Behavioural ecology

An evolutionary route to warning coloration

Tim Caro

Bright colours that signal toxicity can deter predators, but how such colours initially evolve without first endangering conspicuous organisms is a contentious issue. Analysis of amphibians offers an answer to the puzzle.

Understanding how warning colours first evolved has long vexed scientists. Writing in *Science*, Loeffler-Henry *et al.*[1] present data that indicate a hitherto unknown mechanism underlying this phenomenon.

In 1867, Charles Darwin wrote to Alfred Russel Wallace, the co-discoverer of the theory of natural selection, seeking an answer to the question, "why are caterpillars sometimes so beautifully and artistically coloured?" (see go.nature.com/4tdeepk). Conspicuous colourful larvae were a problem for Darwin because the colours of immature animals could not be explained by his theory of sexual selection ("the advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction")[2], given that juveniles don't reproduce.

Wallace realized that to avoid predator attacks, prey with secondary defences (those that are usually used during, or just before, contact between prey and predator) might need traits that help predators to distinguish them from edible prey. Addressing Darwin's caterpillar question, he wrote: "Now supposing that others ... are protected by a disagreeable taste or odour, it would be a positive advantage to them never to be mistaken for any of the palatable catterpillars, because a slight wound such as would be caused by a peck of a bird's bill almost always I believe kills a growing catterpillar. Any gaudy & conspicuous colour therefore, that would plainly distinguish them from the brown & green eatable catterpillars, would enable birds to recognise them easily as a kind not fit for food, & thus they would escape seizure which is as bad as being eaten" (see go.nature.com/3tir8pd).

Darwin was pleased with Wallace's explanation, remarking, "I never heard any thing more ingenious than your suggestion & I hope that you may be able to prove it true" (see go.nature.com/3kttvgb). Wallace incorporated warning as one of the major categories in his classification of coloration[3], although it was his contemporary Edward Poulton who formalized the concept and named it aposematism, defining[4] it as "an appearance which warns off enemies because it denotes something unpleasant or dangerous".

Many species have conspicuous coloration that accurately advertises a defence or other characteristics enabling them to escape predation[5] – but there is an outstanding problem in explaining how this arises. How can a conspicuous form initially evolve in a cryptic (camouflaged) population? Being conspicuous increases the probability of being discovered by a predator, and because conspicuous prey will initially be rare, predators will not learn to avoid the colour signal, and thus the risk of extinction of the conspicuous form will be high.

The geneticist Ronald Fisher recognized this paradox[6] back in 1930. His solution was that aposematic prey often live 'gregariously' in social groups, so a predator might sample an unpalatable caterpillar and then avoid all others nearby that would probably be from the same clutch of eggs. Yet subsequent analysis (by phylogenetic reconstructions of family trees) of the ancestral states of conspicuous and social caterpillars, for example, shows this could not be true. Warning coloration always evolved in solitary species, not in gregarious ones, but gregariousness evolved in species with either aposematic or cryptic larvae[7].

A second solution is that conspicuousness evolves gradually. However, experiments with artificial prey and great tits show that predators do not learn to avoid more-exaggerated conspicuous signals after being trained on a less-conspicuous prey item[8]. A third possibility, that aposematic prey survive attacks, has more traction. When hand-reared (naive) birds were given five types of aposematic insect prey, the overwhelming majority of prey survived, either being dropped unharmed or losing only a leg[9].

A fourth possibility is that predators are simply fearful of trying prey that have an unusual appearance. There is some evidence for this, although predators vary hugely in their pickiness[10]. Now there is a fifth, completely new solution.

Loeffler-Henry and colleagues considered the fact that some cryptic animals, when in competition with other members of their species or when under predatory threat, display a bright colour patch, but only briefly (Fig. 1). In an imaginative leap across various subdisciplines, the authors reasoned that species with conspicuous lower (ventral) surfaces but cryptic upper (dorsal) surfaces might provide a pathway through which full-blown conspicuousness could evolve.

Focusing on frogs, toads and salamanders,

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Figure 1 | A yellow-bellied toad (*Bombina variegata*). This toxic amphibian has a camouflaged upper surface that hides the toad from predators. Its underside is conspicuous, and is revealed when the animal adopts a defensive posture in the face of imminent attack. This response to predators is termed the unken reflex.

some of which are toxic, such as yellow-bellied toads (*Bombina variegata*), California newts (*Taricha torosa*) and poison dart frogs (Dendrobatidae family), the authors mapped a phylogenetic tree of species that had a range of anti-predator characteristics: fully cryptic species; fully conspicuous species; species with conspicuous coloration present as small patches on their ventral surfaces (PV); species with a fully conspicuous ventral surface (FV); and species that had both cryptic and conspicuous forms (polymorphic species). Using two data sets, a large one that lacked information on chemical defences, and a smaller one that included them, the authors uncovered a plethora of evolutionary relationships. What seems to be key to the origin of aposematism is that amphibians with hidden colour signals, specifically the chemically defended FV state, are probably the most important evolutionary precursor of the aposematic conspicuous defended state.

