), yellow (BY), or

midline (CV); bars arranged vertically along the midline orientated horizontally (BV) and vertically (BVV); and bars arranged horizontally about the midline but orientated vertically (BHV) (Fig.1). We also had an unmarked control (C). Planned comparisons tested for an advantage of possessing spots of any type (aggregate of the marked treatments versus the controls), and for a difference between circles and bars (CH versus BH , and CV versus BV). We also tested for an effect of marking position (CH versus CV , and BH versus BV), bar orientation (BH versus BHV), and bar position and orientation (BH versus BVV). If wingspots work due to mimicking eyes , then the treatment with horizontally placed circles (CH) should survive best. If the placement of the stimuli is important in creating an eye-like appearance , then we also expect the targets with horizontally placed and orientated bars (BH) to survive well too. In contrast , if wingspots work simply because they are highly salient signals then there should be no difference in the survival of the targets with different shapes and positions.

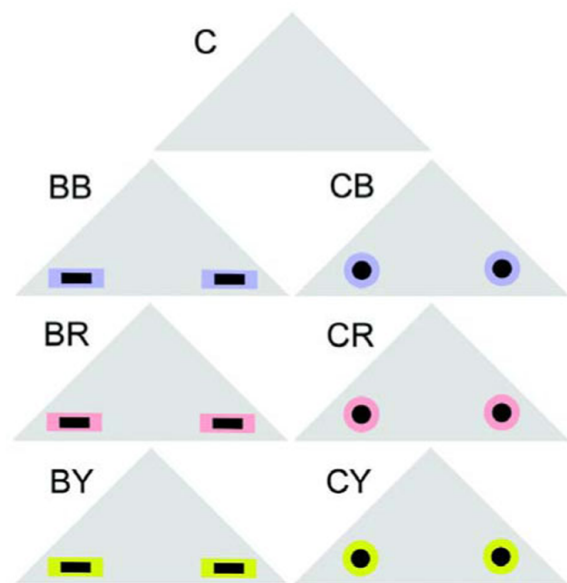


Fig.2 Stimuli used in experiment 2

BY : Bars with a yellow surround. CY : Circles with a yellow surround. BR : Bars with a red surround. CR : Circles with a red surround. BB : Bars with a blue surround. CB : Circles with a blue surround. C : Uniform unmarked control.

blue (BB) surround ; and an unmarked control (C) (Fig.2). Target calibration was according to the log form of a model of avian visual processing , which assumes receptor noise limits visual discrimination (Vorobyev and Osorio , 1998), using the relative proportions of cone types in the blue tit retina (Hart et al. , 2000), and a Weber fraction of 0.05. Although other Weber fractions would alter the model outputs , because the prey were

eaten by multiple bird species, potentially with different Weber fractions, we use this value as a common best estimate. In addition, our prey values are all well above the threshold for discrimination, meaning that all stimuli would be conspicuous even accounting for some error, and different Weber fractions would affect the values of all contrasts for the different colors used. The output of the model is just noticeable differences (‘jnds’), where a jnd value of less than 1.00 indicates two stimuli are likely to be indistinguishable, with increasing values above 1.00 indicating more rapid discrimination and larger differences. To model luminance discrimination, we follow Siddiqi et al. (2004), using photon catches from the double cones as these are likely to be used in avian luminance vision (Osorio and Vorobyev, 2005). Our aim was to create targets with markings of red, yellow, and blue with carefully defined and similar visual contrast to a bird. The color contrast of the red, yellow, and blue components against the grey target background was 9.2, 15.3, and 11.1 jnds respectively, and against the black marking centre 7.8, 14.5, and 14.6 jnds respectively. The luminance contrast of the red, yellow, and blue against the target background was 7.8, 7.1, and 9.3 jnds, and against the black marking centre was 45.5, 52.1, and 42.3 jnds respectively. Although the contrasts were not identical, they were all well above the discrimination threshold of 1.00, and the relative difference between the marking colors was small compared to the respective differences between the colors and the other target values. If the spots work due to conspicuousness, it is possible that progressive increases in contrast make the stimuli progressively more effective in preventing attack. However, it may also be possible that once the stimuli are sufficiently conspicuous and are suprathreshold, that birds categorize the prey in a similar way irrespective of small increases in contrast (cf. Ham and Osorio, 2007).

