*Review*

# Linking the evolution and form of warning coloration in nature

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Many animals are toxic or unpalatable and signal this to predators with warning signals (aposematism). Aposematic appearance has long been a classical system to study predator–prey interactions, communication and signalling, and animal behaviour and learning. The area has received considerable empirical and theoretical investigation. However, most research has centred on understanding the initial evolution of aposematism, despite the fact that these studies often tell us little about the form and diversity of real warning signals in nature. In contrast, less attention has been given to the mechanistic basis of aposematic markings; that is, 'what makes an effective warning signal?', and the efficacy of warning signals has been neglected. Furthermore, unlike other areas of adaptive coloration research (such as camouflage and mate choice), studies of warning coloration have often been slow to address predator vision and psychology. Here, we review the current understanding of warning signal form, with an aim to comprehend the diversity of warning signals in nature. We present hypotheses and suggestions for future work regarding our current understanding of several inter-related questions covering the form of warning signals and their relationship with predator vision, learning, and links to broader issues in evolutionary ecology such as mate choice and speciation.

**Keywords:** aposematism; predation; signal; defensive coloration; vision

## 1. INTRODUCTION

Many animals are toxic, unpalatable, or otherwise unprofitable, and advertise this to predators with conspicuous and/or distinctive warning signals (aposematism [1,2]). Aposematic appearance has been a classical system to study evolution and adaptation for over 150 years, and has received considerable empirical and theoretical investigation. However, research has centred on understanding the initial evolution of aposematism within an originally cryptic population (for which there are a wide range of non-mutually exclusive explanations [1,2]), but these studies generally tell us little about the form and diversity of real warning signals. In contrast, less attention has been given to the mechanistic basis of aposematic markings; that is, 'what makes an effective warning signal?'. As Rowe & Skelhorn [3] argue, studies of animal communication have been dominated by issues of signal cost and reliability, and have largely overlooked psychological mechanisms, which can explain the specific make-up of the vast range of signals that exist [4]. Furthermore, unlike some other areas of adaptive coloration research (such as camouflage and mate choice), studies of warning coloration have been slow to explicitly consider the role of predator vision (and how it will often differ substantially from human vision).

Here, we review current understanding of warning signal form, with an aim to understand the diversity of warning signals in nature. We focus on the following inter-related key issues:

— What makes an effective warning signal in different habitats and contexts?
— Why are some warning signals apparently only moderately conspicuous?
— Why do some aposematic species have intraspecific variation and polymorphism?
— How do other selection pressures (e.g. camouflage, mate choice) influence warning signal form and evolution?

## 2. WHAT MAKES AN EFFECTIVE WARNING SIGNAL?

Signals can be thought of as having a strategic aspect (the information content or 'message') and an efficacy aspect (the form or evolutionary 'design' of the signal) [4]. Signal efficacy relates to the way that a signal is structured in order to effectively influence the response of the receiver under different environmental conditions and constraints. For warning signals to be effective, they need to promote initial avoidance and/or avoidance learning in predators; and strategic and efficacy aspects are important in this. Here, we consider what makes an effective warning signal in terms of efficacy.

### (a) Conspicuousness and contrast

Conspicuousness has long been considered a key aspect of aposematism, and can be both a function of internal contrast of the markings within the body coloration, and contrast of the animal's coloration with the background [5,6]. Conspicuousness may also allow a predator to

detect aposematic prey at a greater distance, perhaps reducing the likelihood of recognition errors [7].

Early experiments showed that non-cryptic colours and patterns promote both unlearnt avoidance and enhance avoidance learning in birds (see review by Ruxton *et al.* [2]), and work with domestic chicks (*Gallus gallus*) found that colour associations seem to be more readily learned than achromatic associations [8], whereas textural discrimination of small objects seems to be mediated primarily by luminance information [9]. Luminance contrast may primarily promote initial avoidance, owing to heightened detection, whereas specific colours (red and yellow) and colour contrast enhances avoidance learning. However, only limited work has tested this with properly controlled stimuli (in terms of predator vision). In addition, work with mantids as predators and milkweed bugs (*Oncopeltus fasciatus*) as prey indicate that achromatic contrast can be important in both the speed and persistence of aversion learning [6]. Most experiments have been carried out under laboratory conditions and we should also consider the visual environment. While achromatic signals may allow high contrast, colour signals may be particularly effective in heterogeneous or changeable environments because chromatic appearance may be relatively resistant to the effects of shadows and illumination changes, owing to visual processes such as colour constancy [10]. Under variable light conditions and heterogeneous environments luminance may become less reliable in avoidance learning and memory.

