Conspicuousness, not eye mimicry, makes "eyespots" effective antipredator signals

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Many animals bear colors and patterns to reduce the risk of predation from visually hunting predators, including warning colors, camouflage, and mimicry. In addition, various species possess paired circular features often called "eyespots," which may intimidate or startle predators preventing or postponing an attack. Most explanations for how eyespots work assert that they mimic the eyes of the predators own enemies. However, recent work has indicated that spots may reduce the risk of predation based purely on how conspicuous they are to a predator's visual system. Here, we use a field technique involving artificial prey marked with stimuli of various shapes, numbers, and sizes, presented to avian predators in the field, to distinguish between the eye mimicry and conspicuousness theories. In 3 experiments, we find that the features which make effective antipredator wing markings are large size and higher numbers of spots. Stimuli with circles survived no better than those marked with other conspicuous shapes such as bars, and changing the spatial construction of the spots to increase the level of eye mimicry had no effect on the protective value of the spots. These experiments support other recent work indicating that conspicuousness, and not eye mimicry, is important in promoting avoidance behavior in predators and that eyespots on real animals need not necessarily, as most accounts claim, mimic the eyes of other animals. *Key words:* antipredator, conspicuousness, eyespots, mimicry, predation, vision. *[Behav Ecol 19:525–531 (2008)]*

Animals utilize a variety of protective markings to reduce the risk of predation from visually hunting predators, including camouflage, warning colors, and mimicry (Wallace 1889; Cott 1940; Edmunds 1974; Ruxton et al. 2004; Stevens 2007). In addition, many species, in particular lepidopterans and various fish, possess "eyespots"; paired circular features often comprising highly contrasting concentric rings. Although eyespots may function in mate choice (e.g., Robertson and Monteiro 2005), they are primarily thought to have a range of antipredator functions, including deflecting the attacks of predators to less vital body regions and, in particular, startling or intimidating predators preventing or postponing an attack (reviewed by Stevens 2005). Indeed, eyespots have long been used as a classic example of adaptive coloration in animals (Tinbergen 1974). Although there is little experimental evidence that eyespots have a deflective role (e.g., see Lyytinen et al. 2003, 2004), evidence that some lepidopteran wing spots possess an intimidating function has been convincingly demonstrated in both peacock butterflies (*Inachis io*) and eyed hawk moths (*Smerinthus ocellatus*), where the spots reduced the probability of attack by avian predators in laboratory trials, particularly with respect to the former species (Vallin et al. 2005, 2007).

Traditionally, in both the popular and scientific literature, wing spots are repeatedly assumed to mimic the eyes of the predator's own enemies (e.g., Blest 1957; Rota and Wagner 2006; Vallin et al. 2007). For example, statements like "Eyespots on the wings of giant silk moths and other Lepidoptera undoubtedly mimic eyes of mammalian predators" by Rota and Wagner (2006) are not uncommon. In fact, most statements assert that lepidopteran eyespots mimic the eyes of avian predators. Regardless, there is no objective evidence that they do so (Stevens 2005), and the only study that has tested why

eyespots work in terms of predator perception has indicated that conspicuousness and contrast effects, rather than eye mimicry, are what produces effective avoidance behavior in predators (Stevens et al. 2007). In a previous experiment, Stevens et al. aimed to distinguish between the eye mimicry and conspicuous signal hypotheses by presenting wild birds with artificial stimuli marked with wing spots of varying characteristics. Stevens et al. (2007) showed that continuously visible wing spots significantly reduced the probability of predation by birds in the field. Changing the level of eye mimicry had no effect on the survival of the prey, provided that the stimuli all had the same relative contrast. For example, spots with a black center (pupil) and white surround (iris) were no more effective in preventing predation than spots with the opposite arrangement (white center and black surround). The only factor that predicted the effectiveness of the spots was the level of contrast of the stimuli. As such, it is important to consider predator perception before making assumptions about how a signal may work (Stevens 2007), and the term wing spot (fin spot for fish), rather than eyespot, is generally used henceforth.

