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Naïve predators and selection for rare conspicuous defended prey: the initial evolution of aposematism revisited

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Many defended prey advertise their defences to potential predators by being conspicuously coloured and/or having distinct bold markings. These colourful warning signals benefit prey since they are easy to learn to avoid, and seem particularly effective as deterrents. However, the evolution of conspicuous coloration is problematic, because if a distinct conspicuous morph arises in a cryptic species, it would be more easily detected and the risk of being attacked would be high. However, this argument assumes that attack probability will be the only factor determining prey survival, but in fact many prey have external defences that predators can detect, allowing prey to survive attacks. This experiment tests whether a rare conspicuously coloured defended morph can have a selective advantage over a cryptic defended morph when defence chemicals can be detected before ingestion by a naïve predator. Using chicks, *Gallus gallus domesticus*, foraging on coloured chick crumbs, we found that naïve birds can learn to avoid defended prey when they are conspicuous but not when they are cryptic. Crucially, when prey ingestion, and not attack probability, was used as our measure of mortality we found that there can be a selective advantage to being conspicuous in a cryptic population. This advantage can occur even in the first avoidance-learning trial, providing an initial selective advantage for rare conspicuous defended morphs. However, this does not occur in all conspicuously coloured prey populations, and it is evident that colour biases are important and their role in the initial evolution of conspicuous colour signals should be considered.

Keywords: aposematism; avoidance-learning; *Gallus gallus domesticus*; predation; receiver psychology; warning signal

Aposematic prey possess antipredatory defences, such as toxins and stings, and advertise them to predators using distinctive conspicuous coloration (Cott 1940; Friedlander 1976; Atkins 1980; Guilford 1988). The conspicuous coloration acts as a warning signal, and seems to be particularly salient, allowing predators to associate the coloration with the defences and avoid aposematic prey before attack (e.g. Gittleman & Harvey 1980; Alatalo & Mappes 1996). In addition, naïve predators can also show an unlearned avoidance of prey that are warningly coloured. Domestic chicks, *Gallus gallus domesticus*, are often used as a model species to look at such unlearned colour biases, and they are known to be not only biased against red and yellow prey, but also against conspicuous prey per se (Smith 1975; Roper & Cook 1989; Roper & Marples 1996; Rowe & Guilford 1996; Lindström et al. 1999, 2001b; Exnerova et al. 2006). Notably, some unlearned biases are only evident when they are used in combination with cues from other sensory modalities (Rowe & Guilford 1996; Rowe & Guilford 1999a, b; Rowe & Skelhorn 2005). Colour biases are also found in hand-reared and wild-caught birds, for example, the avoidance of red and yellow striped prey (Smith 1975) as well as red and black firebugs (Exnerova et al. 2006). The protective benefits of warning coloration against modern-day predators are clear, but identifying the initial benefits to evolving conspicuous coloration has been more problematic.

The key question is how can a conspicuous morph evolve in a cryptic population? Being conspicuous increases the probability of being discovered by a predator, and since conspicuous prey will also initially be rare, predators will not be able to learn to avoid the colour signal, and the risk of extinction will be high. There are three proposed evolutionary scenarios for the evolution of

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aposematism: (1) defences evolved before the conspicuous coloration; (2) conspicuous coloration evolved first and then prey evolved defences; and (3) coloration and defences evolved simultaneously (Guilford 1988; Marples et al. 2005). All three pathways involve cryptic prey losing their ability to hide from predators, the detection costs of which have been well documented (Dawkins 1971; Gittleman & Harvey 1980; Malcolm 1986; Alatalo & Mappes 1996; Lindström et al. 2001a; Riipi et al. 2001). Because of this, the evolution of conspicuous coloration is thought to have most probably arisen in an already defended population (Marples et al. 2005). In addition, there are many insect species that are known to invest in defences but remain cryptic. A recent model by Broom et al (2006) predicts several evolutionary stable strategies (ESSs) whereby this could occur, partly because these prey have a greater chance of surviving a predatory attack (Broom et al. 2006) and so gain an advantage over undefended cryptic prey. It has also been shown experimentally that avian predators can discriminate between cryptic prey based on taste and that they reject cryptic prey more often when they are defended than when they are undefended. Thus there seems to be a selective advantage for a cryptic prey to be defended (Skelhorn & Rowe 2006c).