Given that many animals are thought to have red–green opponent processing mechanisms in their colour vision, this should make longwave (LW)-rich prey colours (e.g. reds and yellows) highly contrasting against many foliage backgrounds. Shortwave (SW)- and ultraviolet (UV)-rich colours are found in some aposematic species, but seemingly relatively infrequently. This may be because shortwave light is scattered more than longer wavelengths, making shadows appear 'blue' (to humans), so that such colours blend in rather than stand out. Although few controlled experiments exist, one study with great tits (*Parus major*) found no evidence that the presence of UV wavelengths would enhance either initial or learnt avoidance of other colours [11], and subsequent field experiments reveal increased predation in moths with UV reflectance compared with moths lacking UV [12]. Separating the role of specific colour types as opposed to contrast is challenging and most experiments looking at contrast have used categories of colour, such as green or brown (low contrast) or red/yellow (high contrast) against green/brown backgrounds. However, these are not testing contrast alone, but rather classes of colour, and work shows that birds group objects into different colour categories (such as 'oranges' and 'blues' to humans [13]). This has implications for the way that predators may categorize groups of aposematic prey based on appearance, and indicates that more than just visual contrast/distinctiveness may affect predator avoidance decisions.

Finally, few experiments have successfully manipulated conspicuousness without altering colour [14], and, in general, findings about prey generalization have been contradictory. This may be because stimuli in learning and memory experiments have rarely been designed with respect to predator vision, making it hard to know how different the appearance of prey types presented are to the relevant non-human predators. Experiments are needed holding colour constant but manipulating contrast. There is good evidence showing that contrast of the overall prey coloration with the background is important in aposematism (reviewed by Ruxton *et al.* [2]), and some recent evidence in studies with chicks suggest that contrast with the background is more important than contrast within the prey [15]. However, most experiments investigating internal contrast simply produce prey with and without patterns (e.g. uniform or striped) and are perhaps better thought of as a test as to whether pattern itself is important or not. A key design that has rarely been implemented is where the pattern is the same but the level of contrast varies in steps that are tightly quantified with respect to predator vision.

## (b) *Pattern*

Relatively little work has been conducted into whether some types of pattern are better than others at enhancing warning signals. Many warningly coloured prey have markings comprising repeated pattern elements. Such arrangements in signal structure may increase redundancy in the signal but improve the likelihood that the strategic component will be detected by the receiver. In addition, repeated elements may be rare in many natural environments, thus increasing conspicuousness of the prey animal. Simple pattern components (such as stripes and spots) may facilitate detection and also speed up avoidance learning if they are easier to memorize. It would be interesting to test initial avoidance and learning when presenting predators with simple and complex pattern types.