One of the frequent arguments used in favor of the idea that wing spots mimic eyes is that they occur in pairs. However, paired occurrence is hardly surprising given that wing spots usually occur on structures on either side of the body, such as on the wings (Stevens 2005). In addition, although often assumed to have a deflective function, many lepidopterans have rows of spots on each wing (especially the hind wings; e.g., the speckled wood butterfly *Pararge aegeria*) or have a single spot on each forewing, such that when the butterfly is resting with its wings closed, it is only possible to view a single spot at a time (e.g., the hedge brown *Pyronia tithonus*). As such, spot number in different species is highly variable. Previous work, investigating the avoidance behavior of domestic chicks *Gallus gallus* and starlings *Sturnus vulgaris* to artificial stimuli, has shown that 2 circular "eyes" are avoided more than singular or triple eyes when the stimuli are surrounded by a model "head" and "beak" (Jones 1980; Inglis et al. 1983). However, (Inglis et al. 1983) found that without the head

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outline, starlings avoided 3 eyes more than a pair; this is crucial given that few lepidopterans with eyespots have any apparent mimicry of the surrounding head region of a predator. It is therefore important to test the effectiveness of different numbers of spots in promoting avoidance behavior in predators in a natural setting with wild predators and with additional controls to account for changes in total stimulus area (3 circles have a larger total stimulus area than 2 circles of equivalent size).

The conspicuous signal hypothesis predicts that features that should strongly stimulate a predator's visual system should be most effective in promoting avoidance behavior, irrespective of the level of eye mimicry. Vertebrate visual systems possess a range of receptive fields with areas of the retina composed of connected receptors often featuring lateral inhibition and characterized by a circular central section and a surrounding concentric ring, frequently linked with other receptive fields to form bar and line detectors (Graham 1989; Wandell 1995). As such, various geometric shapes such as circles, bars, and squares should be highly salient features. Stevens et al. (2007) found that circles were more effective in promoting predator avoidance than triangles but no different in their effect from diamonds. In addition, Blest (1957) found that birds were more startled by circles than they were by other geometric patterns, although shapes such as bars did still have some effect. However, Blest (1957) did not control for factors such as stimulus area, and Stevens et al. (2007) did not test other potentially more salient shapes than triangles, such as bars. Cundy and Allen (1988) undertook similar experiments with markings of different shapes presented to birds, and although there was some evidence that eyespot type markings were more effective than other shapes such as crosses, the results were not clear and the sample size small. As such, it is still not clear whether circles are more effective in promoting avoidance behavior than other shapes or whether circular features are so common in animals because they are simply easier to produce genetically or developmentally. Eyespot development may be explained by the radial diffusion of a morphogen outward forming a concentration gradient, with the epidermal cells producing specific pigments depending on the morphogen concentration (Nijhout 1980, 1990, 1991; French and Brakefield 1992; Dilão and Sainhas 2004), which may explain the frequency of circles and the rarity of other shapes.

An additional factor potentially affecting eyespot performance tested by Blest (1957) was that of complexity and displacement of the spot components toward the midline of the "body." Blest argued that many wing spots have the central elements displaced inward toward the body to create the impression of solidarity of an eye, and popularized descriptions often argue that this effect also creates the appearance of a predator "focused" in, staring at its prey; some lepidopteran wing spots are argued to create this impression. Although Blest (1957) found that displaced complex concentric rings were more effective in intimidating birds, the study did not control for changes in stimulus area, the number of spot rings, and various other factors. As such, it remains to be properly tested whether the displacement of the spot elements enhances the predator aversion.

Here, we undertake 3 experiments designed to further test whether wing spots intimidate predators because they mimic eyes or simply because they are highly salient features to predatory visual systems, promoting neophobia and dietary conservatism (Marples and Kelly 1999; Marples et al. 2005). We do this by testing whether the number (1, 2, or 3), size, and shape of the "spots" are important and whether predator avoidance is also affected by the displacement of spot components toward or away from the target midline.

MATERIALS AND METHODS

The experiment followed the same overall procedure as Stevens et al. (2006, 2007). We created artificial "prey," 65 mm wide and 32 mm high, made from triangular pieces of waterproof paper (HP LaserJet Tough Paper; Hewlett Packard, Palo Alto, CA). These were printed with specific patterns on a Hewlett Packard LaserJet 2200dn printer. Targets were gray scale because these have been effective in previous work in promoting avoidance behavior in avian predators (Stevens et al. 2006, 2007). The targets were composed of a gray "wing" background, which was lighter than the trees to which they were pinned; this was important as other work has shown that rather than being protective, wing spots can actually increase predation when placed on prey that match the background (Stevens M, Stubbins CL, Hardman CJ, unpublished data). On each noncontrol target was placed a pair of stimuli (except in experiment 1 where the number of spots varied between treatments) with a black center and white surround, which differed in a number of characteristics depending on the exact experiment.

