Original Article

The evolutionary significance of butterfly eyespots

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Numerous butterflies have circular patterns called eyespots on their wings. Explanations for their functional value have until recently remained hypothetical. However, several studies in the last few years have supported long-standing hypotheses, and the current paper reviews these recent advances. Large and conspicuous eyespots are thought to be effective by being intimidating to predators and thus reducing predation. This hypothesis has received strong support in different studies. It has been shown that eyespots are intimidating because of their conspicuousness, but experimental support for the idea that eyespots are effective by mimicking vertebrate eyes is at the moment lacking. Studies have also tested the deflection hypothesis, where smaller marginal eyespots are thought to deflect attacks away from the body of the prey, increasing chances of survival with a torn wing. Despite previous negative results, recent work has shown that eyespots can indeed deflect attacks toward themselves under specific conditions. Furthermore, data show that dorsal eyespots are used by males and females as signals during courtship. How the diversity in ventral eyespot patterning has evolved remains a mystery. Future directions and further challenges in understanding the adaptive value of eyespots are discussed. *Key words: Bicyclus anynana*, deflection hypothesis, eyespots, intimidation hypothesis, *Junonia almana*, marginal eyespots. *[Behav Ecol]*

INTRODUCTION

Eyespots on the wings of Lepidoptera have for long evoked interest about their evolutionary significance for their bearers. From the early speculative writings of Poulton (1890), interest in them has resulted in sporadic papers throughout the 20th century, culminating in a series of exciting studies in the last few years that have contributed substantially to our understanding of the functional significance of eyespots. A parallel line of investigation began in the 1980s (Nijhout 1980) with the objective of understanding the developmental and genetic basis governing their formation. This "evo-devo" research has similarly been bolstered by a number of recent studies (e.g., Beldade, Brakefield, et al. 2002; Monteiro et al. 2003; French and Brakefield 2004; Reed and Serfas 2004; Allen et al. 2008). Thus, eyespots have become an important and exciting model study system. Although most common in Lepidoptera, eyespots also occur in other insect groups such as Coleoptera, Fulgoridae, and Orthoptera, as well as in some fishes (Stevens 2005). The highest diversity of eyespot patterns is seen on the wings of butterflies in the family Nymphalidae, and not surprisingly, these have been favorite model organisms for both kinds of studies. Stevens (2005) and Ruxton et al. (2004) provide general overviews of the presumptive role of lepidopteran eyespots. However, the last few years have seen significant strides taking in understanding the function of eyespots in nature, and the current review focuses specifically on findings from these recent studies.

TYPES OF EYESPOTS

The following is a brief description of the kinds of eyespots and their putative function. Readers are referred to Stevens (2005) and Ruxton et al. (2004) for more comprehensive reviews. Given that there are numerous structures ranging from simple spots to crescents similar in morphology to eyespots, it is imperative to first define an eyespot at the outset. Here, following Monteiro (2008), an eyespot is defined as a circular or quasicircular pattern on the wing, with at least 2 concentric rings or with a single color disc and a central pupil. Although this definition encompasses all structures on the wing that are generally considered eyespots, it must be borne in mind that the distinction between eyespots and other structures resembling them is subjective. Thus, some wing features, simple colored discs for instance, may have the same function as eyespots.

Large and strikingly conspicuous eyespots found, for example, in *Junonia almana* (Figure 1) are thought to intimidate predators, thereby reducing the probability of attack on the prey possessing such eyespots. Smaller eyespots such as those found on the wing margins of *Bicyclus* (Figure 2) or *Mycalesis* are thought to function in an opposite manner—they attract attacks toward themselves, thus reducing the likelihood of damage to the vital parts of the body (Poulton 1890; Robbins 1980; Wourms and Wasserman 1985). The 2 kinds of eyespots are not mutually exclusive (Stevens 2005); an eyespot that is intimidating to a small predator can be quite small in relation to a bigger predator and hence serve as a marginal eyespot (Kodandaramaiah 2009). Moreover, the size of such eyespots represents a continuum rather than discrete distributions of large and small. For instance, eyespots on *Lopinga achine* are intermediate in size and cannot be easily classified either as large or small. For the sake of convenience smaller, serially occurring eyespots close to wing margins will be referred to as "marginal eyespots" and larger, isolated ones as "isolated-large." Both kinds of eyespots come in an array of color combinations.