Aronsson & Gamberale-Stille [16] performed experiments with domestic chicks to test the roles of colour and pattern (simple stripes or spots) in avoidance learning and generalization. They found that chicks attended more to colour than pattern, in that during generalization trials chicks would generalize from the stimulus they had learnt to avoid to a new one based on colour similarity, but not for pattern similarity. In other systems, patterns may make effective aposematic signals. Work with Plasticine models of venomous snakes indicates that although their characteristic zig-zag markings do not appear especially conspicuous, they are distinctive enough to promote avoidance behaviour by predators [17,18]. This work has compared different pattern arrangements, such as zig-zags, stripes and nominally disruptive camouflage. However, the experimental stimuli have not yet been related to predator vision or the composition of the background. Studies with dragonflies as predators show that they avoid potential prey with wasp-like black and yellow stripes more than either uniform black or uniform yellow, indicating that pattern is important [19]. However, this does not reveal whether it is the contrast of black and yellow, or the stripes themselves that is most important. In other work with chicks, yellow coloration increased avoidance in inexperienced chicks, but there was no difference between yellow prey with or without wasp-like stripes [20]. However, green prey were avoided more when presented with stripes than without, indicating that striped patterns can increase avoidance when coupled with colours not normally associated with aposematism. In addition, stripes but not colour seemed most important in increasing the speed of avoidance
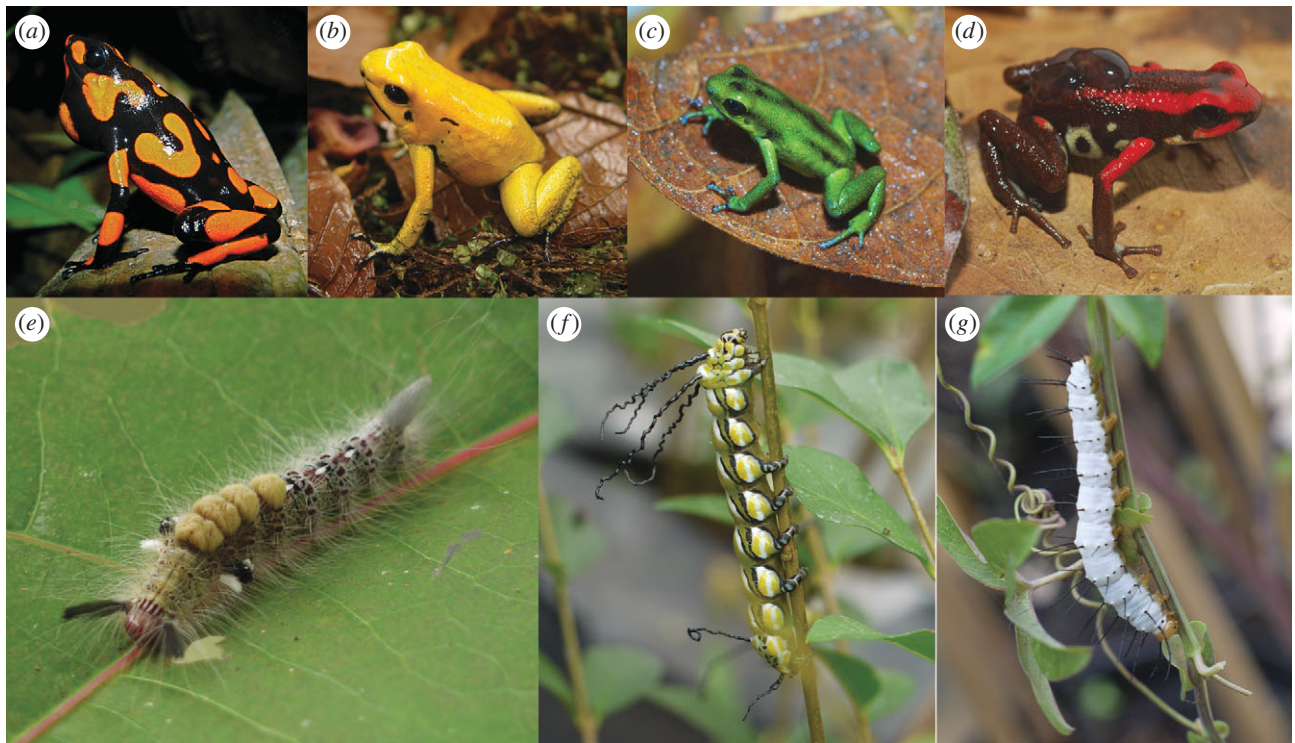
Figure 1. Examples of the diversity of warning signals that exist in two groups, poison frogs and lepidopteran larvae. (*a*) *Oophaga histrionica*, (*b*) *Phyllobates terribilis*, (*c*) *Ranitomeya fulgurita*, (*d*) *Ranitomeya bombetes*, (*e*–*g*) unknown species. (*a,b*) Reproduced with permission from © Adolfo Amezquita; (*c,d*) © Fernando Vargas; (*e*–*g*) © Martin Stevens.

learning, possibly if stripes were more memorable [20]. Therefore, different components of pattern and colour could enhance aspects of initial and learnt avoidance and may also be context-dependent.

One aspect of marking arrangement that has been predicted to influence warning signal efficacy is pattern symmetry, which may enable animals to recognize objects from different positions and orientations. Aviary studies with chicks have shown that birds learn to avoid unpalatable prey more quickly when the prey had symmetrical as opposed to asymmetrically sized markings [21]. Furthermore, chicks' unlearned avoidance of palatable artificial prey was stronger when the prey had markings symmetrical for size, shape and colour [22]. However, the experiments presented prey simultaneously in pairs, whereas most prey encounters in nature will be sequential, and predators will not be able to directly compare and choose between two or more prey individuals. Instead, a predator must decide whether to attack a prey item at all or leave unrewarded. Recent field studies with artificial prey with a pair of markings that were either symmetric or asymmetric for size, shape and placement on the body, found no survival advantage of symmetric over asymmetric markings [23]. The majority of animal markings are symmetric, but perhaps the most parsimonious explanation is that this reflects genetic and developmental constraints rather than an underlying signalling function.

organism adopts an appearance that enhances its distinctiveness to predators from undefended species, that appearance is likely to be conspicuous. This suggestion is not new but has recently been supported by experiments using artificial prey on a computer screen and either human volunteers [24] or artificial neural networks [25] as predators. Under this mechanism conspicuousness is only a commonly selected means of achieving distinctiveness, because it is a trait that undefended prey cannot afford to possess.