In turn, the FV defended state arises from the PV defended state or the undefended FV state, and these FV species themselves evolve from the undefended PV state. The authors report that around 90% of the conspicuous, FV conspicuous or polymorphic species that they analysed are chemically defended, and this is also true for a good proportion of PV conspicuous and cryptic species. This indicates that amphibians are honest signallers rather than species that 'cheat' by mimicking warning colours in the absence of defences. The authors also found evidence that aposematic species can evolve back to cryptic or polymorphic species, mirroring the surprising evolutionary flexibility seen in transitions between mimicry and crypticity in coral snakes[11], an observation that questions the idea that stable evolutionary end points exist.

Remarkably, scientists already knew about mix-and-match cryptic–conspicuous forms of protective coloration in three other contexts. One of these is deimatism, in which cryptic prey briefly flash a hidden conspicuous patch to cause the predator to hesitate — such prey might or might not have chemical defences[12]. Another is flash coloration, whereby prey expose conspicuous patches while fleeing but hide them as soon as they come to rest, causing the predator to search for an inappropriate object[13]. The third is distance-dependent camouflage, in which defended prey are cryptic far off but conspicuous up close[14].

However, none of these three examples was used to solve the aposematism paradox until now. With a new solution at hand, namely that aposematism can evolve without loss of crypsis, it is essential to examine how widespread the phenomenon is by investigating other groups of species with 'dangerous' reputations, such as sea slugs and snakes. Once again, Wallace has led the way, and we mortals simply follow on behind.

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Bioinorganic chemistry

Protein discerns between rare-earth elements

Scott Banta

A protein has been discovered that binds to the lighter members of the rare-earth family of metals more strongly than to the heavier ones — an amazing feat, given the chemical similarities of these elements. **See p.87**

The rare-earth elements (REEs) are indispensable for emerging technologies, yet their chemical similarities make them notoriously difficult to separate from each other. Some specialized microorganisms require REEs for growth, and have evolved biological machinery to process them[1]. On page 87, Mattocks *et al.*[2] report their discovery of a rare-earth-binding protein that shows unrivalled selectivity in its affinity for members of this family of elements. The finding paves the way to the development of bioseparation strategies for isolating individual REEs.

The 17 REEs consist of the lanthanide group (lanthanum to lutetium in the periodic table), as well as scandium and yttrium. The distinctive properties of these elements make them essential for many modern applications, including magnets, batteries, electronics and catalysts[3], and global demand is therefore expected to increase. The lanthanide elements predominantly form ions that carry three positive charges and have similar radii, which decrease with increasing atomic weight. The chemical similarities of the REEs cause them to co-localize in geological deposits, and also complicate their separation from each other. Industrial REE separations are challenging: organic molecules (ligands) are reacted with mixtures of REE ions in acid solution to form complexes, which are then subjected to multiple extraction steps in which the complexes of specific REEs are transferred selectively to a suitable organic solvent[4].

The term 'rare' can be a misnomer for REEs, because most of these elements (other than radioactive promethium) are more abundant in Earth's upper continental crust than is silver (see go.nature.com/3mkkhug). Indeed, lanthanum, neodymium and cerium are about as abundant as copper and nickel, which are not regarded as rare. But REEs can certainly be thought of as rare in the context of proteins — almost half of the protein structures in the Protein Data Bank contain metals, and very few of these are REEs.

However, over the past 12 years or so, REEs have been found to be essential for the biochemistry of bacteria known as methylotrophs[1]. These organisms can use organic compounds that contain just one carbon atom as carbon sources for growth — a talent that requires special metabolic capabilities. The incorporation of REEs into methylotroph proteins probably provides catalytic advantages that aid this distinctive biochemistry, to the extent that some methylotrophs are incapable of growth in the absence of REEs[5]. Investigation of the metabolic capabilities of methylotrophs led to the discovery of the protein lanmodulin in the bacterium *Methylobacterium extorquens*[6]; the protein is probably involved in regulating REE concentrations in this organism.

Lanmodulin is small, consisting of about 112 amino-acid residues, and is unstructured in the absence of REEs. It contains four EF-hand motifs — the amino-acid sequences that are responsible for the binding of calcium ions in many proteins. Workers from the