

Aposematic signals and the relationship between conspicuousness and distinctiveness

Sami Merilaita^{a,*}, Graeme D. Ruxton^b

^a*Department of Zoology, Stockholm University, SE-106 91 Stockholm, Sweden*

^b*Division of Environmental & Evolutionary Biology, Institute of Biomedical & Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*

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Abstract

It has long been recognized that prey that invest in toxic or other defenses often advertise these defenses by means of conspicuously coloured signals. One question that remains unanswered is why conspicuousness is such a universal trait of aposematic signals. Conspicuousness may allow more rapid avoidance learning by predators or improved retention of such learning. An alternative or complementary explanation is that defended species should adopt a conspicuous signal of their defence to make them visually distinct from inconspicuous undefended prey. Here, we use a neural network model of prey detection and attack decision making by a predator in combination with evolving, virtual prey to shed light on the relative importance of conspicuousness against the background and distinctiveness from other species as mechanisms underlying aposematic signalling. Our model suggests that prey conspicuousness may result from selection for distinctiveness, but that selection for distinctiveness does not result in maximization of conspicuousness. On the other hand, our model does not justify the exclusion of the possibility that conspicuousness as such may be a beneficial attribute of warning coloration. It is likely that the relative importance of the two selective forces (for conspicuousness and for distinctiveness) will differ on a case-by-case basis, however there is no empirical or logical reason for the current neglect of evolutionary pressure for distinctiveness. Thus, we suggest that description of aposematism as the teaming of a secondary defence with a conspicuous signal may be overly simplistic; we would rather that the signal were described as conspicuous and/or distinctive.

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1. Introduction

It has long been recognized that prey that invest in toxic or other defenses often advertise these defenses by means of conspicuous signals (Wallace, 1867; Darwin, 1871; Cott, 1940). Examples of such “aposematic” signals are the yellow and black stripes of many wasps and the spotted patterns of many ladybirds. The evolution of aposematic signals has long been an interesting challenge to evolutionary biologists (summarized in Chapter 8 of Ruxton et al., 2004). One question that remains unanswered is why conspicuousness is such a universal trait of aposematic signals (Sherratt, 2002; Sherratt and Beatty, 2003).

Warning signals have been suggested to have various possible functions, such as promoting detection, discrimination or remembrance (Guilford and Dawkins, 1991; Guilford, 1990a; Ruxton et al., 2004). Conspicuousness may be an important trait for some of these functions (e.g. Guilford, 1990a, b). Accordingly, experimental studies have shown that predators more effectively learn to avoid conspicuous than cryptic prey (Gittleman and Harvey, 1980; Roper and Redston, 1987; Lindström et al., 1999). This suggests that conspicuousness may benefit aposematic prey. On the other hand, it has been suggested that it is the colour and not the contrast between the prey and the background as such that predators attend when learning to avoid distasteful prey (Gamberale-Stille and Guilford, 2003). Moreover, it is not self-evident that maximal conspicuousness or detectability of warning signals is favored by selection, because attracting the attention of

*Corresponding author. Tel.: +46 8 164034; fax: +46 8 167715.

E-mail addresses: Sami.Merilaita@zoologi.su.se (S. Merilaita), G.Ruxton@bio.gla.ac.uk (G.D. Ruxton).

predators may simply result in increased predation risk (e.g. Tullberg et al., 2005). In general, it seems that although there are various hypotheses about conspicuousness of warning signals, there are not very many studies that have explicitly investigated why conspicuousness is such a universal trait of aposematic signals.

Conspicuousness of warning signals is easy to understand if there is direct benefit from it. However, there may also be other explanations to conspicuousness, and it is possible that an increased contrast between the prey coloration and its background has resulted as a by-product from selection for characteristics of coloration other than conspicuousness. According to an alternative explanation for the common adoption of conspicuousness in aposematic signals, undefended species should seek to be as inconspicuous as possible, and so defended species should adopt a conspicuous signal of their defence such that predators can tell apart defended prey from undefended prey. For example, Fisher stated that “to be recognized as unpalatable is equivalent to avoiding confusion with palatable species” (Fisher, 1930, p. 148). Such a conspicuous signal may be resistant to cheating because a conspicuous undefended individual would open itself up to higher levels of attack by predators, to which it would have no defence (Sherratt, 2002; Sherratt and Beatty, 2003). In a recent study, Sherratt and Beatty (2003) presented a series of experiments and suggested that it is actually selection for distinctiveness (rather than conspicuousness) that drives the evolution of warning coloration. In the present paper we want, using modelling, to further scrutinize how selection on distinctiveness affects prey coloration. In particular, we will study the relationship between distinctiveness and conspicuousness. We assume that the prey may use cryptic coloration to decrease its predation risk. Further, the prey in question may or may not possess a defence, which we also assume may affect the optimization of the appearance of its colour pattern in relation to the coloration patterns of other, defended or undefended prey that a predator encounters.

We use simple artificial neural networks as predators. Artificial neural networks are increasingly being used as models of natural information processing and decision-making systems (e.g. Arak and Enquist, 1995; Ghirlanda and Enquist, 1998; Merilaita, 2003; Merilaita and Tullberg, 2005) and have the strong advantage over animal (or human) model systems that the structure of the system is entirely under the experimenter’s control, and so we can eliminate concerns that results are artifactual caused by unacknowledged special features of a specific model organism.