The unresolved question is how important selection for distinctiveness has been in shaping conspicuous aposematic signals. To us, its attraction is in its lack of assumptions: it could act even if predators show no special sensory or cognitive biases. It may be that selection for distinctiveness was important in the initial evolution of aposematism, and then once defended prey were conspicuous this drove selection in predators towards the biases that are now seen; these biases may then have driven selection for even higher levels of conspicuousness. An unresolved question is whether natural aposematic signals are more conspicuous than they need to be to ensure distinctiveness from cryptic undefended organisms. For this reason, we feel that exploration of the consequences for prey survival of variation in the strength of conspicuousness (rather than simply comparison of conspicuous with cryptic signals) would be useful.

## (c) *Distinctiveness*

As discussed above, high conspicuousness in aposematic prey may confer advantages in terms of exploiting predator sensory and cognitive systems. However, there is an alternative or additional explanation: undefended organisms are generally inconspicuous, and thus if a defended

## (d) *Why are warning colours often red, yellow and black?*

One of the most immediately apparent things when inspecting the range of warning signals in nature is just how common it is for them to be red, yellow and black (figure 1). Based on the above, we can propose several

(often related) hypotheses about why such colours dominate:

— They provide high contrast against the background (e.g. red/yellow against green foliage), which promotes detection.
— They are resistant to shadows (which are rich in blue-UV), and to changes in illumination (e.g. black should not change during day, whereas white could become 'pink' at sunset and sunrise). Therefore, they provide a reliable signal under varied habitats and light conditions.
— Yellow/red and black has both high chromatic *and* luminance contrast.
— Such colours may allow distance-dependent camouflage if yellow/red and black 'blend' to an average colour that matches the background at a distance when predator vision is no longer sufficient to discriminate individual marking components.
— Such colours are distinctive from profitable species.

These different hypotheses are not mutually exclusive, but it is important to test each of them. One approach is to model changes in colour and luminance contrast over the course of a day and under different light environments in terms of predator vision. We would predict that LW colours should fluctuate less than SW/UV colours as ambient light changes. This approach has been used in a different context to investigate differences between human and avian colour vision [26]. In addition, work should record and quantify more extensively the range of natural backgrounds against which prey are found, and to test in terms of predator vision how much the distribution of warning signals overlaps with background objects. A similar approach could be used to compare warning coloured prey to palatable species. In terms of distance-dependent effects on aposematism and camouflage, work could use models of predator vision, coupled with information on predator acuity (where available), to test the level of conspicuousness and camouflage at different distances of both the individual pattern components, and the average 'colour' of all components combined (as [27]).

## 3. MODERATELY CONSPICUOUS APOSEMATIC SIGNALS

Several recent theoretical papers suggest mechanisms that can select for aposematic signals to be less-than-maximally conspicuous ('weak' warning signals [28–30]; figure 2). These models essentially assume that the benefit of a conspicuous aposematic signal is that it reduces the probability that detection by at least some potential predators leads to death of the prey. It is further assumed that this benefit increases with increasing conspicuousness, but that this increase decelerates and eventually saturates. This seems highly plausible, however, we would welcome empirical demonstration. The further key assumption of these studies is that there is a cost to aposematic signalling, and this cost increases relentlessly (or decelerates more gently than the benefit) with increasing signal conspicuousness. This cost may be imposed by a fraction of predators that attack even strongly signalling prey (perhaps because they have evolved to overcome the prey's defences, or
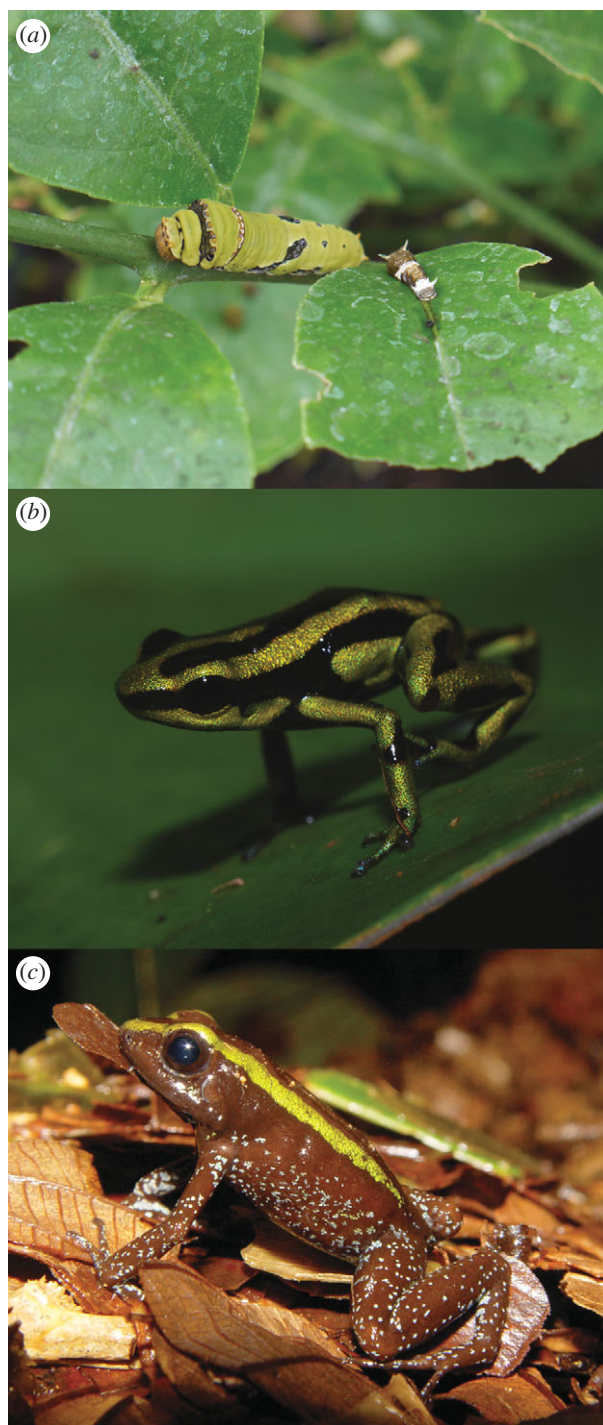


