Scared by shiny? The value of iridescence in aposematic signalling of the hibiscus harlequin bug

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Studies on aposematism have generally focused on the benefits of red or yellow coloration, occasionally in contrast with green or brown, but rarely blue or orange. Furthermore, almost no studies have explicitly studied the utility of iridescent coloration in aposematism. To evaluate the survival benefit of iridescent coloration, we tested the ability of the natural colour extremes of *Tectocoris diophthalmus* jewelbugs to induce initial avoidance, learned avoidance, discrimination from palatable alternatives and broad generalization against avian predators: naïve hand-reared and experienced wild-caught great tits, *Parus major*. Artificial baits were created by hollowing out bugs and inserting pieces of mealworm. Preference tests presented iridescent and orange baits simultaneously, then birds were divided into training groups and sequentially exposed to palatable black baits alternated with iridescent or orange baits made unpalatable by soaking mealworms in quinine solution. This was followed by simultaneous black/coloured discrimination tests, then a generalization test with both previously experienced and novel baits (all palatable). All groups showed a preference for orange baits over ones with iridescent patches. For wild-caught birds, attack latencies of iridescent and orange training groups were statistically indistinguishable, although only orange caused increased attack latency over the sequence. Hand-reared birds showed no change in attack rate/latency towards iridescent bugs over the sequence. In postlearning discrimination tests, all groups showed equally strong preference for palatable black baits and their unpalatable training baits. In generalization tests, hand-reared birds were most averse towards trained baits, but increased avoidance of iridescent-and-black baits suggests iridescence alone can contribute to aposematism. Wild-caught birds showed strong aversion to iridescent and novel orange-and-black baits regardless of training, suggesting birds may be broadly generalizing experience from local red-and-black aposematic bugs. Results suggest iridescent coloration and patterning can be an effective aposematic signal, especially in the presence of alternative palatable prey and/or other aposematic species.

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Aposematism is the phenomenon of prey advertising their unprofitability to potential predators through conspicuous and memorable signals that are often visual in nature (reviewed in Ruxton, Sherratt, & Speed, 2004). There is a rich literature on how predators, particularly birds, respond to visual aposematic signals and their individual components. Several studies have demonstrated that birds attend mostly to colour when learning to avoid aposematic prey (Exnerová et al., 2006; Ham, Ihalainen, Lindström, & Mappes, 2006; Svádová et al., 2009). Other studies have demonstrated the relative value of background contrast (Gamberale-Stille, 2001; Gamberale-Stille & Guilford, 2003), the relative value of internal contrast and patterning (Aronsson &

Gamberale-Stille, 2008, 2009; Hegna, Saporito, Gerow, & Donnelly, 2011), the importance of pattern regularity/symmetry (Forsman & Merilaita, 1999; Stevens, Castor-Perry, & Price, 2009) and the breadth/direction of generalization (Gamberale-Stille & Tullberg, 1999; Svádová et al., 2009) for aposematic signals. Theory and empirical studies also suggest that aposematic patterns tend towards signal uniformity (Greenwood, Cotton, & Wilson, 1989; Lindström, Alatalo, Lyytinen, & Mappes, 2001), and maximal conspicuousness or distinctiveness (Roper & Redston, 1987; Sherratt & Beatty, 2003), but instances of intraspecific variation and/or low conspicuousness are common (Stevens & Ruxton, 2012). Understanding the selection pressures upon these 'non-textbook' aposematic systems is currently an active area of research (Blount, Speed, Ruxton, & Stephens, 2009; Endler & Mappes, 2004; Lindstedt, Talsma, Ihalainen, Lindström, & Mappes, 2010; Speed & Ruxton, 2007).

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However, despite the breadth of the literature, some areas have received considerably less attention. For example, many authors cite the prevalence of red and yellow in aposematic signals (Endler & Mappes, 2004; Ruxton et al., 2004; Théry & Gomez, 2010), but no research has quantified this prevalence. Few authors have experimented with other colours. One paper showed ultraviolet cues are not effective in avoidance learning of great tits, *Parus major* (Lyytinen, Alatalo, Lindström, & Mappes, 2001). While there are few potentially aposematic species with blue components in their signals, seemingly none have been tested empirically (reviewed in Umbers, 2013), although artificial blue/cyan prey have been used successfully by Gamberale-Stille and Guilford (2003) and Aronsson and Gamberale-Stille (2008). The efficacy of green as a colour in avoidance learning is difficult to establish, as green or brown is often used as an 'inconspicuous' control in experiments. However, exposure to pyrazine odours (Rowe & Guilford, 1996) or the bitter taste of quinine (Rowe & Skelhorn, 2005) can cause unlearned biases against yellow or red but not green. However, there is at least one study showing green food as being more resistant to overcoming dietary conservatism than yellow or red (Marples, Roper, & Harper, 1998). Better understanding of the learning value of less common 'warning' colours such as green or blue requires more study.

