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Adaptive constellations of protective marks: eyespots, eye stripes and diversion of attacks by fish

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Coloration of animals often includes spectacular markings that supposedly decrease predation risk. Many vertebrates have eye stripes that have been suggested to conceal the noticeable shape of the eye from predators. Another salient mark in a wide range of taxa that sometimes co-occurs with an eye stripe is the eyespot. Some eyespots divert strikes of attacking predators, but whether the eyelike appearance is essential for the divertive effect is not known. Although numerous species of fish provide iconic examples of spectacular coloration, experimental studies on the protective function of coloration against fish predators are scarce. We investigated the divertive potential of prey marks, using artificial prey and three-spined sticklebacks, *Gasterosteus aculeatus*, as a model for predator perception and behaviour. A significant proportion of attacking fish directed their strike towards an eyespot, but when compared to a square-shaped mark, the bias was not significantly stronger. Importantly, a stripe running through the eyelike mark strongly influenced the attacking fish. When presented simultaneously, the fish directed their strikes towards an intact eyelike shape and away from an eyelike shape disrupted by a stripe. Our results demonstrate that marks of different shapes differ in their divertive potential, and this may contribute to the wide occurrence of eyespots in prey. Importantly, they also show that a stripe can effectively decrease the salience of an eyelike pattern, which provides the first experimental evidence for the adaptive benefit for eye stripes. Moreover, the joint effect of the eyespot and the disruptive eye stripe indicates that prey marks with different functions can form adaptive constellations to manipulate the attack behaviour of predators.

Predation has led to the evolution of a large variety of antipredator adaptations. Although many spectacular colour patterns have been proposed to have a protective function (Cott, 1940; Ruxton, Sherratt, & Speed, 2004; Stevens & Merilaita, 2011), rigorous investigation and evidence for the protective mechanism is still lacking for many types of patterning. Eyespots are colour patterns consisting of concentric circles and have received their name because, at least to humans, they often resemble the vertebrate eye (Blest, 1957; Cott, 1940; Stevens, 2005). Interestingly, eyespots are taxonomically very widespread and occur for example in molluscs, arthropods, fishes (Fig. 1), amphibians, reptiles and birds (Cott, 1940; Edmunds, 1974; Poulton, 1890). Although some eyespots are involved in sexual signalling (Loyau et al., 2007; Robertson & Monteiro, 2005), they are probably more often coupled with antipredator functions. Multiple experiments have shown that some eyespots can intimidate predators (Kodandaramaiah, Vallin, & Wiklund, 2009; Merilaita et al. 2011; Olofsson, Løvlie, Tibblin, Jakobsson, & Wiklund, 2013; Vallin, Jakobsson, Lind, & Wiklund, 2005). Another protective function of eyespots is that they may divert attacks, either towards less vital parts of the prey's body, such as fins of fishes or wing margins of butterflies, or by giving false information about the location of the anterior end and hence the expected escape trajectory of the prey (Kjernsmo & Merilaita, 2013; Prudic, Stoehr, Wasik, & Monteiro, 2015). Divertive (also called 'deflective') marks such as eyespots would thus manipulate where predators direct their strikes, increasing the probability that a prey escapes an otherwise fatal encounter.

Although this popular idea was proposed over a century ago (Poulton, 1890), a divertive function of eyespots has proven difficult to demonstrate (e.g. Lyytinen, Brakefield, & Mappes, 2003; Vlieger & Brakefield, 2007), and rigorous empirical evidence for the divertive effect of eyespots has only started to accumulate over the last few years. Eyespots on artificial prey items drew the attacks of

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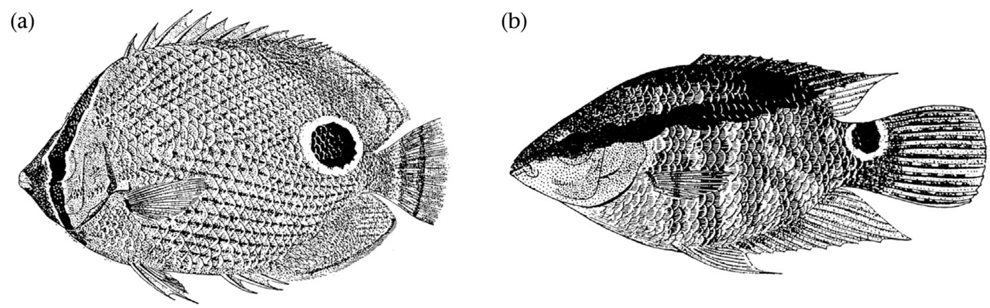

Figure 1. Examples of fish having both an eye stripe and an eyespot in the caudal region. (a) *Chaetodon capistratus* and (b) *Cichlasoma festivum*. Illustrations from Cott (1940) (Figures 76 and 33), published with permission from the Hugh Bamford Cott Archives at the University Museum of Zoology, Cambridge (CAM ZM).

blue tits, *Cyanistes caeruleus*, towards these markings (Vallin, Dimitrova, Kodandaramaiah, & Merilaita, 2011). Eyespots of manipulated speckled wood butterflies, *Pararge aegeria*, diminished the probability that blue tits directed their attacks to the heads of the butterflies (Olofsson, Jakobsson, & Wiklund, 2013). Interestingly, compared with these rather modest effects on passerine birds, eyespots induced a strong divertive effect on attacking three-spined sticklebacks, *Gasterosteus aculeatus* (Kjernsmo & Merilaita, 2013).