Figure 2. Examples of (*a*) Ontogenic changes in morphology and appearance with size in different instars of lepidopteran larvae, where early instars of the lime swallowtail butterfly (*Papilio demoleus*) resemble bird droppings and later instars appear to combine camouflage and warning signals. (*b*) and (*c*) are examples of apparently weak warning signals in *Ranitomeya fulgurita* and *Phyllobates aurotaenia*, respectively. (*a*) Reproduced with permission from © Martin Stevens; (*b*) © Fernando Vargas; (*c*) © Adolfo Amezquita.

simply because they have yet to learn to avoid the prey), and increasing prey conspicuousness increases the prey discovery rate and hence attack rate by such predators [28]. A similar effect of higher conspicuousness leading to higher rates of detection can still select for intermediate levels of aposematism when predators are uniform in behaviour, provided that the probability of such a discovery leading

to attack and prey death is non-zero [29]. Such non-zero attack rates are to be expected even from predators that have learned the aposematic signal if they manage (rather than minimize) their exposure to prey defences (see below). Finally, the cost may be a physiological cost of the compounds required to produce the conspicuous signal [30] or conflict between signalling to predators and other processes influenced by appearance (e.g. interactions with prey, interactions with conspecifics, UV protection or thermoregulation). All these potential costs seem biologically plausible, but more purpose-designed empirical explorations are needed.

One of the most convincing examples of a less-than-maximally conspicuous aposematic signal is that of the European viper (*Vipera berus*). Using Plasticine models, Wüster *et al.* [18] demonstrate that individuals bearing the distinctive zig-zag pattern of this snake were attacked less by wild-living predators than plain models and (since this effect occurred even when models were placed on a plain background) that this effect was due to avoidance of the pattern rather than crypsis. This experiment was carried out in a locality with no other venomous snake species, and no other snake species of similar appearance, suggesting that mimicry or generalization across prey types are unlikely to be important factors. While the appearance of adders to humans is distinctive, it is far from conspicuous, and could be cryptic when viewed from a distance.

It has long been known that viewing distance may greatly influence signal function. Tullberg *et al.* [31] performed a study involving photographs of swallowtail butterfly (*Papilio machaon*) larvae. These caterpillars have a distinctive appearance with brightly coloured dots and have been shown to induce learned avoidance in a range of avian predators. Tullberg *et al.* manipulated photographs of the larvae to make them more or less conspicuous, and then demonstrated that when viewed at close range (by humans) larvae were not maximally cryptic, but were also not maximally conspicuous when viewed from a distance. These observations lead to the hypothesis that (because the resolution of the eye is limited) the same appearance might function as crypsis when viewed from a distance and aposematism when viewed from closer range. These results deserve follow-up study. First, it is important to demonstrate conclusively that larval appearance plays an important role in predator avoidance, through studies that manipulated such appearance. Then work should test if the appearance of such prey could be manipulated to make them more detectable to ecologically relevant predators when viewed from a distance, and less effective in deterring attacks when viewed at close range. At present, only Marshall [27] has explored distance dependent effects on coloration when considering realistic receiver (as opposed to human) vision. Finally, it would be interesting to design studies to explore whether there is a trade-off between the type of appearance that functions well in long-distance crypsis and that which functions well in close-range aposematism.