Although we did not undertake any experiments requiring a careful manipulation of visual contrast, we still calibrated the stimuli in a manner according to previous work (Stevens et al. 2006, 2007) such that the avian-perceived luminance (perceived lightness) of the gray target background lay halfway between that of the white and black of the wing spots on a ratio scale (see Stevens et al. 2007). The targets were calibrated to avian double cones because other work has indicated that it is these which are involved in bird luminance perception (Osorio, Miklósi, and Gonda 1999; Osorio, Vorobyev, and Jones 1999; Jones and Osorio 2004; Osorio and Vorobyev 2005). This was achieved by modeling the cone catch of a blue tit's *Parus caeruleus* double cones (Hart et al. 2000) using reflectance spectra of printed calibration charts (taken with an Ocean Optics, Dunedin, FL, USB2000 spectrometer, with illumination by a PX-2 –pulsed Xenon lamp) and irradiance spectra taken in the study site (e.g., Endler and Meilke 2005). The stimuli gray values were then scaled to correspond to the required luminance values when printed. Because the calibration of the stimuli for contrast was not essential for our experiment, which investigated the effects of shape, size, and spot number, we do not reproduce the full calibration details in depth here; these are outlined in detail by Stevens et al. (2006, 2007). Eyespots were created in Image J (Abràmoff et al. 2004; Rasband 1997–2007) and Photoshop Elements 2.0 (Adobe Systems Inc., San Jose, CA). Although birds are sensitive to ultraviolet (UV) light (Cuthill et al. 2000; Cuthill 2006), this is not a problem with the experimental design because we merely wished to create stimuli that were conspicuous against the background on which they were placed, and the lichen-absent trees to which the targets were pinned reflect minimal UV light (Majerus et al. 2000), resulting in zero internal and external UV contrast for all treatments.

Targets were pinned to trees of various species at a height of 1–3 m in the mixed deciduous University of Cambridge Madingley Woods, Cambridgeshire, UK (0°3.2′E, 52°12.9′N), and checked at approximately 3, 24, and 48 h. Attached to each target was a dead mealworm (*Tenebrio molitor* larvae) to provide an edible component. In experiment 1, the mealworm was attached to the under side of the targets, partially projecting out (as Schaefer and Stobbe 2006), because the manipulations of spot number resulted in some treatments having spots in the center of the target. In experiments 2 and 3, the mealworms were pinned on top in the middle of the targets (as Stevens et al. 2007). The placement of the mealworm underneath or on top of the targets has little effect on overall predation rates. Predation was determined by the disappearance of all or most of the mealworm from the target. The woodland

has a range of avian predators, primarily blue tits, great tits (*Parus major*), blackbirds (*Turdus merula*), European robins (*Erithacus rubecula*), and house sparrows (*Passer domesticus*) (as with similar studies undertaken elsewhere; Cuthill, Hiby, and Lloyd 2006; Cuthill, Stevens, et al. 2006). Other forms of predation could be identified: slugs left slime trails, ants were seen swarming on the target, and spiders left hollow exoskeletons. Nonavian predation, complete disappearance of the target, or the "survival" of the target to 48 h were treated as censored values in the survival analysis (see below; Cuthill et al. 2005).

Each experiment was a randomized block design, consisting of 10 blocks with a sample of replicates of each treatment (the exact number differed between experiments), randomly pinned to trees in July and early August 2007. Each block comprised a nonlinear transect 1–3 km long and 30 m wide, using less than 5% of the available trees, each in a different woodland region on a different date. Treatments were randomly allocated to suitable trees (with never more than one target on a given tree), defined as having little or no lichen cover and a trunk circumference of greater than 0.9 m. The low density of targets within each block and the use of different parts of the wood minimized the chance that any one bird would encounter multiple targets.