Figure 1
Isolated-large eyespots on the dorsal wing surface of *Junonia almana*.

ISOLATED-LARGE EYESPOTS

Isolated-large eyespots are thought to be frightening to predators either by mimicking the eyes of predators' vertebrate enemies (the "eye-mimicry hypothesis") or because of their conspicuousness (the "conspicuous-signal hypothesis"). In the eye-mimicry hypothesis, eyespots are assumed to fool the predator into believing that its own enemy has appeared and refrain the predator from attacking the prey (Blest 1957), whereas in the conspicuous-signal hypothesis, bearers of eyespots are thought to benefit from the innate cautiousness in predators of objects with high contrast and conspicuousness (Dziurawiec and Deregowski 2002; Stevens 2005). They may also be effective due to avoidance of novel features by predators, that is, neophobia (Marples and Kelly 1999), and neophobia could in turn be enhanced by conspicuousness.

No review on the significance of eyespots can be complete without mention of the pioneer work of Blest (1957). He designed a series of experiments using birds as predators to test the intimidation and deflection hypotheses, with both real butterflies and model prey. Although these trials suggested that eyespots were effective in reducing predation,

his results do not pass muster with current scientific standards; they have drawn criticism for poor experimental design and low sample sizes (Ruxton et al. 2004). Moreover, many of the ideas tested by Blest have been investigated recently within a more rigorous experimental framework. Vallin et al. (2005) devised an experiment with the peacock butterfly (*Inachis io*) and Blue Tits (*Cyanistes caeruleus*) to test the intimidation hypothesis. *Inachis io* has isolated-large eyespots on the dorsal surface of its fore- and hind wings. The butterfly has evolved a mechanism wherein, on approach by a potential predator, it flicks its wings open and close, exposing its eyespots abruptly (Blest 1957). Vallin and colleagues presented a group of Blue Tits with butterflies having intact eyespots and another group with butterflies in which eyespots were painted over (Figure 3). Butterflies with intact eyespots survived much better than those without; 13 of the 20 eyespotless butterflies were killed, whereas only 1 of the 34 eyespotted ones was killed. This study was the first to convincingly demonstrate that isolated-large eyespots can increase survival. Vallin et al. (2007) showed a similar effect of eyespots in the eyed hawkmoth (*Smerinthus ocellata*: Sphingidae). This species has its eyespots on the hind wings that are normally hidden under the forewings. When approached by a predator, the forewings are abruptly protracted, thereby displaying the hitherto hidden eyespots. These 2 studies showed that eyespots with a combination of movement and sudden appearance can enhance survival. However, this raised the question of how effective eyespots are when being motionless and continuously visible to the predator.
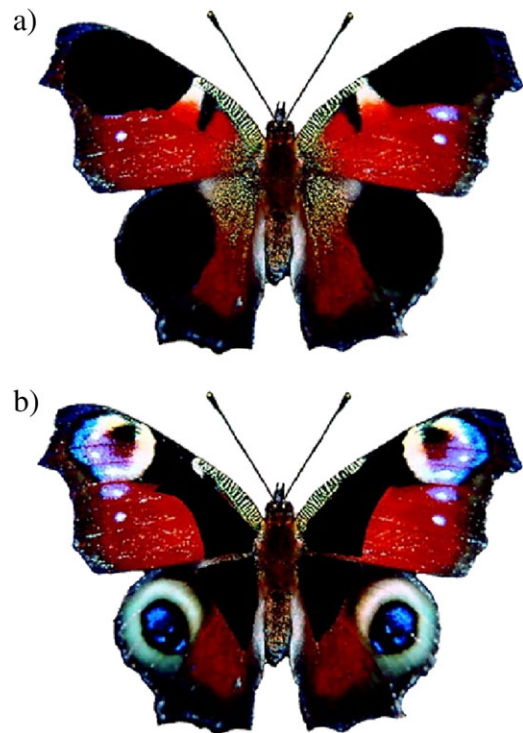

Figure 2
Putative deflective marginal eyespots on the ventral wing surface of *Bicyclus madetes*.

Figure 3
Two treatments used in Vallin et al. (2005) where live peacock butterflies (*Inachis io*) were manipulated to test the effect of eyespots on survival in the face of predation by Blue Tits (*Cyanistes caeruleus*) (a) Treatment where eyespots were painted over using a black marker pen (b) Control, where wings were painted such that the eyespots were visible. Butterflies with visible eyespots survived much better than those with eyespots painted over. Adapted from Figure 1 of Vallin et al. (2005).