## 4. INTRASPECIFIC VARIATION AND POLYMORPHISM IN APOSEMATIC SIGNALS

There seems to be variation in the conspicuousness of aposematic signals both within some species and sometimes between closely related species. Such variation requires explanation because one would expect that uniformity of signalling would aid learning and recognition by predators and thus benefit the prey. In addition, some species may even be polymorphic, with individuals falling into different discrete morphs. There are several potential non-mutually exclusive mechanisms to explain this, including that alternative phenotypes may have equal fitness, they may exist through negative frequency-dependent selection, or heterozygote dominance. Such processes may arise due to environmental variation (spatially or temporally) allowing different morphs to co-occur or to exist at different times or locations, if, for example, backgrounds differ in structure or coloration, which in turn differentially influences conspicuousness of the different morphs. Additionally, seasonal differences in predator communities could lead to variation. For instance, in temperate environments there may be more naive predators in the spring than summer, leading to greater costs of conspicuousness in the spring. Species may also be trading off multiple selection pressures acting on their coloration, only one of which is aposematism. Phenotypic plasticity may allow variation in how the balance between such trade-offs are expressed in individuals. Overall, these processes may lead to true polymorphisms in traditional terms of genetically distinct co-occurring morphs, or more commonly to continuous intraspecific variation or seasonal forms arising under different environmental conditions. Below, we focus mainly on two relatively well-studied systems that illustrate some of the above issues.

The costs and trade-offs of aposematic signal form are well illustrated by studies of the wood tiger moth (*Parasemia plantaginis*). In this species, continuous variation exists in terms of the colour and size of an orange spot on the otherwise black larvae, and also in the hindwings of the females, which vary in terms of how red–orange they are. The adult males, however, come in two morphs, with either white or yellow hindwings. Lindstedt *et al.* [32] demonstrated that larvae reared in cooler conditions had a smaller and duller spot, and this was linked to faster development time and growth rate at low temperatures. However, smaller, duller spots were less effective in promoting avoidance learning of larvae than larger, brighter spots, whereas brighter spots were more likely to incur predator detection [33].

Experiments have also shown that the coloration of adult *P. plantaginis* can be influenced by diet quality [34]. In addition, in aviary experiments with great tits (*P. major*), females with redder hindwings were avoided more than those with more orange hindwings, potentially selecting for reduced variation [35]. In contrast, in field trials, when presented to the entire community of potential predators, there was no significant difference in survival between red and orange forms. This could indicate that under more complex environments selection for a specific signal form is reduced [35]. However, although not significant, there was a trend for higher survival in the red compared with the orange forms. Therefore, more work is needed here, especially as the different colours may have different contrasts against different background types. Finally, in males, both field and aviary experiments show that yellow males are avoided more than white males, but that white males have higher mating success [36]. In *P. plantaginis*, therefore, various factors contribute to the colour variation of both larvae and adults,

illustrating how many selection pressures may be influencing warning signal form.

In other species, variation is manifested in discrete morphs. A widely studied group are the poison frogs of Central and South America. The striking colours of poison frogs are iconic examples of aposematism (e.g. [37,38]), but the colour patterns are also used in mate choice [39]. Some species can be strongly polymorphic, often varying greatly geographically. In the polymorphic strawberry poison-dart frog (*Oophaga pumilio*) females seem to prefer to mate with males of the same colour morph [39,40]. Field experiments by Noonan & Comeault [41] with artificial clay models of *Dendrobates tinctorius* have shown that novel ('immigrant') phenotypes are attacked more by predators than locally occurring forms. Thus, the implication is that the appearance of some poison frogs acts as a 'magic trait', acting in both assortative mating and predation. In *O. pumilio*, incipient speciation may exist, with mating between morphs producing offspring with intermediate appearance phenotypes or inappropriate mate preferences, and thus reduced fitness, potentially leading to reinforcement reducing mating between morphs [42,43]. In populations where more than one morph occurs, females show stronger preferences for their own morph than females in populations with only one morph present, consistent with intraspecific reproductive character displacement [42]. Predation pressure is normally thought to constrain communication strategies used in mating, but here the opposite may be the case and the combination of aposematism and mate choice could be driving geographical polymorphisms.

## 5. THE INFORMATIONAL CONTENT OF CONSPICUOUSNESS

Above, we have discussed how variation in aposematic signal form and polymorphism may occur. Several recent theoretical studies have also explored whether there might be an association between levels of conspicuousness and levels of defence that could potentially provide useful information to predators; hence we ask: are warning signals reliable with respect to the prey's defence levels?

Franks *et al.* [44] demonstrate with a mathematical model that the evolution of mimicry of highly defended model species is easier to achieve because predators will avoid even poor mimics of very strongly defended models. Thus, for a very highly defended species to avoid initially cryptic undefended prey from evolving towards them in appearance they must have a very conspicuous appearance. Intermediate appearances may exist where the costs of raised conspicuousness to the would-be mimic are greater than any benefits from approximate mimicry. Thus, Franks *et al.* predict a positive correlation (across species) between levels of defence and conspicuousness.