Here, we use such a predator model in combination with evolving, virtual prey to simulate simple scenarios related to the evolution of prey appearance. Although most previous studies have for practical reasons focused on one anti-predator strategy at the time, recent studies suggest that it is important to consider the costs and benefits of all the alternative strategies when studying the

evolution of protective coloration (Merilaita and Tullberg, 2005; Speed and Ruxton, 2005). Therefore, in this study we will simultaneously investigate the effects of selection for camouflage, mimicry and conspicuousness as well as selection against mimetic resemblance on prey appearance. More specifically, we aim to shed light on the relative importance of conspicuousness against the background and distinctiveness from other species as mechanisms underlying aposematic signalling. We use an essentially similar scenario to Sherratt and Beatty (2003). They studied how the coloration of two virtual prey species, defended or undefended, evolved. Sherratt and Beatty (2003) drew the interesting conclusion that “the most fundamental reason why defended prey tend to be conspicuous is because it sets them apart from undefended prey”. It is, however, possible to increase the reality of some aspects of the evolutionary process in their model. Accordingly, the main difference in our model is that instead of using only few, pre-determined appearance phenotypes, which may affect the process (by limiting the possible routes through the adaptive landscape) and the result of evolution, we let the prey appearance, described by a multidimensional variable, freely evolve along all the dimensions of the variable. This yields a more flexible representation of the relationship between conspicuousness and distinctiveness.

Our model should be viewed as a strategic tool for exploring the general concepts of difference from the background and difference from non-defended prey as alternative or complementary mechanisms underlying aposematism. Our aim is not to mimic the likely course of the evolution of aposematism, only to evaluate the logical plausibility and implications of these distinct mechanisms. As such, our model does not attempt to describe visual appearance, the genetics underlying appearance or co-evolution between species. Rather, we adopt the simplest defensible representations of these, so as to allow clearer identification of the processes of interest.

2. Methods

2.1. General assumptions of the model

This model consists of two species of prey, a predator and a background. Because of morphological similarity between the prey species, the predator cannot tell them apart if they have identical appearance (involving all aspects of coloration, patterning and morphology). We are interested in how the appearance of one of the prey species, called the focal prey, evolves in relation to the background and to the other prey species, called the reference prey. For ease of interpretation, the reference prey has a fixed, unchanging appearance. At the beginning of a simulation, the two prey species have similar appearance, and we describe the outcome of the evolution of appearance of the focal prey in terms of both conspicuousness (i.e. visual

deviation from the background) and distinctiveness (i.e. visual deviation from the reference prey).

We study the evolution of appearance of the focal prey species under four main scenarios. In scenario one, the focal prey is unpalatable and the reference prey is palatable. Scenario two is the reverse situation: palatable focal prey and unpalatable reference prey. In both of these scenarios a predator's avoidance learning about an appearance type is only affected by the proportion of individuals of that appearance type that are unpalatable but is independent of how common that appearance type is. The third scenario is like the first scenario, but now the predator's avoidance learning is affected by both the proportion of unpalatable individuals within an appearance type and the proportion of the appearance type within the focal species. In the final scenario, we make the same assumptions as scenarios one and two but now both prey species have a defence of equal strength.

Within each scenario we vary two more aspects of the model. We vary the visual difference between the reference prey and the background. The greater the difference from the background the more conspicuous the reference prey is. At the start of a simulation we assume that both prey types have the same appearance, although the focal prey has the flexibility to evolve away from that appearance. Because the initial appearance of the evolving focal prey is similar to the appearance of the reference prey, this also means that in varying the appearance of the reference prey, we simultaneously vary the initial appearance of the focal prey. In addition, we assume that the maximal benefits from crypsis and predator avoidance as a result of aposematism are asymmetric and we vary these benefits. This is to increase the realism of the model. We find it highly unlikely that the maximal benefits or the benefit to cost ratios would be equal between crypsis and aposematism, and such inequality may affect the outcome of evolution. Specifically, in some cases we assume that perfectly matching the background allows an individual to have a 100% chance of avoiding predation whereas maximizing avoidance learning through aposematism or mimicry only provides 90% protection. In other cases, these probabilities are reversed. With this model we study how the evolution of the focal prey appearance is affected by selection for crypsis, conspicuousness, ease of recognition, and mimicry. We replicated each case 50 times.

2.2. Predation and appearance of prey and background

Visual similarity between an object and its background is a major determinant of the probability of detection, and we have assumed that the only thing that affects the probability of detection of a prey is the difference between background and prey appearance. Further, we assume that the probability that a predator attacks a prey with a given appearance depends on the proportion of individuals (of both the species) with that appearance that are unpalatable (and the proportion of individuals with the given appear-

ance within the focal species in the third scenario). In other words, a prey may be protected by camouflage (low probability of detection) and by aposematism or mimicry (avoidance learning based on previous encounters between the predator and prey). Predator behaviour was simulated with artificial neural networks (see below).