Survival analysis was performed via Cox proportional hazards regression (Cox 1972; Lawless 2002; Klein and Moeschberger 2003; Cuthill et al. 2005), which can accommodate nonuniform changes in predation risk with respect to time of day. Cox regression assumes that all survival functions have the same shape, and so checking the targets at regular and constrained time intervals (3, 24, and 48 h) ensures that this assumption is valid in this form of experiment. Survival analysis, such as Cox regression, is ideally suited to accommodate censored values (in this instance comprising nonavian predation, lost targets, and survival to 48 h), which, rather than being treated as missing data in the analysis, are included and still provide information on those replicates up until the time point at which a nonavian predation event occurred. Such values would normally have to be included as missing data in most "conventional" statistics. Experiments 1, 2, and 3 had 150 (25%), 149 (21.3%), and 86 (14.3%) censored values, respectively. Significance was tested with the Wald's statistic (abbreviated *W*) and unplanned pairwise contrasts used to compare specific treatments. Effect sizes are odds ratios (ORs), being the ratio of the probability of predation in one treatment to the probability of predation in another treatment, such that a value of 1.00 occurs when 2 treatments have the same survival probabilities.

Experiment 1: spot number and size

In experiment 1, we manipulated the number of spots on the targets, from 0 (control, C), 1 (single small, SS), 2 (double small, DS), and 3 (triplet, T) spots, where each small spot was approximately 9 mm in diameter. However, because increasing number of spots of the same size results in increased total stimulus area and because we also wanted to investigate the effect of stimulus size, we also needed 2 additional treatments with 1 (single large, SL) and 2 spots (double large, DL), each approximately 15 and 11 mm in diameter, respectively, but with the same overall area as the 3 small spots on treatment T combined (Figure 1). As such, treatments SL and DL had the same overall stimulus area as treatment T, but the number of spots and spot size varied, whereas SS and DS had spots of the same size as T but with different numbers. Because symmetry may be an important factor in creating aversive stimuli (Forsman and Merilaita 1999; Forsman and Herrström 2004), all treatments were symmetrical about the midline (e.g., tar-
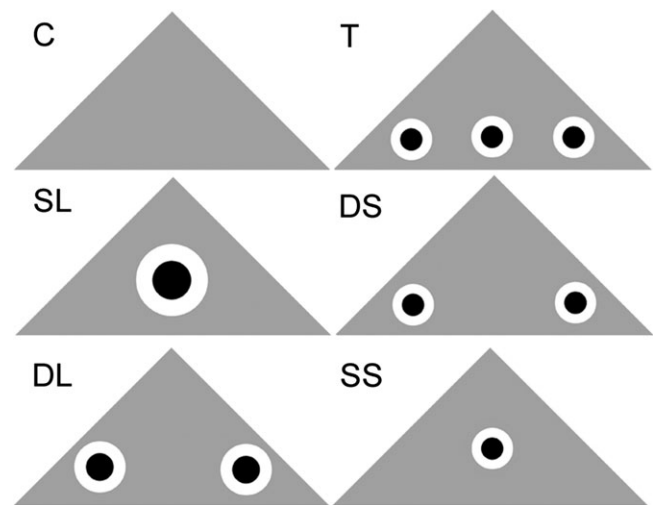

Figure 1
Treatments used in experiment 1: uniform gray control (C), 3 small spots (T), 1 large spot with the same combined area as all the spots on treatment T (SL), 2 large spots with the same combined area as all the spots on treatment T (DL), 2 small spots each with the same size as those on treatment T (DS), and 1 small spot with the same size as those on treatment T (SS).

gets with a single spot were placed in the center). Each block contained 10 targets of each treatment (total sample = 100 per treatment).

If wing spots are effective because they mimic eyes, we would expect those treatments with 2 spots (DS and DL) to survive best. However, if it is conspicuousness which is important, then we would expect that the treatments with larger spots should survive best (SL then DL) because these will be conspicuous from a greater distance. This may be followed by the treatments with 3 (T), 2 (DS), and 1 (SS) small spots because here the overall stimulus area and spot number is decreasing.

Experiment 2: spot shape

Experiment 2 tested the effectiveness of different shapes in reducing predation. In addition to an unmarked control treatment, there were 6 other treatments (Figure 2), each marked with a pair of stimuli: 1) two 9-mm-diameter circles with a center and surrounding ring (R; the same as treatment DS in experiment 1), 2) squares of the same area as the spots on treatment 1 (SA), 3) squares with the same diameter as treatment 1 (SD), 4) squares with the same perimeter as treatment 1 (SP), 5) bars with the same perimeter as treatment 1 (BP), and 6) bars with the same area as treatment 1 (BA). Each block contained 10 targets of each treatment (total sample = 100 per treatment).