Little is still known about the function of divertive eyespots. Eyespots in general are characterized by their eyelike appearance, but the importance of this appearance for their function is still discussed (Janzen, Hallwachs, & Burns, 2010; Merilaita et al. 2011; Stevens & Ruxton, 2014), and it is unclear whether eyelike appearance is even necessary for the divertive effect. This is because the wide occurrence of the eyespot pattern might also be explained by the relatively simple developmental mechanism generating the shape (Beldade & Brakefield, 2002; Dilão & Sainhas, 2004).

Another mark that characterizes, for example, many fishes, amphibians, birds, reptiles and mammals is the eye stripe (e.g. Cott, 1940; Edmunds, 1974). An eye stripe is a bar that runs through the eye, matching the eye's (or part of the eye's) colour (Cott, 1940). Many fishes have an eye stripe that blends into the colour of the pupil and disrupts the iris whereas the rest of the iris often blends into the facial colour adjacent to the eye (Fig. 1). The appearance of eye stripes varies substantially, and in fish their shape and angle have been suggested to depend on the head and body shape of the species (Barlow, 1972). Eye stripes have often been assumed to decrease the salience of the eye and increase the camouflage of a species through a disruptive effect (Barlow, 1972; Cott, 1940). According to the principle of coincident disruptive coloration, some colour patterns can change the apparent shapes of salient body parts such as eyes or extremities by visually joining discontinuous surfaces and breaking up continuous surfaces (Cuthill & Székely, 2009; Stevens & Merilaita, 2009, 2011). Cuthill and Székely (2009) have demonstrated that coincident disruptive coloration can decrease the risk of a prey being detected or recognized by predators. However, so far there has not been experimental evidence for such a concealing effect of eye stripes.

Concealment of salient features, such as the eyes, may increase prey survival directly by decreasing the risk of being detected by predators, but it might also strengthen the divertive effect of an eyespot. If a divertive eyespot mimics the eye to give a false indication of the location of the anterior end or escape direction of the prey, then the actual eye is a feature that the eyespot needs to outcompete, in terms of gaining the attention of the predator. Instead of or in addition to adjusting the size or contrast of the eyespot, which might also result in increased detectability of the prey, selection might act on the salience of the actual eye (Cott, 1940; Kelley, Fitzpatrick, & Merilaita, 2013). This suggests that despite their very distinct effects (concealment and misdirection of visual attention), eye stripes and eyespots may, in some cases, form a constellation of protective marks in which their effects interact for a common purpose. Similarly to eye stripes, eyespots are also found in many species of fishes. A comparative study on butterflyfishes (family Chaetodontidae) showed that although eyespots appear evolutionarily labile, all species with eyespots also had eye stripes (Kelley et al., 2013). This association supports the suggestion that eye stripes may facilitate the effect of eyespots.

To better understand the wide occurrence of eyespots and eye stripes in prey, their function and why they may co-occur, we conducted a series of predation experiments. Relatively few experiments have addressed protective coloration in aquatic environments, and therefore we used the visually hunting three-spined stickleback as a model for predator cognition. Our aim was to investigate the predator's behavioural response to prey marks, and more particularly how various marks may be used to manipulate where attacking predators direct their strikes. To this end, we presented the fish with artificial prey items to study their innate response towards prey marks. Given an otherwise identical behaviour of prey, the ability to misdirect or divert predator strikes can decrease the risk that a strike will have fatal consequences and increase the probability that a prey escapes and survives an attack (Cooper & Vitt, 1985; Cott, 1940). To understand the appearance of prey marks and colour patterns and why they have evolved, it is necessary to know how various marks influence predators' attack behaviour. In the present study on eyespots and eye stripes, we first tested the significance of the eyelike appearance for the divertive function of body patterning. Second, we investigated whether an eye stripe can effectively decrease the salience of eyelike shapes. Third, we wanted to investigate the idea that constellations of protective marks with distinct functions, in this case divertive eyespots and disruptive stripes, could jointly manipulate predator behaviour.

METHODS

The Predators

In this study we chose the three-spined stickleback as a model of fish cognition and behaviour for the following reasons. First, sticklebacks are primarily visual predators, and can therefore serve as a general model of visually oriented predatory fish (e.g. Kjernsmo & Merilaita, 2013). Second, practical reasons, such as the relatively small size of the species and the large amount of information that is available on it, make it particularly useful in behavioural experiments. Finally, in nature they commonly forage on small crustaceans, insect larvae and fish fry (Hart & Gill, 1994; Litvak & Leggett, 1992), and it is therefore unlikely that sticklebacks have evolved any counter adaptations towards protective