The predator visually samples its habitat. These visual samples, and thus also the prey and background appearance, are described by vectors consisting of four cells, each cell being occupied by one of eight possible appearance elements denoted by the numbers 0, 1, 2, 3, 4, 5, 6 or 7. An appearance element can be considered as a colour or a feature such as a stripe or a spot. We assumed that the background was uniform in the sense that every 4-cell sample of it was equal. Such visually homogeneous habitat allowed an easy way of improving prey camouflage through an increase in background matching. The appearance elements constituting the sample of the background were chosen randomly at the beginning of each simulation. The appearance of the background, focal prey and reference prey can all be considered as points in the same four-dimensional space, with positions determined by their 4-cell appearance vectors. The distinctiveness of the focal prey is defined as the Euclidean distance between focal and reference prey in this four dimensional space, whereas the conspicuousness is the Euclidean distance between focal prey and the background.

The focal prey underwent evolution until it had reached an evolutionarily stable appearance. We considered that it had reached evolutionarily stable appearance when the most protected 50% of the population had attained a stable monomorphic appearance (i.e. was not invaded by another appearance) for 30 successive generations. Note that there was never polymorphism within the top 50% of the population when the simulation was stopped. This does not exclude the possibility that in some replicates there may have been (transient) polymorphism before the simulation reached its termination.

2.3. The prey populations

The focal prey and the reference prey had equal population sizes, namely 32 individuals. In the beginning of each simulation, the reference prey was assigned a randomly chosen four-cell appearance vector at the Euclidean distance of 0, 1, 3 or 6 from the background. The initial appearance of the focal prey was identical to the appearance of the reference prey. A simplified haploid genetic system (cf. Merilaita, 2003; Merilaita and Tullberg, 2005) coded for the appearance phenotype of the evolving focal prey. Thus, the focal prey had four loci, one for each cell of the appearance. For each locus there were eight possible alleles, each corresponding to one of the eight possible appearance elements.

Because the reference prey did not evolve, it was necessary to simulate predation on and reproduction of the focal prey only. Thus, during each generation the

individuals of the focal prey population were ranked according to their fitness (see later), and the half of the population with the lowest fitness was removed before reproduction took place. Then, each individual of the half with the lowest predation risks contributed two offspring to the next generation. Consequently, because the generations were non-overlapping, population size remained constant between generations. Genetic variation necessary for evolution of appearance was produced in each generation by three point mutations and one recombination event among the offspring, which were otherwise exact genetic copies of their parents. The three mutations each changed an allele in a random locus of a random individual. The recombination event took place between two randomly chosen individuals and resulted in the individuals exchanging a set of appearance alleles ranging from a randomly chosen locus to the last locus.

2.4. Artificial neural networks

We used two different kinds of artificial neural networks to simulate predation on the focal prey. A radial basis function network (Bishop, 1995; Haykin, 1999) with only one neuron simulated the ability of the predator to detect deviations from the visual background (cf. Merilaita and Tullberg, 2005). Such a network compares an input vector with a template vector of equal size, and its output is determined by the difference between the two. More precisely, the output is given by the function $f(x) = e^{-x^2}$, where $x = |\text{dist}| \times [-\log(0.5)]^{1/2}/2$. Here, $|\text{dist}|$ is the Euclidian distance between an input vector and the template vector, and it is multiplied by a constant that determines the width of the bell-shaped function. The template vector was a visual sample of the background, and the prey appearances of the current populations were presented as input vectors. Because $|\text{dist}| \geq 0$, it follows that $x \geq 0$ and $0 < f(x) \leq 1$. Thus, the output value for a given prey appearance could be used as the probability of escaping detection (i.e. the complement probability of the risk of detection) on a given visual background. For increasing difference between the prey appearance and background (i.e. increasing conspicuousness) this probability decreased, asymptotically approaching zero.

The second neural network was used to simulate the decision-making of the predators about attacking a prey. It consisted of four consecutive layers, i.e. a layer of four input cells, the first intermediate layer of neurons, the second intermediate layer of neurons, and the one-neuron output layer. It was a feed-forward network, and thus, the signals from the input cells traversed only in one direction through the intermediate layers to the output layer (Bishop, 1995; Haykin, 1999). The neural network was saturated such that each neuron was connected to every neuron or input cell of the adjacent layers. Each neuron consisted of specific weights for every incoming connection,

a bias and a transfer function. The signals coming into a neuron were first multiplied with the connection-specific weights. The sum of the weighted signals and the bias formed the input to the transfer function. The output from the transfer function was then forwarded to the neurons of the next layer. All transfer functions were log-sigmoid (i.e. smooth threshold) functions except the transfer function of the output neuron, which was a linear function. Because the number of different output values was potentially larger and, thus, the categorization task was more demanding in scenario 3 (where we add a frequency-dependant advantage to aposematism) than in scenarios 1, 2 and 4, the number of neurons in the intermediate layers was larger in scenario 3. In scenarios 1, 2 and 4 the number of neurons in the first and the second intermediate layers were 8 and 3, whereas they were 16 and 5, respectively, in scenario 3.

The response of a feed-forward neural network to a given input depends on its weight and bias values. When such a neural network is trained, these values are adjusted such that for a given input a desired output is received. Such a training procedure corresponds to the learning of natural neural systems. Training also affects the neural networks generalization ability, i.e. the ability to correctly categorize data that it has not encountered previously (Bishop, 1995; Enquist and Arak, 1998; Haykin, 1999). The training was based on a back-propagation algorithm with an adaptive learning rate (Demuth and Beale, 2000). The network was trained once during each generation of the focal prey. During each prey generation the presentation of the training set was continued until the mean square error of the output of the network was smaller than 10^{-4} , or until the training set had been presented 600 times in scenarios 1, 2 and 4 and 800 times in scenario 3. Predation on the prey did not begin until training was complete.