We compared if markings provide a survival advantage (controls versus the aggregate of all marked

treatments), for a difference between possessing bars and circles (CY, CR, and CB, versus BY, BR, and BB), and for a difference between yellow and red (CY and BY versus CR and BR), yellow and blue (CY and BY versus CB and BB), and blue and red markings (CB and BB versus CR and BR). If wingspots work by mimicking eyes, targets with circles of a black centre and yellow surround should survive best. If the markings simply mimicked warning signals, there should be no difference between the bars and circles with yellow surrounds, and there should also be a strong advantage conferred by red and black markings. If the markings are effective because they are unfamiliar and induce neophobia and dietary conservatism, then markings with the blue surrounds should also survive well.

2 Results

2.1 Experiment 1

There was a significant effect of treatment ($W_6 = 20.706$, $P = 0.002$; Fig. 3), but not block ($W_6 = 5.709$, $P = 0.457$), with 30.9% censored values (events not corresponding to avian predation). Marked treatments survived significantly better than the unmarked controls ($W_1 = 8.154$, $P = 0.004$, OR = 1.597). There was no difference in survival between targets with horizontally arranged circles (CH) and bars (BH) ($W_1 = 0.063$, $P = 0.801$, OR = 1.061), or between vertically placed circles (CV) and bars (BV) ($W_1 = 0.155$, $P = 0.694$, OR = 0.913). There was no significant difference between targets with horizontally and vertically placed circles ($W_1 = 0.426$, $P = 0.514$, OR = 1.161), or horizontally and vertically placed bars ($W_1 = 0.001$, $P = 0.997$, OR = 0.999). There was no difference between targets with horizontally placed and orientated bars and horizontally placed-vertically orientated bars (BHV) ($W_1 = 0.594$, $P = 0.441$, OR = 1.196), or between those with horizontally placed and orientated bars and vertically placed and orientated bars

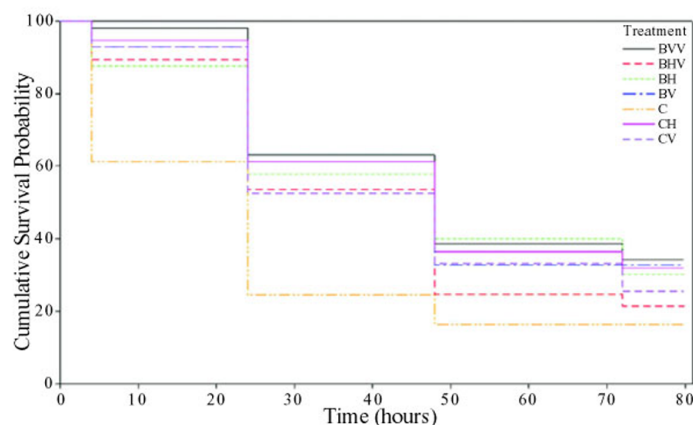


Fig. 3 Non-parametric survival plot of the treatments in Experiment 1

Curves are the probability of surviving bird predation. Only the controls survived worse than the other treatments.