If eye mimicry is primarily important in creating aversive stimuli, then we would expect the circles (R) to produce the highest survival. Conversely, if conspicuousness is what matters, then we predict no difference in the survival between all the marked stimuli.

Experiment 3: spot component displacement

In experiment 3, each noncontrol target had a pair of spots 9 mm in diameter (Figure 3). One treatment had spots with the central black component placed in the center of the spot (M), whereas the other 2 treatments had the central component marginally displaced either toward (internally, I) or away
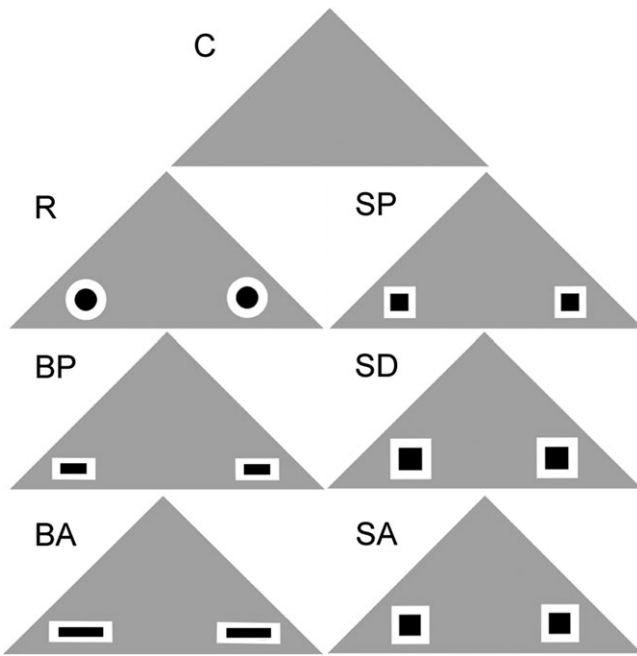
Figure 2
Treatments used in experiment 2: uniform gray control (C), 2 spots (R), 2 squares with the same perimeter as treatment R (SP), 2 squares with the same width as treatment R (SD), 2 squares with the same area as treatment R (SA), 2 bars with the same perimeter as treatment R (BP), and 2 bars with the same area as treatment R (BA).

(externally, E) from the midline (Figure 3). These treatments had 3 quarters of the outside white ring width on one side (3.35 mm) and one quarter of the width (1.15 mm) on the other side of the spot. This created the impression of spots either "looking" inward or "outward" in these 2 treatments. Therefore, treatment I creates the impression of eyes focusing inward and is more eye-like than M, whereas treatment E creates an unnatural appearance less like eyes than M. As with the previous experiments, we also had a nonmarked control treatment. Each block contained 16 targets of each treatment (total sample = 160 per treatment).

If eye mimicry creates effective stimuli, then we would expect survival of the treatments in the following order (highest to lowest): I > M > E > C. Conversely, if it is conspicuousness
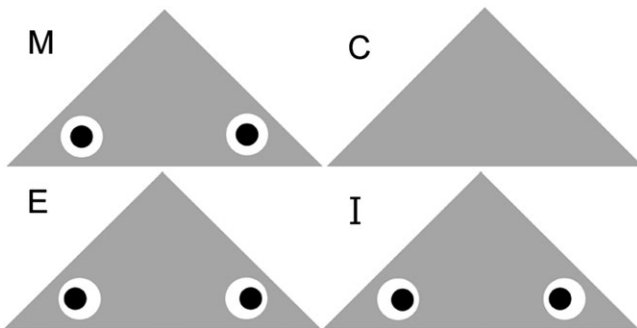

Figure 3
Treatments used in experiment 3: uniform gray control (C), 2 spots with the central black component in the center (M), 2 spots with the central black component displaced outward (externally) from the target midline (E), and 2 spots with the central black component displaced inward to the target midline (I).

that is important, then we would expect no difference in survival between the spotted treatments. Although previous work has indicated that asymmetry may impair the protective value of simple spots (Forsman and Merilaita 1999; Forsman and Herrström 2004), our previous work does not support this when the level of asymmetry is small (Stevens et al. 2007). Therefore, because the level of asymmetry within each spot on treatments I and E is minor and because the overall symmetry of all targets is the same, we do not expect this to influence the results.