The linear transfer function of the output neuron enabled avoidance responses varying in strength that could be treated as attack probabilities instead of binomial decisions about attack. In other words, the training of the second neural network produced a relationship between appearance phenotypes and attack probabilities. This relationship determined the output of the neural network (i.e. the attack probability) both for appearance phenotypes that had been used in the training and for novel appearance phenotypes that were produced by mutation or recombination that the network had not encountered before. We included the appearance of each individual of the focal prey population and the reference prey population as input values in the training data set. The corresponding target output values were calculated as follows: In scenarios 1, 2 and 4 the training target value for each appearance phenotype was the proportion of defended individuals among all individuals with the appearance. In scenario 3, the training target values were calculated as the product of the proportion of defended individuals among all individuals with given appearance

and the abundance of the given appearance phenotype in the focal species. The abundance was calculated as the sum of individuals with a given appearance divided by 29 (29 was used instead of 32 since due to the 3 mutations, the maximum number of individuals with the same appearance in the population of the focal prey was 29). Note that although our population sizes are modest, previous experience with similar models suggests that our results would not be changed quantitatively by higher population sizes or lower mutation rates.

2.5. Calculation of fitness

The fitness of a prey individual was determined by its prospects of surviving to reproduction. Thus, fitness was in effect equal to the probability of escaping predation (i.e. the complement of the probability of a detected prey being attacked) and its calculation was based on the outputs of the two neural networks. More precisely, taking into account the asymmetric benefits from camouflage and warning coloration, the fitness function was: $\text{Fitness} = 1 - \{ [1 - a \times (\text{probability of escaping detection})] \times [1 - b \times (\text{probability of escaping attack})] \}$, where $a = 1.0$ or 0.9 , and $b = 0.9$ or 1.0 , depending on the direction of the benefit asymmetry. Probability of escaping detection was given by the first neural network and probability of escaping attack was given by the second neural network.

3. Results

3.1. Scenario 1: prey with secondary defence

In Fig. 1a, the defended focal prey (and the undefended reference prey) starts at the point (0,0), being maximally cryptic. In this case, when crypsis allows greater protection than aposematism (squares), we find (unsurprisingly) that the focal prey always remain in their initial state of being maximally cryptic. However, in the reverse situation, in which greater protection can be obtained through aposematism, the prey evolve a more conspicuous appearance (circles). The apparent correlation between conspicuousness and distinctiveness in Figs. 1a and b is caused by auto-correlation: If the reference prey is cryptic then there will be an auto-correlation between conspicuousness and distinctiveness, because in this case similarity to the background also means similarity to the reference prey. Consequently, this illustrates the fact that it may not be possible to separate distinctiveness from conspicuousness, if the reference prey is highly cryptic. Similarly, if the reference prey is not cryptic and the focal prey becomes more similar to the background, then it may be difficult to tell whether the change is due to selection for distinctiveness or selection for crypsis (or both).

Regardless of the starting point, when camouflage yields higher benefit than aposematism, appearance will evolve to the cryptic optimum or at least very close to it