Franks *et al.* [44] also speculate that highly defended prey might be more conspicuous because they can offset the cost of increased encounter rates with predators if their defence allows them to better survive attacks or speeds aversion learning by predators. However, the theoretical work of Leimar *et al.* [45] predicted a negative correlation across species between conspicuousness and potency of defence. This arises in their model because prey with better defences can better survive attacks, and so benefit from reducing their investment in costly conspicuous signalling even if this decreases the rate of learning. Speed & Ruxton [30] demonstrate that the optimal combination of investments in aposematism and defences depends critically on how the cost and benefits are affected by the values of these two traits, and are also affected by population density and aspects of life-history strategy. Both negative and positive correlations (as well as no correlation) can be predicted under different sets of model assumptions. Thus, interspecies correlations between conspicuousness and potency of defences cannot be expected to be strong unless the species concerned are very similar in ecology.

Blount *et al.* [46] predict a negative correlation between conspicuousness and potency of defences between individuals within the same population, based on a model whose critical assumption is that both defence and appearance compete for some limited resource. They argue that for toxic defences, such a resource is likely to be anti-oxidant molecules that serve both as pigments and in protecting the individual from oxidative stress as they accumulate toxins within their body. Another important model assumption is that a predator's decision to attack a prey individual is influenced by the combined (interacting) levels of defence and conspicuousness and not the two independently. We would very much welcome the behavioural study of such attack decisions in real predators, and physiological study that might evaluate the importance of the presumed physiological competition between defence and signalling.

Speed *et al.* [47] present a model that offers another mechanism that could lead to a within-species positive correlation. They assume that individuals vary in level of defence and that this is environmentally conferred rather than being genetically determined (as might happen if individual host plants of herbivorous insect larvae vary in the levels of secondary chemicals that the larvae can sequester). They also assume that an individual can select its appearance once its defence level is determined, with greater conspicuousness attracting more encounters with predators. A predator's decision to attack is based on knowledge of the mean level of defence (across the prey population) associated with different levels of conspicuousness. The probability of the individual surviving any attack increases with its level of defence. Simulated prey populations evolved such that prey of each defence level varied in appearance, but on average there was a positive correlation between defence level conspicuousness, with predators being more likely to avoid attacking more conspicuous individuals. Thus, predators benefit from conspicuousness being at least a partially reliable signal of defence level.

Clearly, further empirical study is required and a number of pioneering studies may suggest suitable study groups. Cortesi & Cheney [48] report a positive correlation between conspicuousness and toxicity across 20 species of marine opisthobranches. This might be a particularly suitable group for further study since the animals themselves have limited vision and so their appearance is unlikely to be strongly influenced by within-species interactions. The next useful step would be to demonstrate that an ecologically relevant predator is sensitive to the measured variation in signal conspicuousness.

Summers & Clough [38] reported a positive correlation across species of dendrobatid frogs between conspicuousness to humans and alkaloid concentration from skin secretions. However, the situation in this group appears more complicated, since Darst *et al.* [37] report that closely related species appear to have either increased conspicuousness or toxicity, but not both, and that various work indicates that appearance is important in mate choice decisions (see above). Further progress with this study group may require overcoming the significant logistical challenges of exploring predation threats in the wild.

Within a single ladybird population, Bezzerides *et al.* [49] report strong variation in the extent of red coloration on the wing elytra that was correlated with levels of alkaloid defences. Again, exploration of whether predators are sensitive to this variation would be very worthwhile, although it will be important to remember that elytra coloration has been shown to be important in mate choice in the species concerned. Finally, some defended prey have minimal warning signals and may even be cryptic. Work with pine sawfly larvae (*Neodiprion sertifer* and *Diprion pini*), which are chemically defended, indicates that conspicuousness does not evolve because detectability costs of more conspicuous signals increases predator attacks and also because the chemical defences are not costly to produce [50]. We would welcome further tests of this finding in other groups.

## 6. INTEGRATING WARNING SIGNALS MORE GENERALLY INTO BEHAVIOURAL AND EVOLUTIONARY BIOLOGY

Warning signals do not exist in isolation from other traits that influence the fitness of individuals, and there are still interesting issues to explore to understand how such interactions occur. One area that would benefit from further study is predator foraging strategies and how they affect the evolution of signal form. For example, in some laboratory experiments, predators do not behave so as to minimize their exposure to toxic prey but rather to manage their exposure. Skelhorn & Rowe [51] and Barnett *et al.* [52] demonstrate that predators that have learnt an association between toxin load and appearance of a given prey type modulate their use of that prey type in ways that can be understood in terms of strategic utilization of the nutrients contained in that food while managing exposure to toxins. This suggests that predators do not simply categorize a particular prey as 'bad' but rather have a more nuanced representation of the costs and benefits. We would be interested in the expansion of this work to more complex prey environments involving many different prey types, in order to explore to what extent predators categorize prey, how they generalize between different types, and how aspects of aposematic signals influence this. It has also long been known that predators show innate unlearned biases against certain signalling traits and some of those can be associated with the appearance of dangerous animals (such as snakes) for which learned avoidance would be too costly. An interesting strand of research seeks to integrate such phenomena within a general theory of dietary conservatism by predators [53]. It is clear that predators can have a long-term reluctance to sample novel food