RESULTS

Experiment 1: spot number and size

There was a significant effect of treatment ($W = 42.116$, $P < 0.001$, degrees of freedom [df] = 5; Figure 4) and block ($W = 35.952$, $P < 0.001$, df = 9), the latter of which relates to differences in average predation rates in different parts of the woods on different dates and is not relevant to our hypotheses. All spotted treatments survived significantly better than the unmarked controls (e.g., the worst surviving spotted treatment SS vs. C; $W = 10.394$, $P < 0.001$, df = 1, OR = 1.610; Figure 4). Treatment SL survived no better than DL ($W = 0.057$, $P = 0.811$, df = 1, OR = 1.039), but both SL and DL survived significantly better than all the other treatments except T (e.g., DS vs. DL; $W = 3.922$, $P = 0.048$, df = 1, OR = 0.732). Treatments DL and SL survived qualitatively better than T (e.g., DL vs. T; $W = 1.778$, $P = 0.182$, df = 1, OR = 1.234). There was no difference in survival between any of the small spotted treatments, although T survived qualitatively better (e.g., SS vs. T; $W = 0.890$, $P = 0.346$, df = 1, OR = 0.866).

Experiment 2: spot shape

There was a significant effect of treatment ($W = 41.913$, $P < 0.001$, df = 6; Figure 5) and block ($W = 37.811$, $P < 0.001$, df = 9). All marked treatments survived significantly better than the plain controls (e.g., C vs. the worst surviving marked treatment SP; $W = 14.819$, $P < 0.001$, df = 1, OR = 0.556). Treatment BP survived better than all the other treatments (e.g., BP vs. the next best surviving marked treatment SD; $W = 4.306$, $P = 0.038$, df = 1, OR = 1.423 and BP vs. the worst surviving marked treatment SP; $W = 6.187$, $P = 0.013$, df = 1,
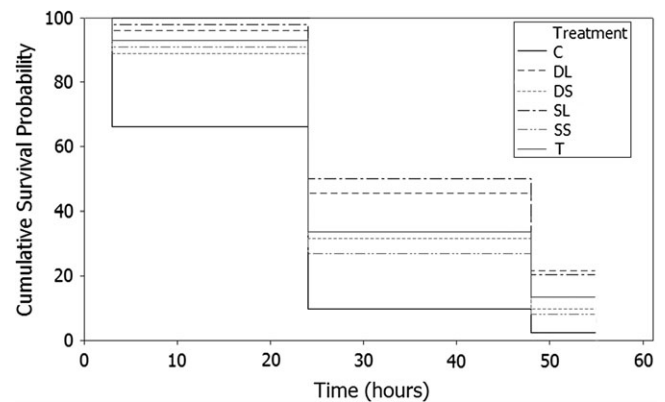

Figure 4
Survival plot of the treatments in experiment 1, with curves being the probability of surviving bird predation as a function of time (hours) based on Kaplan–Meier estimates to account for censoring due to nonavian predation and survival to the end of the study period (48 h). Survival top to bottom: SL, DL, T, DS, SS, and C.
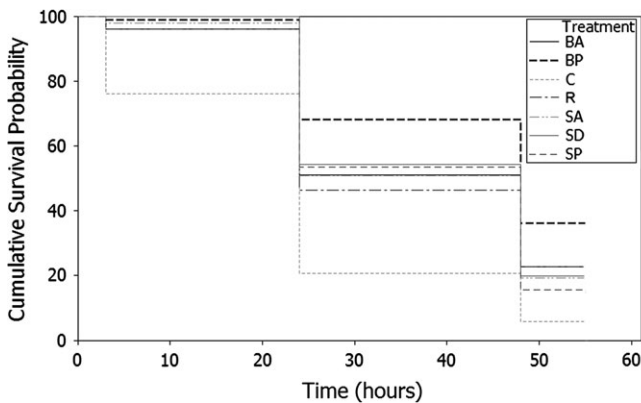
Figure 5
Survival plot of the treatments in experiment 2, with curves being the probability of surviving bird predation as a function of time (hours) based on Kaplan–Meier estimates to account for censoring due to nonavian predation and survival to the end of the study period (48 h). Survival top to bottom: BP, SD, BA, R, SA, SP, and C.

OR = 1.524). However, there was no difference in survival between any of the other marked treatments (e.g., SD vs. SP; $W = 0.185$, $P = 0.667$, df = 1, OR = 0.934).