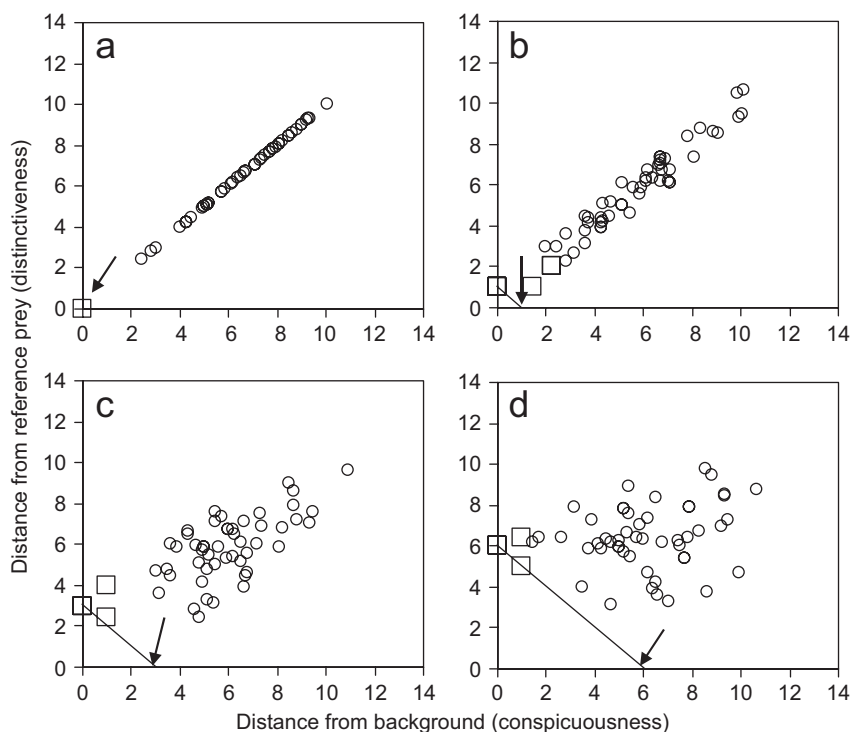


Fig. 1. The outcome of the evolution of appearance in relation to the background and the reference prey when the focal prey had a secondary defence (Scenario 1). The four panels differ in the appearance of the reference prey and hence in the initial appearance of the evolving focal prey indicated by the arrow: conspicuousness = (a) 0; (b) 1; (c) 3; (d) 6. For \square , the maximum probability of avoiding detection/attack was 1.0 due to crypsis and 0.9 due to aposematism (50 replicates). For \circ , the maximum probability of avoiding detection/attack was 0.9 due to crypsis and 1.0 due to aposematism (50 replicates). Because of the relationship between the background and the reference prey it was impossible to produce values below the dashed line.

(Fig. 1: squares). On the other hand, if it is possible to achieve higher benefits from aposematism than from camouflage, then the outcome of evolution will be a warning appearance (Fig. 1: circles). The warning appearance will be both conspicuous (i.e. deviate from the background) and distinctive (i.e. deviate from the reference prey). Similarity to the reference prey is favoured in the focal prey only when the reference prey is highly cryptic and the benefit from crypsis is higher than the cost from visual similarity to the undefended reference prey (Fig. 1a: the square). On the other hand, selection for conspicuousness depends on the pay-offs from camouflage and aposematism. If camouflage is more rewarding than aposematism, then even an unpalatable prey will evolve towards camouflage.

The important point with Figs. 1c and d is that because the auto-correlation between conspicuousness and distinctiveness is low in them, it is possible to distinguish the effects of these two factors and draw conclusions about their respective roles in selection on appearance of the focal prey. From the cases where aposematism is more beneficial than crypsis (Figs. 1c and d: circles) it is obvious that the distasteful, focal prey has evolved to become distinct from the palatable reference prey. This is because, due to the lack of auto-correlation, there would now be ways to be distinct from the background but still similar to the reference prey and (despite the fact that their initial state is one of these ways) prey always evolve at least some distinctiveness from the reference prey. This can be quantitatively verified by using each of the variables distinctiveness and conspicuousness in Fig. 1d as the independent variable on turn, fitting a regression line on the data, and analysing the intercepts. The intercept of the regression line is positive and significant only when distinctiveness is the independent variable and conspicuousness is the dependant variable ($y = 0.085x + 5.93$; $F_{1,98} = 1571.9$, $p < 0.001$). When conspicuousness is the independent variable and distinctiveness is the dependant variable the intercept is negative and non-significant ($y = 0.78x - 1.69$; $F_{1,98} = 1571.9$, $p = 0.36$). Accordingly, all the points depart significantly from the distinctiveness axis but not from the conspicuousness axis. To conclude, our focal prey always evolved to some degree of distinctiveness from the other prey type but not necessarily always some degree of conspicuousness against the background.

Generally, there are fewer ways to be similar to something than to be different from it. In our model, there is only one appearance that yields maximal crypsis (i.e. the appearance that is equal to the background), but many different appearances that can yield maximal aposematic effect. Also, there is only one appearance that corresponds to the fitness minimum due to mimetic resemblance of the reference prey. Consequently, it should be very easy to evolve so as to become distinct from the reference prey. On the other hand, it is less likely that mutations and recombination produce an appearance that maximizes crypsis than that they produce an appearance that

maximizes aposematism. However, if the pay-offs from crypsis are higher than the pay-offs from aposematism, then even a slightly sub-optimal cryptic appearance can provide a better protection against predation than aposematism does (Figs. 1c and d).

3.2. Scenario 2: edible prey

In the second set of simulations the focal prey was edible and the reference prey inedible (Fig. 2). In this case, the prey appearance evolved towards the cryptic optimum (maximizing similarity to the background) or to the mimetic optimum (maximizing similarity to the defended reference prey). The maximum benefits given by the two strategies strongly affected, but did not solely determine, the course of evolution. For example, in Fig. 2b (in which the Euclidian distance between the initial appearance and the background was 1), when crypsis yielded higher maximum benefits than aposematism, 50 times out of 50 the prey evolved to the cryptic optimum. Conversely, when it was possible to benefit more from aposematism than crypsis, the prey could escape from the mimetic optimum to the more beneficial cryptic optimum (remember that mimicry dilutes the benefit from aposematism for the mimics and the models) in only two replicates out of 50. However, when the Euclidian distance between the initial appearance of the focal prey and the cryptic optimum was 6 (Fig. 2d), the focal prey found its way to the cryptic optimum only 4 times when crypsis was more beneficial than aposematism, and 6 times when aposematism was more beneficial than crypsis. Thus, increase in the distance between the mimetic and the cryptic optima decreased the probability of transitions between them.

3.3. Scenario 3: prey with secondary defence and with a frequency-dependant benefit from aposematism

In the third set of simulations the focal prey had a secondary defence. In addition, the benefit from aposematism is now increased with increasing frequency of the particular appearance phenotype in the total population. It is a natural consequence that such frequency-dependency restricts the evolutionary change of the appearance of the unpalatable prey. This makes it difficult for the focal prey to avoid mimicry by the reference prey and to gain maximum benefit from aposematism (Fig. 3). However, it is possible to escape mimicry (i.e. the initial appearance of the focal prey) with the help of camouflage (Figs. 3b–d). In other words, mutations or recombination must produce an appearance phenotype for which camouflage compensates for its initial rarity, but (as Fig. 3 shows) such events are still possible in our model.