Solutions to overcome the initial detection problem have been sought by considering the role of prey aggregations and kinship groups (Alatalo & Mappes 1996; Speed 2001; Lindström et al. 2004; Despland & Simpson 2005), the possibility of gradual change from cryptic to conspicuous coloration (Lindström et al. 1999, 2001b), the idea that aposematic prey might survive attacks (Wiklund & Järvi 1982; Sillén-Tullberg 1985), or that dietary conservatism and visual neophobia in educated predators might allow conspicuous coloration to evolve (Marples et al. 1998; Speed 2001; Thomas et al. 2003, 2004). Curiously, despite this interest in the initial evolution of conspicuous coloration, there has been no direct test of whether a rare conspicuous morph arising in an already defended cryptic population can actually enjoy better survival than its cryptic and more abundant conspecifics.

Alatalo & Mappes (1996) showed that aposematic defended morphs have an initially higher attack rate than cryptically defended morphs, and that avian predators only learnt to avoid defended prey when they were distinctively patterned and conspicuous. Although there has been criticism of this paper (Tullberg et al. 2000), it stands as the only experimental test comparing the survival of defended cryptic and conspicuous morphs (Alatalo & Mappes 1996, 2000). However, in their experiment, the defended cryptic and conspicuous prey occurred with equal frequency, which potentially risks overinflating the initial costs of being conspicuous by increasing their encounter rate. But perhaps more importantly, mortality was measured as the number of attacks made by predators on each type. This does not take into account the fact that many prey secrete their defences (e.g. Eisner & Meinwald 1966; Pasteels et al. 1983; de Jong et al. 1991), and that avian predators are able to taste prey and discriminate among defended prey on the basis of their level of defence (Wiklund & Järvi 1982; Sillén-Tullberg 1985; Gamberale-Stille & Guilford 2004; Skelhorn & Rowe 2006a, c). Therefore, the predatory behaviour of birds foraging on rare conspicuous prey with externally detectable toxins may provide a route by which costly conspicuous signals might evolve by individual selection as a prey could survive an attack if it is rejected after being tasted (e.g. Sillén-Tullberg 1985).

This study tests the hypothesis that the psychology of birds offers a selective advantage to a rare defended conspicuous prey-type over an equally defended cryptic form. Frequency-dependent selection can occur in prey selection by predators, where they preferentially predate the rare morph (antiapostatic selection: e.g. Lindström et al. 2001a) or the more common morph (apostatic selection: e.g. Skelhorn & Rowe 2006b). We currently do not know how birds differentially predate rare aposematic morphs in comparison with more common cryptic conspecifics when defence chemicals are externally detectable (Lindström 1999). We used newly hatched domestic chicks, *Gallus gallus domesticus*, as naïve predators, foraging on artificial prey-types to test how rare conspicuous morphs might enjoy a survival advantage when predators can detect defences before ingestion. This gave us the opportunity to control the conspicuousness and the defence levels of prey, and also to know the foraging history of the predators. By measuring the numbers of prey attacked and ingested, we could specifically look for a survival advantage for conspicuous defended prey in this evolutionary scenario.

EXPERIMENT 1: CAN RARE CONSPICUOUS MORPHS HAVE A SELECTIVE ADVANTAGE OVER CRYPTIC CONSPECIFICS?

We modelled the situation where a conspicuous morph arises at a low frequency in a population of cryptic prey that is already defended, and where predators can detect defences before ingestion. By measuring the attacks made on each prey-type, as well as the numbers of each type consumed, we could explicitly look for a survival advantage for conspicuous defended prey in this evolutionary scenario.

Methods

Experimental subjects and housing

Fifty-five day-old domestic chicks, *G. gallus domesticus*, of either sex were hatched in the laboratory and housed in cages measuring $100 \times 50 \times 50$ cm and maintained at a room temperature of 25°C. They were kept on a constant 14:10 h light:dark cycle, using fluorescent lights with no UV component. Water was available ad libitum, as were unmanipulated (brown) chick starter crumbs, except during specific experimental periods when food deprivation was necessary (see below). All chicks were marked with nontoxic marker pens on day 1 to make them easily identifiable. This did not appear to affect the chicks in any way and they did not attempt to peck at other chicks' markings. They were also weighed every day throughout the experiment so that they could be monitored for