Experiment 3: spot component displacement

There was a significant effect of treatment ($W = 21.790$, $P < 0.001$, df = 3; Figure 6) and block ($W = 26.977$, $P < 0.001$, df = 9). All marked treatments survived significantly better than the plain controls (e.g., C vs. I; $W = 10.849$, $P < 0.001$, df = 1, OR = 0.657). However, there was no difference in survival between any of the spotted treatments (e.g., M vs. E; $W = 0.202$, $P < 0.653$, df = 1, OR = 1.063; E vs. I; $W = 0.133$, $P < 0.715$, df = 1, OR = 0.952).

DISCUSSION

In experiment 1, we found that the treatments with larger spots survived the best, followed by those with 3 small spots and then those treatments with either 2 or 1 small spot. As with previous work (Stevens et al. 2007), all spotted treatments (in each experiment) survived better than the uniform gray
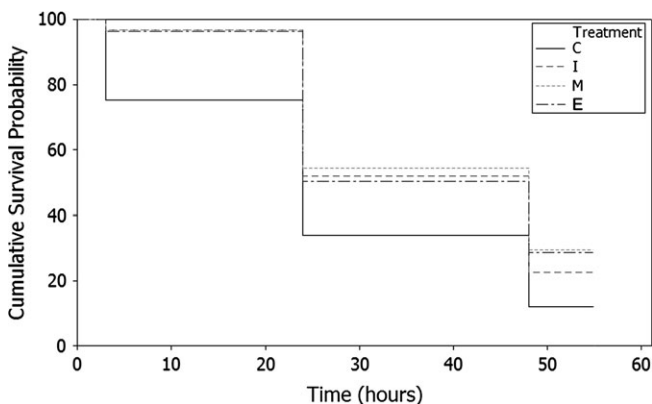

Figure 6
Survival plot of the treatments in experiment 3, with curves being the probability of surviving bird predation as a function of time (hours) based on Kaplan–Meier estimates to account for censoring due to nonavian predation and survival to the end of the study period (48 h). Survival top to bottom: M, I, E, and C.

controls. The primary factor in promoting effective concealment was the size of the stimuli, followed by the number of stimuli; the results being entirely in support of the conspicuous signal hypothesis and not in favor of eye mimicry as the paired spots survived worse (or no better) than those treatments with either spots of larger area or a higher number. Experiment 2 also supported the conspicuous signal hypothesis. Here, we found that there was, with the exception of one treatment, no difference in survival between any of the targets with different shaped spots. The treatment with bars of the same perimeter as the circles survived significantly better than all the other treatments, but otherwise all shapes, including circles, had equal survival. Experiment 3 also favors the conspicuousness theory in that spots where the central element had been displaced inward, to create more eye-like stimuli, did not survive better than any of the other spotted treatments. In fact, the manipulation of displacing the central spot component had no effect on survival; all spotted treatments had near-identical survival probabilities as shown by their OR comparisons.

The role of wing spots as antipredator signals has been considered for more than 150 years (Stevens 2005), but there has been a recent resurgence in work into their function. In particular, recent work has shown both that the wing spots on real butterflies are effective signals to startle predators when suddenly exposed (Vallin et al. 2005, 2007) and that continuously visible spots can intimidate predators, prolonging the survival of artificial prey (Stevens et al. 2007). However, although it is well established that spots do startle/intimidate predators and are effective antipredator signals, it is still contentious as to why they work. Most descriptions, both popular and scientific, of wing spots (or fin spots in fish) assert that they mimic eyes, giving the impression that one of the predator's own enemies has suddenly appeared. However, until now, only Stevens et al. (2007) have systematically tested whether wing spots work because they mimic eyes or merely because they are signals highly effective in stimulating a predator's visual system, promoting avoidance behavior (Stevens 2005). In fact, Stevens et al. (2007) found that the factors that made wing spots most effective were high contrast and conspicuousness and not eye mimicry. The results from these experiments further support the conspicuousness theory.