3.4. Scenario 4: both prey types are defended

Here, we made the same assumptions as in scenarios 1 and 2 except that now both the prey species were equally

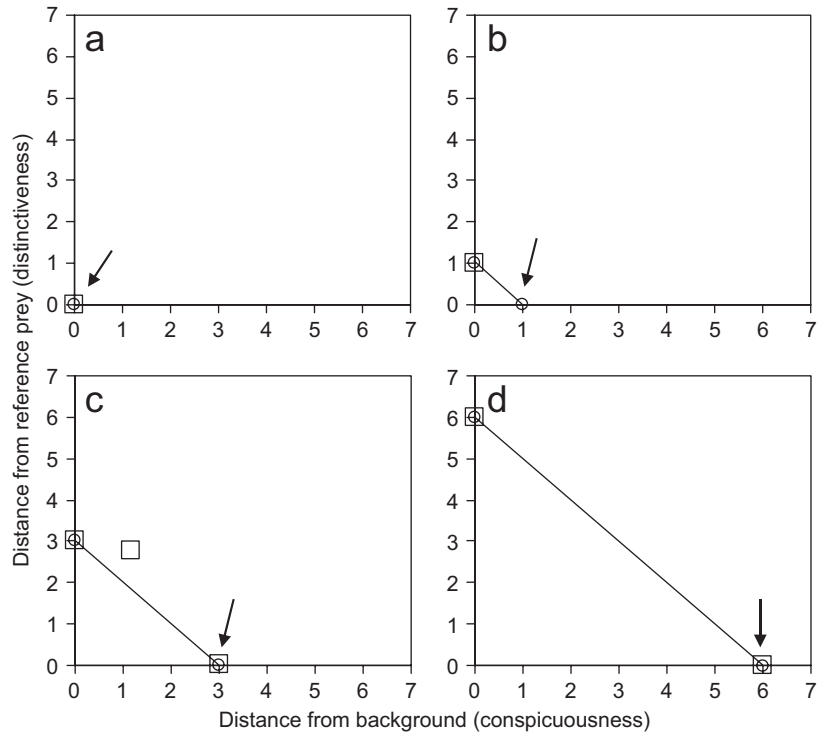


Fig. 2. Exactly as Fig. 1 except now the focal prey is undefended and the reference prey defended.

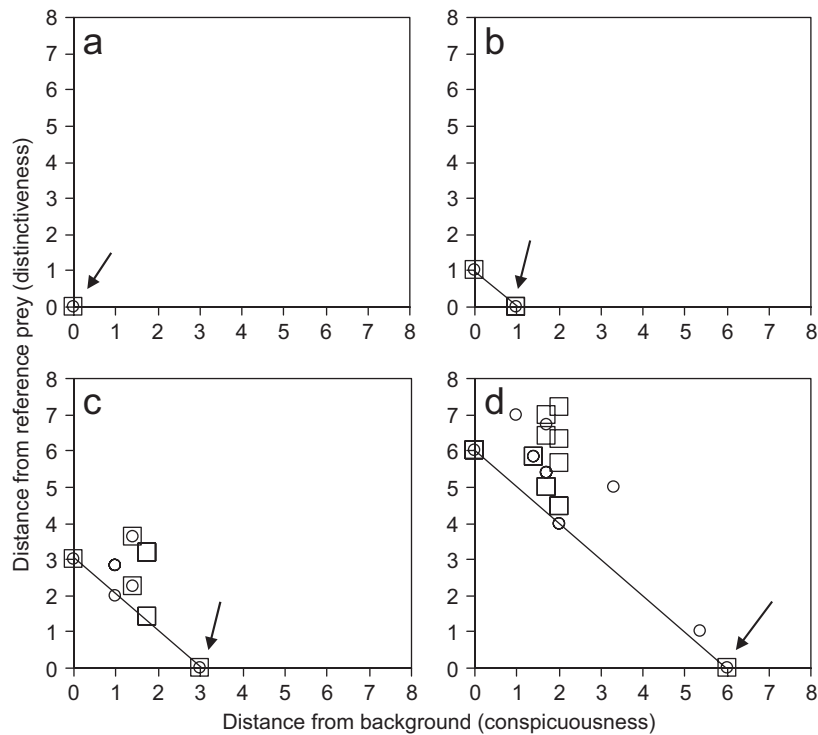


Fig. 3. Exactly as Fig. 1 except that the benefit from aposematism increases with the frequency of the appearance phenotype in the total prey population. The focal prey escaped mimicry (i.e. changed from the initial appearance indicated by the arrow) in (a) 0, (b) 97, (c) 30 and (d) 25 replicates out of 100.

defended. Because both species were equally defended, there was no selection for mimicry avoidance (distinctiveness) (Fig. 4). A comparison between Figs. 1 and 4

demonstrates quite different predictions in the two cases, and thus provides further evidence that selection for distinctiveness had an important effect on evolution in