Later studies (Stevens et al. 2007; Stevens, Hardman, et al. 2008; Kodandaramaiah et al. 2009) answered this question. The latter 2 studies used artificial "prey," triangular pieces of waterproof paper with specific designs printed on them, and were not intended to mimic any particular species. A mealworm was placed either at the center of the triangle or underneath it. In addition to other results (discussed in the following paragraph), one of the results from these field-based experiments was that eyespot-like patterns reduced predation risk on the prey. Kodandaramaiah et al. (2009) took a slightly different approach, using wings from real butterflies instead of artificial paper prey to specifically test whether continually visible and motionless eyespots are beneficial by being intimidating. Their prey consisted of wings from peacock pansy butterflies (*J. almana*; Figure 1) glued onto a piece of cardboard in a manner similar to a basking butterfly, with the difference being that a dead mealworm was placed in place of the butterfly body. A bird predator, the Great Tit (*Parus major*), was offered a choice between 2 prey, one with eyespots painted over and the other without. Mealworms on the prey without eyespots were attacked more frequently. All trials were filmed, and they reported that birds actively avoided eyespotted prey. Thus, neither the sudden display of eyespots nor any movement associated with them is a prerequisite for them to be intimidating. The studies on *I. io* and *J. almana* were conducted with Blue Tits and Great Tits, both small passerine predators. It would be interesting to know whether the intimidating effect of these eyespots is reduced when attacked by much larger predators.

EYE MIMICRY VERSUS CONSPICUOUS SIGNAL

The eye-mimicry hypothesis has been the most cited explanation for the intimidating nature of eyespots, although this has not been backed up by genuine experimental evidence. Blest (1957) concluded that eyespots are most effective when they are circular, composed of concentric elements and appear to be 3D. He based this partly on his own results that suggested that eyespots with their centers shifted to a side so as to render them more 3D in appearance were more "scary" to birds. These features are characteristic of vertebrate eyes and thus appear to indirectly support the eye-mimicry hypothesis. Stevens et al. (Stevens et al. 2007, 2009; Stevens, Hardman, et al. 2008) performed a suite of experiments to test the importance of conspicuousness. Stevens et al. (2007) showed that all patterns—circular, triangular, and rhomboidal—that had strong internal contrast and also contrasted highly with the target background were more effective in reducing predation risk compared with no pattern. Thus, conspicuousness per se can be beneficial against predation. Two subsequent studies (Stevens, Hardman, et al. 2008; Stevens et al. 2009), both using similar setups, were designed expressly to test whether more eye-like patterns are better than less eye-like ones. In Stevens, Hardman, et al. (2008), they found that the shape of the pattern—circular, rectangular, and square, in this case (Figure 4)—did not matter, but the overall size and number of the patterns were factors that decreased predation risk on prey. This supports the conspicuous-signal hypothesis because the area of stimulus increases with size and higher numbers. They found no evidence suggesting that circular patterns in pairs (therefore more similar to a pair of eyes) were more effective than other arrangements—the combined size of the stimuli was the determining factor. Furthermore, their results did not support the idea that patterns with their centers displaced inward, thus appearing 3D and eye like, were better than patterns without displacement. Stevens et al. (2009) demonstrated that eyespots present in arrangements mimicking real eyes were not more effective
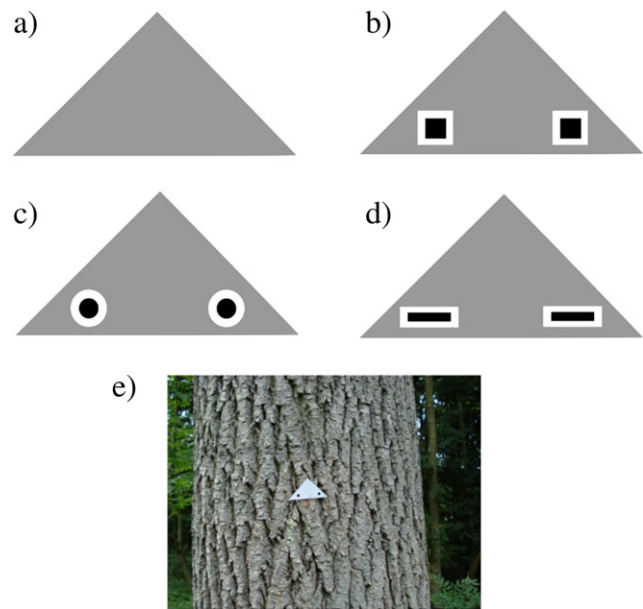