If wing spots work because they mimic eyes, then the treatments in experiment 1 with 2 spots should have had the highest survival because they resembled a pair of eyes. This was not the case, as the factor which enhanced the protective value of the stimuli was primarily spot size, followed by spot number. The survival of the paired treatments fell neatly in the order predicted by this interpretation (see Figure 4), and these findings are also consistent with previous work. Studies with domestic chicks, involving artificial prey with an aversive taste, found that large spots were effective in promoting avoidance learning (Forsman and Merilaita 1999; Forsman and Herrström 2004). In addition, other work with starlings has found that 3 spots promote avoidance behavior more effectively than 2 spots, provided that the stimuli are not placed on a head shape (Inglis et al. 1983). In a study with peacock butterflies and eyed hawk moths, Vallin et al. (2007) found that the wing spots on the peacock butterflies were more effective in preventing predation by birds than those on the eyed hawk moths and interpreted this as the form of the peacock eyespots being more effective than those on the hawk moths. However, peacocks have 4 spots (2 on each wing), compared with just one pair in the eyed hawk moths, and so it could equally be that spot number is of key importance here. Overall, experiment 1 supports the theory that conspicuousness, and not eye mimicry, is of primary importance in promoting effective avoidance behavior in predators.

If wing spots are effective because they mimic eyes, then one would expect that circular stimuli should provide a higher survival than other shapes. Conversely, because vertebrate visual systems are highly receptive to a range of geometric shapes, such as circles, bars, lines, and so on, one would expect that circles should not survive better than various other conspicuous shapes of similar dimensions. Here, we find that circles do not survive better than bars or squares of equal width, area, or perimeter. In fact, between most of these shapes, the survival was almost identical as indicated by the effect size measurements. The exception was that bars with the same perimeter as the circles survived better than all the other treatments. This is a puzzling result because it was only this treatment that showed a higher survival probability. We think it is unlikely that this treatment survived better because it resembled something in the natural environment because there was no parallel increase in survival conferred by the other bar-shaped markings. Because the area of the less effective bars was larger, it is possible that above a certain size the barred stimuli become less effective (though still highly aversive), but this is speculative and does not seem to fit in with the general findings of experiment 1, although it merits further investigation. In other work, circles have been shown to be more effective in intimidating birds (e.g., Blest 1957), but these experiments have generally not controlled for differences in area, perimeter, or width. Previous work by Stevens et al. (2007) found that circles were no more effective than diamonds of the same area but were more aversive than triangles. However, as Stevens et al. (2007) stated, it is possible that other shapes, such as bars, would be more effective in stimulating a visual system. Here, we find that circles are no more effective than a range of other salient shapes in preventing predation, indicating that the widespread occurrence of circular spots on lepidopterans, fish, and other animals may be because they are developmentally easier to produce. However, because the work of Blest (1957) involved suddenly exposing stimuli of varying shapes when a bird approached, whereas here we use continuously visible stimuli, it would be useful to repeat Blest's experiments with various new controls.

Our third experiment showed that making the spots more eye-like by displacing the central black component inward had no effect on the protective value of the spots. In this experiment, all the spotted targets survived equally, regardless of the manipulation of spot structure. The lack of an effect of this manipulation may be because the differences between the different treatments were subtle. For example, in previous work, Stevens et al. (2007) found that small variations in asymmetry did not affect the protective value of the wing spots. This is in contrast with other work with domestic chicks, which has shown that large variations in size and color of the spots affect the protective value of the signals on artificial prey (Forsman and Merilaita 1999; Forsman and Herrström 2004). The main difference here was that, even though the displaced spots were individually asymmetrical, overall each target was still symmetrical about the midline, and so this may explain the lack of any asymmetry effects. Although this experiment does not support the eye mimicry theory, more work with greater structural manipulations to alter the level of eye mimicry would be valuable.

The results from this study, coupled with those of Stevens et al. (2007), provide firm evidence that the antipredator function of eyespots can be based purely on conspicuousness effects. The traditional explanation of eye mimicry seems unjustified because there is no objective evidence in favor of this theory from carefully controlled experiments. As such, we suggest researchers adopt the terms "wing spot," "fin spot," and so forth when referring to such features on animals instead of the term eyespot. It would be useful to repeat experiments such as these with stimuli presented in startle displays, which more or less resemble eyes, while controlling for conspicuousness effects because other features may become important under such circumstances. Clearly, there is more work to be done, and although it may be difficult to determine the role of higher levels of "receiver psychology" (Guilford and Dawkins 1991), considering predator perception can help to elucidate the mechanistic basis of protective signals (Stevens 2007) without the need for anthropomorphic conjecture.

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