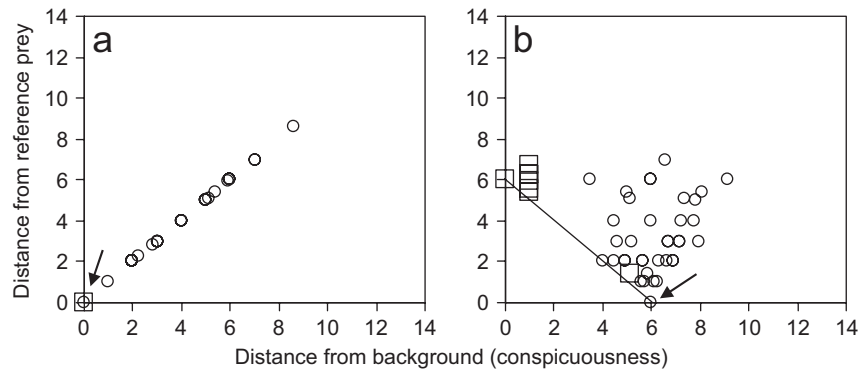


Fig. 4. Exactly as Fig. 1, but now both prey species are equally and maximally unpalatable. The two panels differ in the appearance of the reference prey and hence in the initial appearance of the evolving focal prey: conspicuousness = (a) 0; (b) 6.

scenario 1, and that the results in that case cannot simply be explained solely by selection for conspicuousness against the background.

4. Discussion

One important general prediction of our model is that defended prey need not always evolve to advertise their defence with conspicuous aposematic colouration. Many models of the evolution of aposematism start with the scenario of a population of cryptic but defended prey in which a mutation conferring conspicuousness arises (see Chapter 8 of Ruxton et al., 2004 for a recent review). Such models have been criticized over the apparent implausibility of prey that are protected by camouflage investing in secondary defences, and other works explicitly assume that cryptic species will be undefended (Ruxton and Sherratt, 2006 and references therein). However, here we show that defended prey that start off being less than maximally cryptic can evolve to become maximally cryptic (e.g. Fig. 1d). Hence, our work lends credence to the possibility of investment in secondary defences without these defences necessarily triggering evolution away from cryptic appearance (Broom et al., 2006). In our model, defences were fixed and cost free.

Previous studies have indicated the importance of constraints and costs and benefits of alternative strategies in the evolution of prey coloration (Merilaita and Tullberg, 2005; Speed and Ruxton, 2005). Our study lends further support for this as changes in cost to benefit ratio (i.e. the benefit asymmetry) affected whether the prey evolved towards cryptic or signalling coloration.

We must also address the realism of the undefended but less than maximally cryptic reference prey used in the simulations shown in Figs. 1b–d. There may be many possible reasons for an undefended prey type not to be maximally cryptic. Firstly, it is important to remember that there are likely to be constraints and costs to camouflage. These may take the form of costs of pigment production, or opportunity costs related to behavioural aspects of camouflage or physiological costs associated with the

constraints on morphology imposed by camouflage (see Chapter 5 of Ruxton et al., 2004 for a recent review). Alternatively or additionally, one could imagine that the prey live in a heterogeneous environment and have adopted an appearance that makes them less than maximally cryptic against one of the backgrounds that they encounter, which happens to be the background used in our study (Merilaita et al., 1999). Hence, there is nothing ecologically implausible about the undefended but conspicuous reference prey used in scenario 1.

Another important implication of Fig. 1 is that when aposematic signalling was selected, then there was always great variation between the 50 simulations in the end-point of evolution. Since these 50 replicates differed only in the random events related to mutation and recombination, this model suggests that the end-point of evolution for aposematism can be dependent on random factors, and hence this model provides one explanation for the variety of aposematic signals that we see in the natural world around us. There may be some mechanisms such as Müllerian mimicry, sensory bias and developmental constraints that act to restrict the variety of possible aposematic signals. However, it is clearly the case that not all species have converged on the same warning signal. Here, we present a plausible explanation for the existence of factors driving such variation, not just between species but even between sub-populations of one species.

In general there has been a tendency to consider the function of aposematic signals as being to allow defended prey to stand out against their background. An important conclusion that can be drawn from Figs. 1c and d is that this need not be the only function of such signals and indeed it may not even be the primary one. Specifically, Figs. 1c and d suggest that a key driver of appearance in our focal prey is the need to differentiate themselves from the undefended reference prey. We are by no means the first to suggest this mechanism (Fisher, 1930; Sherratt and Beatty, 2003), but we do hope that our demonstration of this effect in a formal qualitative mathematical model will encourage greater consideration of this neglected mechanism. However, care must be taken in interpreting our

results. It is true that our model shows that conspicuousness can result even without direct selection for conspicuousness. However, we cannot exclude the possibility that conspicuousness may indeed yield some form of direct benefit (e.g. a memorability benefit), a benefit that was not included in our model.