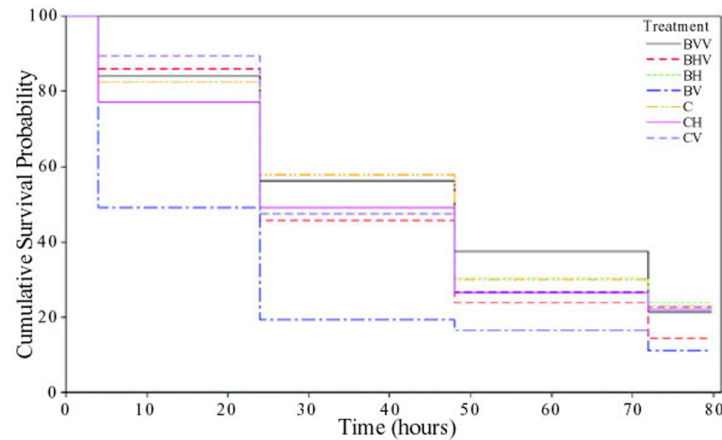


Fig.4 Non-parametric survival plot of the treatments in Experiment 2

Curves are the probability of surviving bird predation. Only the controls survived worse than the other treatments.

(BVV) ($W_1 = 0.265$, $P = 0.594$, $OR = 0.880$).

2.2 Experiment 2

There was a significant effect of treatment ($X_6 = 29.069$, $P < 0.001$; Fig.4), and block ($X_6 = 18.511$, $P = 0.005$). Censored values amounted to 22.5% of the targets. Spotted treatments survived significantly better than the unmarked controls ($X_1 = 27.495$, $P < 0.001$, $OR = 2.066$), but there was no difference in survival between treatments marked with circles or bars ($X_1 = 0.032$, $P = 0.858$, $OR = 0.983$). Targets with yellow and black markings did not survive differently from those with either red ($X_1 = 0.574$, $P = 0.448$, $OR = 1.134$) or blue ($X_1 = 0.231$, $P = 0.631$, $OR = 0.947$) markings , and there was no significant difference between targets with blue and red markings ($X_1 = 1.617$, $P = 0.204$, $OR = 1.197$).

3 Discussion

In both experiments , conspicuous wingspots conferred a significant survival advantage compared to the unmarked targets. In experiment 1 , there was no difference in the survival of targets marked with circles and bars , and no significant effect of changing the arrangement and orientation of the markings. In experiment 2 , there was also no difference in survival of targets with bars and circles , and no significant difference between treatments with red- , yellow- , and blue and black markings. Our current experiments , in line with previous work (Stevens et al. , 2007 , 2008a , b , 2009) , illustrate how effective continuously visible wingspots are in reducing the risk of predation against avian predators. It has been widely believed for over 100 years that wingspots mimic the eyes of the predators own enemies (reviewed by Stevens , 2005). However , recent experiments have shown that high visual salience in wingspots is the crucial factor in promoting predator avoidance (Stevens et al. , 2007 , 2008a). Our current results are in line with this .

As with previous work (Stevens et al. , 2008a) , we find that more eye-like shapes (circles) do not provide a survival advantage over non-eye-like but still highly conspicuous shapes (bars). Stevens (2005) suggested that circles may be particularly effective in scaring birds because they may be good at stimulating circular receptive fields in vertebrate visual systems. However , such circular receptive fields are just one aspect of spatial vision , and there are a range of features which encode a variety of information about contrast in a visual scene. For example , so called ‘ simple cells ’ , with excitatory and inhibitory regions often form elongated areas highly sensitive to lines , bars , and gratings of a range of orientations and spatial frequencies (Graham , 1989 ; Rolls and Deco , 2002). In addition , sharp changes in intensity at the boundary of a shape , object , or pattern will be encoded as edges by the visual system , which are likely to play a key role in representing local image features and subsequent object recognition (De Valois and De Valois , 1980 ; Graham , 1989). Therefore , a range of shapes should be effective in stimulating aspects of the visual system if these are composed of high contrast patterns. This is consistent with the survival results of our current and previous work. Thus , it appears that the frequent circularity of most wingspots seemingly stems from developmental processes (e. g. Brakefield , 2001).