Figure 4
Examples of treatments used as part of studies conducted by Stevens and colleagues to test the effectiveness of different shapes on prey survival. The prey consisted of triangular pieces of gray card with specific patterns printed on and a mealworm placed in the center (either on or below the card). (a) Uniform gray control; (b) Prey with 2 squares; (c) Prey with 2 circular eyespots; and (d) Prey with 2 rectangles. Mealworms in all treatments with printed patterns survived better than the control, although there was no difference between different patterns. Adapted from Figure 2 of Stevens, Hardman, et al. (2008). (e) Photograph of a prey in the field.

than those occurring in non–eye-like arrangements. Black and yellow are color components common in the eyes of birds of prey. Thus, the eye-mimicry hypothesis predicts enhanced survival of the prey with a combination of these 2 colors. However, Stevens et al. (2009) found that eyespots with this combination were just as effective as those with a red and black or blue and black combination.

Based on these strong results, Stevens and colleagues have convincingly argued that conspicuousness, and not eye mimicry, is what makes eyespots effective. However, eyespots in several species exhibit some kind of displacement of elements, which gives them the appearance of being 3D (Figure 1). The fact that Stevens, Hardman, et al. (2008) did not find patterns with displaced centers to be more effective than patterns without displacement might possibly be simply because the differences were subtle, as the authors themselves admit. Further work is needed before concluding that such patterns are not better than simpler concentric circular ones. Whether more 3D is equivalent to more eye like is a related question that will perhaps remain unanswered. More importantly, the 2 hypotheses are not necessarily mutually exclusive explanations—eyespots might be effective primarily due to conspicuousness, whereas eye mimicry or 3 dimensionality could add to their effectiveness under some circumstances.

POTENTIALLY MALADAPTIVE?

Stevens, Stubbins, et al. (2008), using a similar setup as in the above experiments, showed that circular patterns reduce predation when the artificial prey was conspicuous against the background, whereas they increased predation when the prey color matched the background. This suggests that eyespots make camouflaged prey more conspicuous, being maladaptive in

certain conditions. It has also been shown that the evolution of eyespot patterning in *Junonia* and related butterflies has not been directional with respect to either eyespot number or size (Kodandaramaiah 2009). Species in Junoniini (Nymphalidae: Nymphalinae), which includes *Junonia*, possess an array of eyespot patterning, with eyespot numbers ranging from 1 to 7. In some clades, there is a trend toward decreasing eyespot number and a concomitant increase in eyespot size, making the eyespots more conspicuous and presumably more intimidating, *J. almana* being an excellent example (Figure 1). However, there are also instances of shifts from a pattern of such isolated-large eyespots to a series of much smaller marginal eyespots. This suggests that large conspicuous eyespots are not necessarily beneficial in all scenarios. The habitat of the butterfly and the general background may influence the effectiveness of eyespots. An eyespot pattern that is protective against predation in a certain region can therefore be harmful in another region (Janzen 1985; Kodandaramaiah 2009). Moreover, not all predators react in the same way to a particular eyespot (Vallin et al. 2007). If predators are not intimidated by eyespots, the conspicuous nature of eyespots might make them more easily detected (Stevens, Stubbins, et al. 2008). Consequently, the net reaction of predator communities can differ considerably, imposing differential selection pressures on eyespot patterning. Ruxton (2005), in a commentary on Vallin et al. (2005) remarked that a major challenge for behavioral ecologists is to explain how the potentially maladaptive behavior of predators' reaction to *I. io* has persisted in nature. We have answered the question partially; it does not necessarily persist.

Bicyclus anynana, like many of its related satyrine species, is seasonally dimorphic with respect to ventral eyespot patterning. Eyespots are larger in the wet season when butterflies are more active and inconspicuously small in the dry season when butterflies are more sedentary (Brakefield and Larsen 1984). Lyytinen et al. (2004) showed that the cryptic dry season form is better protected against a background of brown leaves, which is typical of the dry season. However, both morphs were equally conspicuous against a background of green leaves. Similar results were obtained in a field-based release–recapture study (Brakefield and Frankino 2007) where wet and dry season *B. safitza* (which exhibits seasonal polyphenism similar to *B. anynana*) butterflies were released in dry and wet seasons. Dry season butterflies survived better during the dry season, whereas there was no difference in survival in the wet season. These results further support the idea that the protective value of eyespots varies with the environment.

