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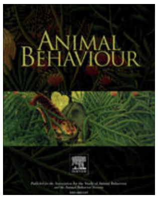

Role of different colours of aposematic insects in learning, memory and generalization of naïve bird predators

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Among the various properties of visual warning signals, colour seems to be especially important for avian predators. We tested the role of particular colours of an aposematic insect (firebug, *Pyrrhocoris apterus*; Heteroptera: Pyrrhocoridae) in unlearned avoidance, learning, memory and generalization of a naïve avian predator (great tit, *Parus major*). The wild type of the firebug is aposematic, red-and-black, and its colour mutants (white, yellow, orange) retain the same black pattern; the bug can be made artificially nonaposematic (painted uniformly brown). Wild-caught great tits avoid the firebug depending on colour, and their reaction to variously coloured prey is a result of avoidance learning and may vary according to their experience. We trained naïve great tits to avoid firebugs of different colours, and then gave some birds a memory test with firebugs of the same colour and other birds a generalization test with firebugs of a different colour. Naïve, hand-reared great tits showed no initial avoidance and attacked firebugs irrespective of colour. They learned to avoid all the colour forms at a similar rate. The generalization was asymmetric: birds that learned to avoid red firebugs did not generalize their experience to yellow or white mutants whereas birds that learned to avoid yellow mutants generalized their experience to red firebugs. The red colour thus represents a more effective signal than the yellow; predation by birds could have played a crucial role in selectively favoured evolutionary transitions from yellow to red coloration in pyrrhocorids.

Aposematic animals advertise their defensive mechanisms to predators by conspicuous signals (Poulton 1890; Ruxton et al. 2004). Some predators possess innate biases against conspicuous signals of aposematic prey such as specific colours and odours (Smith 1975; Schuler & Hesse 1985; Roper 1990; Rowe & Guilford 1996).

Predators with no innate responses to warning signals have to learn the association between the warning signal (conditioned stimulus) and unpalatability (unconditioned stimulus) during encounters with aposematic prey (Järvi et al. 1981; Sillén-Tullberg 1985a; Cardoso 1997; Exnerová et al. 2007). To be effective, aposematic signals should (1) accelerate avoidance learning, (2) prevent or delay forgetting, and (3) facilitate accurate recognition of the prey (Ruxton et al. 2004).

Avoidance learning can be enhanced by a specific quality of the warning signal, such as odour (Rowe & Guilford 1996; Roper & Marples 1997), taste (Hilker & Köpf 1994; Gill et al. 1998), sound (Rowe 2002), size (Marples 1993; Gamberale-Stille 2000; Riipi et al. 2001), pattern symmetry (Forsman & Merilaita 1999), and especially coloration (Sillén-Tullberg 1985a; Roper & Redston 1987; Avery & Nelms 1990; Marples et al. 1994; Lindström et al. 1999b; Gamberale-Stille & Guilford 2003). Retention of the signal in the predator's memory may be influenced by prey coloration (Roper & Redston 1987; Roper 1994; but see Ham et al. 2006) or by the presence of specific odours (Avery & Nelms 1990; Roper & Marples 1997). Surprisingly, studies focused on those features of aposematic signals that enhance the signal memorability are rare (Ruxton et al. 2004).

Once the learning process is completed, particular features of aposematic prey may facilitate its recognition by the predator (Guilford 1986; Gamberale-Stille 2001) or may influence the scope

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and direction of generalization (Ruxton et al. 2004). Generalization is a response to a particular novel stimulus resulting from previous experience with another stimulus (Lieberman 2000). The ability of predators to generalize their experience with aposematic prey is important for the evolution of warning signals and mimetic associations (Leimar et al. 1986; Yachi & Higashi 1998; Balogh & Leimar 2005; ten Cate & Rowe 2007). Generalization may be narrow or broad; the scope is predicted to depend on the strength of the previous negative experience with the aposematic prey (Lindström et al. 1997) and on the number of prey species involved (Beatty et al. 2004). Cases of both narrow (e.g. Sillén-Tullberg et al. 1982) and broad (e.g. Evans et al. 1987) generalization have been demonstrated in experiments with living aposematic prey. The generalization gradient may be asymmetrical, that is, generalization is easier in one direction of the stimulus dimension than in the opposite one. Such asymmetry is usually considered to result from the peak shift: a product of discrimination learning in which the peak of response is shifted away from the training stimulus (Spence 1937). There is growing evidence that some features of aposematic signals may be generalized asymmetrically. Domestic chicks, *Gallus gallus domesticus*, generalized their experience with larvae of aposematic heteropterans towards larger, older instars (Gamberale & Tullberg 1996), larger groups of individuals (Gamberale & Tullberg 1998) and species with more intensive red coloration (Gamberale-Stille & Tullberg 1999) than the prey they learned to avoid. Similarly, great tits, *Parus major*, that were trained to avoid artificial food with various visual markings generalized their experience towards more conspicuous items (Lindström et al. 1999b). On the other hand, when trained to associate visual signals with a positive unconditioned stimulus (food), chickens either remembered the particular colour precisely (Osorio et al. 1999) or generalized towards a colour intermediate between the two different colours they were trained to prefer (Baddeley et al. 2001; Jones et al. 2001). It is possible that rules for generalization of positive and negative signals may be different.