Compared to the study by Sherratt and Beatty (2003), our study agrees that selection for distinctiveness (mimicry avoidance) is important for defended prey. However, there are also differences between the studies. For example, both studies suggest that distasteful prey may evolve to crypsis, but while Sherratt and Beatty (2003) emphasized selection for distinctiveness (mimicry avoidance) as the reason, our study suggests that it can simply be caused by selection for concealment (see also Sherratt and Franks, 2005). This also points out an important difference between the models. Because we enabled prey appearance to evolve freely instead of only allowing switches between a few alternative phenotypes, in our model there is a large set of different possible outcomes. This enables us to more easily distinguish the effect of different selective forces such as selection for mimicry avoidance (i.e. distinctiveness) and selection for camouflage, or selection for mimicry avoidance and selection for conspicuousness. Importantly, our model suggests that despite the importance of selection for mimicry avoidance it is not sufficient to explain whether the prey turns out to be conspicuous or cryptic because there are many different ways to be conspicuous. Instead, if the protection given by cryptic appearance is stronger than the protection given by conspicuous appearance that signals for the defence then even a defended prey will evolve to a cryptic appearance (independently of whether there are undefended prey with that cryptic appearance). On the other hand, if crypsis does not provide good protection, then a defended prey will evolve towards a conspicuous appearance, or more correctly, away from cryptic appearance. In this case, selection for mimicry avoidance causes the conspicuous appearance to be different from the appearance of undefended prey. The result of scenario 2 (where the focal prey was undefended) shows the value of evolutionary simulations such as those presented here. In every situation that we modelled, the optimal strategy for the prey was either to be maximally cryptic or to be maximally alike to the reference prey. In any single simulation one of those alternatives yielded a higher fitness than the other, yet although the prey always evolved to one of these two local optima, it did not always evolve to the best one. This emphasizes the caution that must be used when applying optimality principles to biology (Alexander, 1996), it may sometimes be difficult for prey to evolve from one local maximum to a global maximum because of the challenges of crossing the phenotypic space in between them. In the natural world, some undefended prey have evolved to be Batesian mimics and some have not and have adopted camouflage. The route that a given species has gone down will in part be determined by the opportunities it has had (e.g. the

availability of suitable model species to mimic) but may also have been determined in large part by chance, or more specifically by the microevolutionary processes controlling mutation and the chance effects that control the success or failure of mutations.

It could be argued that change of appearance in scenarios 1 and 2 is unrealistically easy, because the protection afforded by mimicry was independent of the number of individuals sharing a particular appearance (being dependant only on the fraction of these individuals that are defended). Although little is known about the relationship between frequency of a defended prey type and avoidance response of its predators in natural communities, there is laboratory evidence suggesting that frequency is important for aposematic prey (e.g. Lindström et al., 2001). However, the advantage of higher numbers is the basis for the most-commonly accepted explanation for Müllerian mimicry (see Chapter 9 of Ruxton et al., 2004). Hence, we included this feature into scenario 3. As expected, this does decrease the frequency with which crypsis evolves, but importantly it does not decrease it to zero. One other aspect that can be seen by comparing Figs. 1 and 3 is that the numbers-dependence of Fig. 3 makes intermediate forms (that are neither maximally cryptic or maximally aposematic) a more likely outcome. Remember that the probability of mutation producing a phenotype with a given level of conspicuousness (or difference from the background) increases dramatically with increasing level of conspicuousness, because there is only one way to be similar (i.e. match the background), but several ways to be different. Accordingly, the probability of a mutation producing a phenotype with zero conspicuousness is $1/8 * 1/8 * 1/8 * 1/8 = 1/4096$ but for conspicuousness equal to one it is up to eight times higher ($1/512$). Thus, it is much more likely that a novel form produced by a mutation will be a moderately cryptic intermediate form than a form that perfectly matches the background. This together with the fact that number-dependence hinders gradual evolution towards the cryptic optimum in part explains why the intermediate forms are so common in Fig. 3. Another condition that needs to be satisfied for an intermediate form to invade the initial population is that the intermediate form must be cryptic enough to have higher fitness than the initial form.

However, as comparison of Figs. 1 and 4 emphasizes, the most important of our model results is that aposematic signals should be seen as having potentially two different functions: making the prey conspicuous in its environment and aiding predators in discriminating between that type and sympatric undefended prey types. Specifically, our models suggest that it is possible that in some cases conspicuousness may be a by-product of evolutionary pressure for distinctiveness. On the other hand, our model does not justify the exclusion of the possibility that conspicuousness as such may be a beneficial attribute (a function) of warning coloration. It is likely that the relative importance of the two mechanisms will differ on a

case-by-case basis; however, there is no empirical or logical reason for the current neglect of evolutionary pressure for distinctiveness.

In summary, our study suggests the following. Selection for distinctiveness can result in increased prey conspicuousness. However, it does not select for maximization of conspicuousness, and we note considerable empirical evidence that not all aposematic species have maximally conspicuous signals (Endler and Mappes, 2004; Tullberg et al., 2005). Further, selection for distinctiveness may even select for camouflage, depending on the appearance of other prey in the local environment and the relative benefits of camouflage and warning coloration. Finally, it may in practice be difficult to distinguish whether conspicuousness of a prey has been caused by selection for distinctiveness or selection for conspicuousness, if there is (or has been) another, edible and cryptic prey present in the community. We hope that the results provided in this paper stimulate further consideration of the importance of selection for distinctiveness. However, perhaps our work and that of Sherratt and Beatty (2003) already provide sufficient evidence to suggest that description of aposematism as the teaming of a secondary defence with a conspicuous signal may be overly simplistic; we would rather that the signal were described as conspicuous and/or distinctive.

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