MARGINAL EYESPOTS

In contrast to the intimidation hypothesis, the deflection hypothesis did not garner much experimental support until very recently. Wourms and Wasserman (1985) found evidence for the related false-head hypothesis. Many butterflies, especially in the family Lycaenidae, have 1 or 2 simple spots or eyespots at the margin of the wing, along with lines radiating toward it, and extensions of the wing close to the eyespot that presumably imitate antennae. Such eyespots are referred to as "false heads" (see Figure 5 for an example) and thought to draw the attention of predators from the real heads, increasing the chances of surviving with a torn wing (Van Someren 1922; Robbins 1980, 1981; Tonner et al. 1993; see Cordero 2001 for a different perspective). Wourms and Wasserman (1985) tested the hypothesis using dead *Pieris rapae* (Cabbage white) butterflies and Blue jays (*Cyanocitta cristata*). *Pieris rapae* does not possess the false-head pattern in nature. Butterflies painted with the 3 pattern elements constituting the false head (spots, lines, and wing-extensions) and combinations of these 3 pattern elements were presented to the birds along

Figure 5
The "false-head" pattern in the lycaenid butterfly *Deudorix epijarbus*.

with normal butterflies as a control group. Spots, when occurring by themselves, were effective in drawing attacks toward themselves, but ineffective when present in combination with either of the remaining pattern elements. These results were the first to provide evidence that an eyespot can deflect attacks away from the body. However, the spots or eyespots in false-head patterns are found either singly or in pairs and are presumed to mimic the head of the prey. Marginal eyespots, on the other hand, are present in varying numbers along the margin and have not been explicitly hypothesized to mimic either the eyes or the head of the prey. These eyespots are simply thought to draw the attention of predators toward themselves by being more conspicuous than other parts of the wing, but not large enough to intimidate them. Additionally, marginal eyespots are thought to be advantageous when present on the ventral surface because they are visible to predators at rest. Examples of nymphalid genera with such eyespots on the ventral surface include the genera *Bicyclus*, *Mycalesis*, *Pararge*, and *Euptychia*; *Lopinga*, *Lasiommata*, and some *Junonia* species possess such patterns both on the dorsal and ventral sides.

Lyytinen et al. (2003) tested whether the serial eyespots found on the ventral surface of *B. anynana* could deflect attacks by lizards (*Anolis carolinensis*) and birds (Pied Flycatcher; *Ficedula hypoleuca*). Predators were presented with butterflies of wet and dry season morphs, the latter of which has highly reduced eyespots. Eyespots did not influence the position of attacks on the butterfly by either predator. In a follow up experiment, Lyytinen et al. (2004) used naïve flycatchers with the same setup and reported that butterflies with eyespots survived better compared with the previous experiment where birds were adult and experienced. Vlieger and Brakefield (2007) repeated the experiment with a slightly modified setup, using both adult and naïve lizards. They again found no evidence that eyespots influenced the position where butterflies were grabbed. In summary, the 3 experiments with *B. anynana* have found no hard evidence for the deflection hypothesis, although there is weak evidence suggesting that eyespots may be beneficial against predation by naïve birds (Lyytinen et al. 2004).