Warning signals may be multimodal, that is, they may consist of any combination of visual, olfactory, gustatory, acoustic or behavioural components which reinforce themselves or act synergistically (Rowe & Guilford 1999), but for avian predators coloration is considered to be an essential component of multimodal signals (Sillén-Tullberg 1985b; Roper 1990; Marples et al. 1994; Rowe & Guilford 1996). It is not clear which aspects of visual warning signals (colour, pattern, contrast between differently coloured body parts, contrast against the background) are most important for learning and memory; however, the colour itself plays an important role (Sillén-Tullberg 1985a, b; Gamberale-Stille & Guilford 2003; Exnerová et al. 2006). Most studies have focused on comparison of typical warning colours (red, orange, yellow and white) with a nonwarning one (e.g. Sillén-Tullberg 1985a; Roper & Redston 1987; Roper 1990; Lindström et al. 1999a; Rowe & Guilford 1999; Exnerová et al. 2003; Gamberale-Stille & Guilford 2003) whereas studies comparing reactions of birds to different warning colours are scarce. Exnerová et al. (2006) compared reactions of wild-caught birds to yellow, orange and red aposematic insects while Ham et al. (2006) did the same with artificial prey items. Wild-caught great tits readily avoided red-and-black individuals of the firebug, *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae; Exnerová et al. 2003, 2006) and were able to generalize their experience with red or yellow unpalatable prey to novel orange prey (Exnerová et al. 2006; Ham et al. 2006). However, the behaviour of wild-caught birds in the experiments may have been affected by their previous experience.

In this study we tested (1) the effect of different warning colours on the rate of predator avoidance learning and on the memorability of the learned warning signal, and (2) the ability of predators to generalize different warning colours. More specifically, we investigated the response of naïve (hand-reared) great tits lacking any previous experience to aposematic prey. The effectiveness of different warning colours was compared in live insect prey, the adult red-and-black, wild-type firebugs versus their laboratory-born, yellow and white mutants sharing the black pattern with the wild type (Exnerová et al. 2006), and with brown-painted firebugs whose brown colour is not aposematic (Exnerová et al. 2003). Birds were trained to avoid firebugs of a particular colour to assess whether the rates of avoidance learning are similar for different warning colours and more rapid than for the brown colour. We gave some birds a memory test with firebugs of the same colour they had learned to avoid. This memory test showed whether the birds had remembered their experiences equally well. Other birds had a generalization test with firebugs coloured differently from those they had learned to avoid. Using this test we could detect (1) whether the birds generalized different warning colours, and if they did, (2) whether the generalization was symmetrical.

METHODS

Prey

Brachypterous adults of the red-and-black, wild-type firebug and the white and yellow mutants of this species were used as the warningly coloured, prey. These firebugs are gregarious and phytophagous, warningly coloured and widely distributed in Europe; they are unpalatable for small passerines (Exnerová et al. 2003). The warning coloration of the wild type is formed by red erythropterin-based epidermal pigmentation and a black cuticular melanin pattern. Mutants differ from the wild type only in the composition of the epidermal pteridines. The yellow colour of *yellow* mutants results from a low amount of erythropterin and the presence of xanthopterin; the white colour of *albinotic* mutants is produced by the absence of erythropterin and the presence of xanthopterin and leucopterin (Socha & Němec 1992; Bel et al. 1997). Other characteristics (e.g. size, body shape, locomotion, black melanin pattern) are the same as in the wild type. The defensive secretion produced in the metathoracic glands of wild-type adults contains 35 chemicals, mainly short-chain aldehydes (Farine et al. 1992); the secretions of albinotic and yellow mutants are very similar to those of the wild type (J.-P. Farine, A. Exnerová, P. Štys & R. Socha, unpublished data). For more details about the colour mutants, their occasional occurrence in the wild and the origins of laboratory strains see Exnerová et al. (2006). 'Non-aposematic' firebugs were obtained by painting the wild-type firebugs with dark brown watercolour dye and chalk (as in Exnerová et al. 2003) to cover the original colour and pattern. This treatment did not impair the firebugs' chemical defence; painted individuals were able to release the secretion as well as the non-painted ones. According to our pilot tests with painted house crickets, *Acheta domestica*, the dye used is not aversive to birds in any way. The colour forms are henceforth referred to as the red wild type/form, yellow and white form/mutant and brown (-painted) form.

The wild-type firebugs were collected in the field (populations feeding on small-leaved lime trees, *Tilia cordata*) in Prague, Czech Republic. White and yellow mutants came from strains reared for several years in the laboratory (by R. Socha). All experimental firebugs were reared on linden seeds (*T. cordata*) and water ad libitum, under a long-day photoperiod 18:6 h light:dark, at a temperature of 26 ± 1 °C. Mealworms (larvae of *Tenebrio molitor*) were used as a familiar and palatable control prey to check the foraging motivation of birds during the experiments.

Reflectance spectra of all colour forms of the firebug (Fig. 1) were measured using equipment that allowed for measurements of reflectance in the UV and visible wavelengths from very small areas