Similarly to short wavelengths, iridescent colours in aposematism are an understudied phenomenon. By definition, the hue of iridescent colours changes with viewing angle, owing to the arrangement of multiple reflecting layers creating a coherent Bragg mirror (Seago, Brady, Vigneron, & Schultz, 2009). Additionally, small variations in the layer spacing can result in a large change in hue or saturation (Kurachi, Takaku, Komiya, & Hariyama, 2002). These aspects of iridescent coloration may affect their efficacy in providing a reliable aposematic signal. However, iridescent and other structural colours can be very bright (Seago et al., 2009), and are capable of creating short-wavelength hues such as blues and ultraviolets uncommon in pigments (Doucet & Meadows, 2009; Umbers, 2013). Paired with pigmentary colours, this ability to create chromatic and/or luminance contrast may enhance the conspicuous and distinctiveness of the aposematic signals (Doucet & Meadows, 2009; Endler, 1992). Therefore, there is great value in studying the role of iridescence in aposematic learning.

There are scattered studies documenting a role for iridescent colours in aposematic signals in beetles (Schultz, 2001) and butterflies (Bowers & Larin, 1989; Pegram, Lillo, & Rutowski, 2013; Rutowski, Nahm, & Macedonia, 2010), but none for true bugs. Iridescence is relatively common in some families of true bugs (Heteroptera), particularly in the assassin bugs (Reduviidae), plant bugs (Miridae), leaf-footed bugs (Coreidae), burrower bugs (Cydnidae), shield bugs (Pentatomidae and Scutelleridae) and some minor families (P. Štys, unpublished data). In many of them, iridescence is combined with yellow, orange or red pigment coloration producing a potentially aposematic effect. This type of coloration is sometimes limited only to larvae for poorly understood reasons (P. Štys, unpublished data).

Tectocoris diophthalmus (Heteroptera: Scutelleridae), the hibiscus harlequin bug, is an ideal study system to engage with questions of intraspecific variation and learning value of iridescent coloration. Rather than employing the more 'typical' aposematic colour scheme of red or yellow with black markings, *T. diophthalmus* bugs display a matte orange background overlaid with bright metallic blue-green iridescent patches. These patches are highly variable between individuals, ranging in size from almost covering the dorsal surface to being entirely absent (Fabricant, Kemp, Krajíček, Bosáková, & Herberstein, 2013). Like other true bugs, *T. diophthalmus* produces defensive secretions (Staddon, Thorne, & Knight, 1987), and has been demonstrated to be capable of inducing avoidance learning in chickens (Fabricant & Smith, 2014). The species lives in Australia and Malesian and Melanesian islands, and is arboreal and mainly phytophagous, feeding on *Hibiscus*, *Gossypium* and *Lagunaria* species (Malvaceae; Cassis & Gross, 2002).

The aim of this study was to test the efficacy of the iridescent coloration of *T. diophthalmus* in inducing initial aversion, avoidance learning and generalization. We tested this bug, a native to Australia, against European great tits, an allopatric species which has no experience with *T. diophthalmus* or any other iridescent species of true bug. We tested both wild-caught and hand-reared birds; hand-reared great tits are completely naïve to noxious prey, and have been shown in experiments to lack innate bias against red-and-black coloured bugs (Exnerová et al., 2007; Svádová et al., 2009), but wild-caught birds are likely to have experience with noniridescent true bugs, including local red-and-black aposematic species (Hotová Svádová, Exnerová, Kopečková, & Štys, 2010). We compared wild-caught and hand-reared birds with respect to their initial wariness towards palatable iridescent and noniridescent (orange) bugs, and in their behaviour towards unpalatable iridescent bugs across a learning sequence, discrimination tests and generalization tests using novel bugs sharing features of conditioned bugs. Because we could not control the previous experience of wild-caught birds, we also compared the behaviour of wild-caught birds exposed to unpalatable iridescent bugs with that of birds exposed to unpalatable orange bugs that lacked iridescent patches.

Our specific questions were as follows. (1) Do the iridescent patches of *T. diophthalmus* increase initial avoidance? (2) Are iridescent patches efficient in inducing avoidance learning and discrimination from palatable alternatives? (3) Do birds generalize broadly among different colour phenotypes of the bugs, including to more 'typical' orange-and-black bugs? (4) Do experienced and naïve birds differ in their patterns of initial avoidance, learning to avoid unpalatable iridescent prey, and generalization of novel bug phenotypes? By comparing the results of these experiments to previous studies, we aimed to elucidate what components of the visual warning signals of *T. diophthalmus* are most salient to birds, and how variation may affect their survival.

METHODS

Avian Predators

Great tits are small, predominantly insectivorous passerine birds inhabiting mostly woodlands, parks and gardens (Cramp & Perrins, 1993). They are frequently used as model predators in studies of warning signals and mimicry (Lindström, Alatalo, & Mappes, 1997; Sillén-Tullberg, Wiklund, & Järvi, 1982; Svádová et al., 2009) and are suitable for studies involving novel prey, because they are less neophobic than other European species of Paridae, and their avoidance of aposematic insects is mostly based on individual learning (Exnerová et al., 2007).

Hand-reared Birds

We used 20 hand-reared great tits as naïve predators, and all were trained only with iridescent bugs. Juvenile birds were taken from their nestboxes in spring 2012 when 12–16 days old. At this age the nestlings have no visual experience with their food. The nestboxes were placed in parks and orchards in the outskirts of Prague, Czech Republic. No more than two nestlings were taken from the same brood. The nestlings were transported to the laboratory in the same artificial nests they were housed in (plastic boxes for keeping small animals with textile and tissue-paper lining for insulation and comfort). The length of transport did not exceed 30 min. Nestlings were