The recent study on marginal eyespots of *L. achine* (Olofsson et al. 2010) is arguably the most exciting study with respect to the deflection hypothesis. This satyrine species has a series of marginal eyespots on the ventral surface, each with a white ultraviolet (UV)-reflective pupil (Figure 6). Olofsson and colleagues presented the ventral wing surface of mounted specimens of *L. achine* to Blue Tits under 3 different light conditions. They found that the birds directed attacks toward
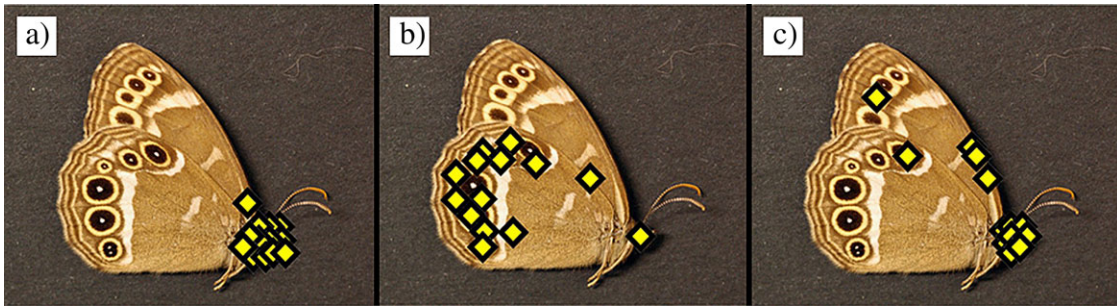
Figure 6
Trials in Olofsson et al. (2010) that showed that marginal eyespots on the ventral wing surface of the Woodland Brown butterfly (*Lopinga achine*) can deflect attacks from birds (Blue Tits; *Cyanistes caeruleus*) under certain light conditions. (a) Distribution of bird attacks under high light intensity in the presence of UV (b) Distribution of bird attacks under low light intensity in the presence of UV (c) Distribution of bird attacks under low light intensity in the absence of UV. Figure taken from Olofsson et al. (2010); Figure 4 in the original paper.

the marginal eyespots under low light conditions with elevated UV levels but instead attacked the head at higher light intensity or low light intensity with low UV level (Figure 6). This is the first study with results showing a clear propensity for predatory attacks to be directed toward naturally occurring eyespots, albeit under some specific conditions. The authors contend that deflecting eyespots are likely to be effective during dawn and dusk, when the ambient light is richer in UV wavelengths. The authors also reason that the activity of predators such as birds is at its peak during early dawn, and, moreover, lower temperatures during dawn and dusk may inhibit escape by flying away. However, the light regime in their treatment where eyespots were deflective does not correspond to that under dusk or dawn environments in nature. The relative difference between the intensities of low (which includes the UV spectrum) and high wavelengths was exaggerated compared to natural levels. Furthermore, UV spectral irradiance varies across latitude (Diffey 1991). Hence, it still remains to be seen whether marginal eyespots can be effective under natural conditions.

If such specific light conditions are indeed a prerequisite for marginal eyespots to be effective, the negative results in previous studies with *B. anynana* are explained by the fact that they used daylight mimicking light regimes. However, the experiments also differed in the predators used and in the manner in which the prey were presented. It would be interesting to test whether eyespots in other species, including *B. anynana*, elicit a deflective effect under the same set up as in the Olofsson et al. study. Intriguingly, the simple artificial spots used in Wourms and Wasserman (1985) were effective without using special light conditions. This suggests that the effectiveness of marginal eyespots with different morphologies may be influenced by light, background, and other conditions. Vallin et al. (2011) tested whether the effectiveness of deflective eyespots varies with background. They used an indoor set-up where blue tits were presented triangular pieces of artificial prey items with small (3 mm diameter) and large (6 mm) eyespots. They also used 2 different backgrounds—one that concealed the prey and one where the prey was conspicuous. They found that birds did direct attack toward smaller eyespots, but this effect was not seen in the case of the large eyespots. However, the deflective effective of small eyespots was independent of whether the prey was concealed or conspicuous.

Although it is unambiguous that natural marginal eyespots can deflect attacks under specific laboratory settings, further work is needed to understand under what conditions they are effective. Laboratory trials with controlled manipulations of the light spectrum, and different kinds of predators are one option. Such trials may also be used to manipulate different components of eyespots (the UV-reflective pupil and the surrounding rings) to test which of these is important. Alternatively, field-based investigations can be designed to test this. For example, dead specimens of butterflies can be planted in their natural resting position and predation events videotaped. Hill and Vaca (2004) compared 3 species of *Pierella* butterflies, 1 with a putative deflective patch on its hind wing (*P. astyoche*) and the other 2 without. They found that wing tear weight (a measure of how easily parts of the wings can tear) in the region of the putative deflective patch was lower in *P. astyoche*. They concluded that the region of the wing with deflective markings is relatively weak and hence should have a higher tendency to tear when handled by a predator. Such comparisons can be undertaken within butterfly clades comprising species with and without eyespots to test whether wing tear weights are lower in species with marginal eyespots. Similarly, one can also test whether eyespotted areas of the wing are more easily torn compared with regions without eyespots within the same species. Another hypothesis worth exploring is that marginal eyespots occur at regions of the wing where a tear is less likely to affect flight ability significantly.