Manipulating the orientation and placement of the markings into arrangements that are less eye-like in form also had no significant influence on the effectiveness of the markings. An alternative experimental approach would have been to randomly place the spots on some treatments to create even less eye-like positions. However , whilst this would be worthwhile , a problem may be that it could be hard to distinguish between the effects of eye-likeness and symmetry. Although results testing the importance of symmetry in the value of a conspicuous protective signal are conflicting (cf. Forsman and Merilaita , 1999 ; Forsman and Herrström , 2004 ; Stevens et al. , 2009) , if

symmetry does play a role in the effectiveness of conspicuous signals it may be difficult to elucidate the underlying reason for any advantage of signals of different shapes along different axes of symmetry. For example, symmetry detection in humans is influenced by orientation effects, whereby symmetry is more salient when occurring either side of a vertical axis than with a horizontal axis (see Wenderoth, 1995; Herbert and Humphrey 1996; Herbert et al., 2002). Overall, as with shape, the arrangement of wingspots may stem from a paired placement of two features either side of a bilaterally symmetrical animal being easiest to produce during development, rather than there being a specific advantage to having features with the same arrangement as a pair of eyes. One drawback with the current experimental design is that we cannot be certain as to what angle(s) or position(s) the birds approach the targets from, and this may render the placement of the spots on the prey less important. However, because the targets are flat against the tree they can still essentially only be seen from the front, meaning that the angles at which the markings are observed will be relatively conserved. Either way, any effect of different angles of observation further supports the notion that the paired arrangement of the signals may not be a crucial feature of their effectiveness.

Experiment 2 is consistent with the first experiment, with no difference in survival between targets with markings of different shapes and colors. The eye mimicry theory specifically predicted an increased survival to circles with yellow and black components. In fact, qualitatively, the treatment with the highest survival was that with blue and black bars. In our field site there were some aposematic species, particularly wasps *Vespula vulgaris*, with (to human eyes) yellow and black stripes, hornets *Vespa crabro*, with reddish-brown-yellow, and black stripes, and tiger moths *Artia caja* with red and black hind wing markings. It is possible that the value of the yellow and black and red and black treatments was influenced by the level of experience of birds in the field towards warning colored prey. For example, the survival of the yellow and black bars may have benefitted from a resemblance to the banding of wasps. However, we are not aware of any study systematically testing warning signal effectiveness of different shapes and colors, directly controlling for the level of color and luminance contrast to a bird's visual system. As such, it is difficult to make direct predictions about how effective different combinations of shapes and colors are as warning signals (for either learned or innate avoidance). Whilst the conspicuous signal hypothesis clearly predicts that increases in conspicuousness should increase the effectiveness of the signal initially, whether relatively small increases in conspicuousness should have an effect on already suprathreshold stimuli (as in our study) is less certain. For example, the responses of domestic chicks

Gallus gallus to certain colors can be influenced not just by discrimination values, but by other factors too, such as categorization (Ham and Osorio, 2007). In our study, although non-significant, targets with red markings survived approximately 0.13 times worse than those with yellow markings, and 0.2 times worse than those with blue markings. The modeled discrimination values for color contrast are broadly consistent with these results, because the differences in jnd values between the blue and yellow colors were small, but the red color generally had the lowest contrast against the spot centre and wing background. As such, our results imply that comparatively small increases in contrast for color can provide a further benefit to already suprathreshold stimuli. It would be worthwhile to conduct experiments along the lines of the current study, gradually increasing the level of color and/or luminance contrast to determine the exact benefits of increasing contrast. Qualitatively, the highest surviving treatment was marked with blue and black bars, which neither resemble warning signals or eyes, and so it seems likely that the survival value of the wingspots stemmed from them producing neophobic reactions and dietary conservatism (Marples and Kelly, 1999) in the foraging predators.