items that can be interpreted in terms of controlling the risk posed by prey defences, and that the deactivation and reinstatement of such avoidance behaviours can be influenced by predator state, previous experience and environmental cues [54]. We would welcome further study into what drives variation in neophobia between predatory individuals, and the role of prey traits in influencing this behaviour.

Higginson & Ruxton [55] provide an attempt to predict how aposematism might be expected to change over the lifetime of an individual, and how it might be affected by such life-history traits as longevity and reproductive strategy. However, this work did not consider interactions between individuals with different strategies. This is important when it is known that aposematic signals and defences can both vary within a population. Further, predators' responses to a given signal are likely to be influenced by previous experience, which in turn is likely to be influenced by the signalling and defences of other prey in the same locality. Finally, individual prey can share limited resources and resource depletion is likely to often be an important mechanism by which individuals influence those around them.

Cryptic animals are often restricted to certain backgrounds and individuals may only be active at certain times because movement is often antipathetic to good crypsis. These opportunity costs are not generally considered to apply to aposematism. Speed *et al.* [56] make the important point that greater freedom to exploit resources may be an important selective pressure encouraging evolution of aposematism. We would also argue that aposematism might influence other aspects of life-history through this mechanism. For example, if aposematism allows greater freedom of movement and/ or access to new resources then it might also allow faster growth rates and earlier maturation or larger size at maturation. There is a need to clarify these arguments in theoretical development (perhaps using Higginson & Ruxton's work as a foundation), but some of these ideas are already clear and logically sound enough to justify empirical testing. Ontogenic colour change (figure 2) may provide a useful phenomenon to study opportunity costs of crypsis for the adoption of aposematism, since it has been argued that organisms switch to aposematism in life-history stages where foraging or mating demand extensive movement that would reduce the effectiveness of crypsis [57,58]. In contrast, warning signals may be ineffective when body size (and thus signal size) is very small. In this instance, crypsis may be more effective.

Finally, aposematism and mate choice can interact in ways that have implications for macroevolution (see also discussion of poison frogs in §4). For instance, *Heliconius* butterflies are a diverse group with closely related species often differing greatly in appearance, while distantly related species often converge in appearance owing to Müllerian mimicry against predators (see reviews by Jiggins [59] and Mallet & Joron [60]). Jiggins *et al.* [61] have shown that two sister species, *Heliconius melpomene* and *Heliconius cydno*, differ in coloration and habitat use and have recently diverged to mimic two different models (*Heliconius erato* and *Heliconius sapho*, respectively). The colour patterns in each species are important in mate choice, and when the populations occur in sympatry individuals are less likely to court members of the other species

than when found in allopatric populations. When hybridization does occur, intermediate phenotypes lack effective mimicry of either model, and are thus likely to be vulnerable to predation. At interspecific contact zones reinforcement may occur, leading to character displacement of mating preferences to prevent hybridization [62]. However, although mark–release–recapture experiments with different morphs of *H. erato* in different locations indicate that foreign morphs have lower survival than resident morphs owing to predation [63], predator selection against hybrids has rarely been explicitly demonstrated or even tested [59] and this remains a key piece of work needed to 'complete' the story. Finally, *Heliconius* butterflies can also provide important information about the genetics underpinning warning signal form. For example, recent work shows that a single gene (*optix*) drives variation in the red colour wing patterns across several species of *Heliconius* [64]. It will be valuable to discover more in a range of species about how specific genes may allow both diversification in signal form across populations and species, and how some colour types may be constrained.

## 7. CONCLUSION

There is growing consensus that we now have a robust understanding of the mechanisms underlying the evolution of aposematic signalling [1]; hence, we feel that the time is ripe to extend this understanding to the diversity and functioning of naturally occurring aposematic signals. We have summarized the areas of current understanding and provided suggestions as to how the gaps within and between these areas might be bridged. We have also provided pointers as to how aposematism can be incorporated most effectively into the study of life-history strategy and how its relevance to foraging ecology and speciation might most usefully be explored. Despite many years of study, aposematism has much still left to tell us about evolution and ecology.

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