FIELD-BASED VERSUS LAB-BASED EXPERIMENTS

It is interesting to contrast the 2 schools of eyespot research, one that uses controlled laboratory trials and the other, field-based experiments. The first approach has advantages in that the mechanism by which eyespots are effective can be observed, and the predator is known. Predation events on butterflies are rarely observed in nature, which makes it difficult to ascertain the relative pressure from different kinds of predators. Vertebrate predators, especially birds, are generally thought to be the most common predators (see Vlieger and Brakefield 2007 and references therein), although this is based largely on circumstantial evidence. An advantage of field-based methodology, used by Stevens and colleagues for instance, is that the effectiveness of eyespots is determined by the predator community rather than a single predator. Nevertheless, predators that do not normally attack butterflies, ants for instance, may feed on artificial prey in trials. This could potentially bias results, although efforts have been made in studies so far to statistically control for non-avian predators. Outdoor trials have so far mainly used artificial prey (an exception is Brakefield and Frankino 2007), whereas indoor trials have tended to use real butterflies (but Vallin et al. 2011 used artificial cardboard prey). Although real prey may be preferable in some studies, one advantage of artificial prey is that they allow manipulation of patterns that

can be used to disentangle the relative effects of different components of eyespots. Overall, both field and laboratory approaches have their relative merits and weaknesses and complement each other well.

MATE CHOICE AND OTHER SELECTIVE FORCES

Hingston (1933) observed that eyespots in most species are located on the outer wing section where they are most visible to competitors and hence could be of importance in intra-sexual selection as threat signals. This idea has not been tested thus far. Stevens (2005) remarked that the possible role of eyespots in sexual selection should be investigated before concluding that eyespots function solely as antipredator devices. Four studies on *B. anynana* (Breuker and Brakefield 2002; Robertson and Monteiro 2005; Costanzo and Monteiro 2007; Prudic et al. 2011), all using laboratory populations with the same founders, have shown that isolated-large eyespots on the dorsal surface are involved in sexual selection. Breuker and Brakefield (2002) found that females preferred males with larger eyespots. However, these results were challenged in Robertson and Monteiro (2005), which was a more comprehensive study that tested the possible role in sexual selection of all eyespots in the species. In this study, females chose males based on the size and UV reflectance of the white pupil of the isolated-large dorsal eyespots (Figure 7), whereas the size of eyespots did not matter. The effect of eyespot size found in the Breuker and Brakefield study is explained by the manner in which eyespot size was manipulated—individuals with large eyespots had the UV-reflective pupils, whereas the ones with experimentally reduced eyespots did not. The results in Robertson and Monteiro (2005) were further corroborated in Costanzo and Monteiro (2007). Interestingly, Prudic et al. (2011) found that female choice based on the UV-reflective pupil is restricted to those with the wet season morphology—dry season females did not discriminate between males with or without the pupils. *Bicyclus anynana* has additional, smaller dorsal eyespots on the hind wings, and it is unclear what their role is. Similar experimental studies on other eyespotted species and perhaps also on wild populations of *B. anynana* are needed to understand how pervasive the phenomenon of sexual selection on dorsal eyespots is and the variation across species. The possible interplay between sexual selection and selection by predation on eyespots remains to be tested.

Figure 7
Dorsal wing surface of a *Bicyclus anynana* dry season female, illustrating the isolated-large eyespot on the forewing. Experiments (Robertson and Monteiro 2005; Costanzo and Monteiro 2007; Prudic et al. 2011) have shown that both males and females use the size and UV reflectance of the central white pupil of the large eyespot in choosing mates.

There is surprisingly little discussion in literature on sexual dimorphism with respect to eyespot patterning. There are several examples of species in which sexes possess different eyespot number or size. For example, in *J. orithya*, females have larger eyespots (Kusaba and Otaki 2009), whereas males of some subspecies of *Hypolimnas bolina* lack eyespots. Females of a newly described *Euptychia* species possess dorsal eyespots on the hind wing, but the males do not (Pulido et al. 2011). Oliver et al. (2009) and Oliver and Monteiro (2010) provide more examples within the genus *Bicyclus*. Interestingly, in almost all these examples, eyespots are smaller or absent in males (note that this is not a comprehensive survey), suggesting the possible role of male mate choice or an effect of differential predation pressure on sexes in these species. Indeed, the recent experiments on *B. anynana* by Prudic et al. (2011) have shown that dry season males choose females based on the UV-reflective pupils, although wet season males showed no such preference. Hence, in this species, wet season females and dry season males use the eyespot pupil to choose mates. Pupil size and UV reflectance are higher in wet season males compared with dry season males, but females of the 2 morphs differ only in UV reflectance, with dry season females having higher reflectance percentage (Prudic et al. 2011). These results indicate a complex interplay among dynamic selective forces acting on dorsal eyespots.