If yellow is not more advantageous than other conspicuous colors in wingspot effectiveness, and the arrangement does not mimic eyes, why do many wingspots often have this coloration? It is likely that this is simply a good way of creating strong luminance and chromatic contrast, with contrast between adjacent objects an important aspect of visual processing, edge detection, and image segmentation (Graham, 1989). In addition, the white 'specs' often seen in the centre of lepidopteran eyespots, often assumed to mimic specular reflection off the predator's eyes, have been shown to have a role in mate choice in *Bicyclus anynana* butterflies, based on the ultraviolet component (Robertson and Monteiro, 2005). Furthermore, various wingspots are not yellow and black, but rather have a range of other striking colors, such as the blues and reds of peacock butterflies and eyed hawk moths. Provided the colors are conspicuous, wingspots can be highly effective. Indeed, conspicuous markings *per se* can be effective in preventing predation (e.g. Götmark, 1992). Finally, an alternative approach would be to manipulate the appearance or color of real lepidopteran eyespots to determine any change in effectiveness in scaring predators.

There are various convincing examples of mimicry in nature. For example, it has been argued for a long time that some spiders mimic the form of certain ants (Poulton, 1890) to reduce the likelihood of being eaten by birds, convincingly demonstrated in recent work (e.g. Nelson and Jackson, 2006). However, quantification or tests of mimicry hypotheses in real animals are still rare, and many apparent examples of mimicry in nature

are based on human assessment. Although our experiments (Stevens et al. , 2007 , 2008a) have not involved real prey animals , the set-up allows general principles about the evolution and mechanistic basis of wingspot function to be derived , and the previous and current studies fail to support the eye mimicry hypothesis. The next step is to replicate the experiments with stimuli used in startle displays , as this is where many wingspots function (Vallin et al. , 2005). Studies should also investigate whether spots found in other animal groups , such as fish , work due to eye mimicry or conspicuousness. Some owls also have conspicuous spots located on their nape , such as pygmy owls *Glaucidium* , that seem to deter mobbing attacks to the front of the owls (Deppe et al. , 2003). However , the basis for this response is untested. Conspicuous spots are also found in caterpillars , such as elephant hawk moth *Chaerocampa elpenor* larvae , which have long been thought to combine changes in morphology to resemble a snake (Wallace , 1889). However , no experiments have tested this idea and it remains speculative with behavioral experiments needed (Stevens , 2005). It is also important to try and investigate the cognitive processes involved in the relevant predators , because this too , along with their perceptual characteristics , will influence how they respond to their prey. Recent work , investigating how changes in ' emotional ' state can influence the way in which information is processed (' cognitive bias ') , have shown that starling *Sturnus vulgaris* responses to eyespots are not affected by their state , indicating that the starlings did not respond to eyespots in a manner as if they resembled predatory eyes (Brilot et al. , 2009). Further work in this area would be very revealing in terms of the basis of eyespot function. In addition , although there are reasons why predators should avoid unfamiliar or novel prey items , especially if they are conspicuous (they could be toxic) , more information regarding how long such neophobic and conservative behavior persists in different species would be valuable , and how it links with predator experience in general (see for example Lindström et al. , 2001). There is evidence that such behaviors can persist for at least several days , and possibly months (Marples et al. , 1998) although the effect of other cues , such as odor , may complicate things (Siddall and Marples , 2008). In addition , avoidance behaviors may be deactivated by experience of similar , but not identical food items , but this also depends on the type of experience (Marples et al. , 2007). For example , observations by domestic chicks *Gallus gallus domesticus* of other individuals eating a novel food type are not sufficient to overcome avoidance behavior , but rather direct contact with the food may be needed (Marples et al. , 2007). Further information about the potential transfer of information between individual birds regarding target edibility would be welcome. Conspicuous spots in

animals may also function in mate choice (e. g. Breuker and Brakefield , 2002 ; Robertson and Monteiro , 2005) , and may be linked to changes in sexual maturity in some fish (Gagliano , 2008). As such , there are many avenues for exploring the function of spots in other animals and contexts , and eyespots make a valuable model system for exploring both proximate and ultimate questions regarding adaptive coloration , signaling , and behavior.

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