Oliver et al. (2009), in their comparative work on eyespots in the genus *Bicyclus*, found that rates of evolution of eyespot patterning on the dorsal surface were higher compared with ventral surface patterns in the group. It has been speculated that butterflies utilize their dorsal surface in mate signaling during courtship. Oliver and colleagues posited that sexual selection acting on dorsal eyespots has permitted elevated rates of evolution in terms of eyespot number, whereas evolution of ventral eyespots is driven by strong stabilizing selection, probably by predation.

Although a protective role is often invoked to explain the presence of ventral eyespots, this cannot, by itself, explain the huge diversity of eyespot patterns on the ventral surface of butterflies. For instance, in some satyrine groups, closely related species in the same region can differ in features as subtle as the relative placement of a single eyespot or in having 5 versus 6 eyespots. Predation pressure per se is insufficient to explain such variation between species. Species-specific patterns are, however, much more conservative and museum specimens from a century ago tend to have the same patterns. This points to species-specific selective forces, and predation could be one of a suite of selective agents.

Janzen (1985) suggests that the adaptiveness of a trait such as an eyespot can vary across the range of a species distribution, being adaptive in one region, maladaptive in another, and neutral in yet another. If eyespots are neutral and have no selective advantage or disadvantage, eyespot size and number can be free to vary extensively merely by genetic drift. This perhaps explains some of the variation in eyespot patterns, both ventral and dorsal. Studies on *Maniola jurtina* and related species (Dowdeswell and Ford 1952; Creed et al. 1959; Frazer and Willcox 1975) are worth noting in this regard. These surveys have shown a considerable degree of geographic variability in ventral eyespot numbers in several species, and these patterns have been studied extensively in *M. jurtina*. As such, this species might be a good system to investigate whether differences among populations are adaptive in their respective environments.

EYESPOTS IN OTHER GROUPS

Eyespots are known to occur in several fish species, coral reef fishes for instance (Randall et al. 1997). Both the intimidation

and deflection hypotheses have been postulated to explain the presence of eyespots in such fish (Cott 1957; Neudecker 1989; Meadows 1993). Eyespots considered to have a deflective effect are located on areas of the body, such as tails or fans, that allow survival after attack (Neudecker 1989). However, there is very little empirical evidence supporting either the deflection or the intimidation hypothesis (Gagliano 2008). Further studies are needed to understand the adaptive value of eyespots in fish and other non-insect organisms.

SUMMARY AND FUTURE DIRECTIONS

The last 5 years have seen significant strides taken in understanding the functional value of eyespots in butterflies. It is clear that certain eyespots on butterfly wings can intimidate predators and secure survival of the eyespotted prey. It is also evident that conspicuousness makes eyespots effective, but there is little evidence to support the idea that more eye-like or 3D patterns are more advantageous. Recent work has also shown that smaller marginally placed eyespots can deflect attacks toward themselves under certain conditions, thereby giving the butterfly a chance to escape with a torn wing. There is mounting evidence supporting the role of dorsal eyespots in mate choice. Evo-devo studies suggest that adaptive forces, rather than developmental constraints, are shaping how eyespot patterns are evolving (Brakefield 2001; Brunetti et al. 2001; Beldade, Koops, et al. 2002; Evans and Marcus 2006). A suite of selective agents has given rise to the stunning diversity of eyespot patterns in nymphalids, and deciphering these forces will be a challenge. Speciose satyrine (Nymphalidae: Satyrinae) groups such as *Bicyclus*, *Mycalesis*, and *Ypthima* are amongst the most prominent groups possessing eyespots. Comparative methods present the possibility of testing the intriguing idea that eyespots are linked to diversification processes. The potential effect of different predator communities or habitats on the value of eyespots can be tested in field studies. Significant insights are likely to come from controlled laboratory experiments on more eyespotted species. Testing the role of eyespots in both inter- and intrasexual signaling, and in species discrimination in different species will be especially exciting. Field-based and laboratory approaches have their respective strengths and both will prove important in shedding further light on eyespot evolution.

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