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The Evolution of Warning Signals as Reliable Indicators of Prey Defense

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abstract: It is widely argued that defended prey have tended to evolve conspicuous traits because predators more readily learn to avoid defended prey when they are conspicuous. However, a rival theory proposes that defended prey have evolved such characters because it allows them to be distinguished from undefended prey. Here we investigated how the attributes of defended (unprofitable) and undefended (profitable) computer-generated prey species tended to evolve when they were subject to selection by foraging humans. When cryptic forms of defended and undefended species were similar in appearance but their conspicuous forms were not, defended prey became conspicuous while undefended prey remained cryptic. Indeed, in all of our experiments, defended prey invariably evolved any trait that enabled them to be distinguished from undefended prey, even if such traits were cryptic. When conspicuous mutants of defended prey were extremely rare, they frequently overcame their initial disadvantage by chance. When Batesian mimicry of defended species was possible, defended prey evolved unique traits or characteristics that would make undefended prey vulnerable. Overall, our work supports the contention that warning signals are selected for their reliability as indicators of defense rather than to capitalize on any inherent educational biases of predators.

Keywords: warning signals, aposematism, signal reliability, receiver bias, mimicry.

It has long been recognized that prey that possess significant defenses against predators tend to be conspicuous in some way (Wallace 1867; Darwin 1871; Poulton 1890). The contemporary explanation for this phenomenon, termed "aposematism" (Poulton 1890), is that there is

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"something special" (Guilford 1990; Speed 2000) about the educational properties of conspicuous traits as a signal of defense (Guilford 1988; Endler 1991; Mallet and Joron 1999). For example, it has been repeatedly shown that predators learn to avoid unpalatable prey more quickly when they are conspicuous than when they are cryptic (Gittleman and Harvey 1980; Gittleman et al. 1980; Roper and Wistow 1986). This theory for the evolution of aposematism is plausible, but there is an important caveat. Whatever the underlying cause of aposematism, it is likely that predators would evolve an enhanced psychological predisposition to learn to avoid conspicuous prey precisely because such prey tend to be defended (Turner 1984; Sherratt 2002).

While the idea that prey signals evolve to capitalize on the preexisting psychological biases of predators has become one of the most popular theories for the evolution of aposematism, there are several alternative theories (Guilford 1988; Endler 1991; Mallet and Joron 1999). One important rival explanation is that conspicuous traits simply make defended prey less liable to be confused with undefended prey, which are typically cryptic (Wallace 1867; Fisher 1930; Edmunds 1974; Turner 1975; Chai 1996). The idea was first hinted at by Alfred R. Wallace (Wallace 1867; Guilford 1990) but articulated most explicitly by R. A. Fisher (1930, p. 148), who compared the evolution of warning signals to the tendency of unpalatable models to evolve away from palatable mimics: "To be recognized as unpalatable is equivalent to avoiding confusion with palatable species." The advantages of looking different from undefended prey are clear: predators are more likely to learn to react appropriately to a signal if it is a reliable one (Zahavi 1993), and being distinguishable is essentially creating a reliable signal. However, it is less obvious why selection to avoid confusion might act on conspicuousness and not some other distinguishing characteristic such as pattern. One possibility is that such traits are among the least exploitable by undefended cheats (since nonmimetic conspicuous mutants of undefended prey would be readily detected and attacked) and therefore are the most persistent in evolutionary time. In sum, conspicuous traits may

have evolved as stable warning signals because these characteristics help distinguish defended prey from undefended prey and are difficult to exploit by undefended prey.

To test Fisher's theory of evolution of warning signals as a means of avoiding confusion and its corollary (namely, that defended prey evolve traits that are difficult to exploit by undefended prey), we investigated how the morphological and behavioral attributes of undefended (profitable) and defended (unprofitable) computer-generated prey evolved when subject to selection by foraging humans (see Bond and Kamil 1998, 2002 for an analogous approach to crypsis using blue jays as predators). Human models have long been used to test and refine ideas relating to predation (Holling 1959; Dill 1975; Knill and Allen 1995; Glanville and Allen 1997), and here their use was desirable not just because of the flexibility they allowed but also because participants would have few preconceptions concerning the profitability of novel prey they encountered (a "novel world"; cf. Alatalo and Mappes 1996; Lindström et al. 1999; Riipi et al. 2001).

Our computer experiments allowed us to independently control both the reliability of a given trait as a signal of defense and its conspicuousness. Many experiments have shown that conspicuous defended prey are attacked less frequently than cryptic conspecifics (e.g., Sillén-Tullberg 1985), particularly after a period of learning (e.g., Alatalo and Mappes 1996). However, we know of no experimental study on warning signals that has attempted to formally evaluate the relative importance of signal reliability compared to signal conspicuousness or one that has simulated changes in morph frequencies of prey from generation to generation as a result of selection.

Methods

To address the contrasting theories we have outlined, we conducted 14 related experiments, termed "trials" (table 1). Each trial was replicated a minimum of five times using different human volunteers as predators. The volunteers (more than 100 undergraduate students) had no knowledge of the experimental aims (the majority were non-biologists), and no individual participated in more than one replicate or trial. In each replicate, a single predator typically foraged for prey until 30 prey generations were complete. We therefore assumed that predators live longer than prey and that predators can potentially remember their experiences across prey generations (cf. Servedio 2000; Bond and Kamil 2002).

All experiments except trials 1 and 10 involved a single species of artificial undefended prey and a single species of defended prey (trial 1 had no defended species; trial 10 had an additional undefended species). When attacking

an undefended prey item, a human predator added b ($=1$) points to its total displayed score, while when attacking a defended prey item, a predator lost c ($=1$) points from its score. To enhance the stimulus (Rowe 2002) and to allow predators to know what they had attacked without having to view their score, attacks on undefended and defended prey generated distinct sounds (undefended: high pitch, rising scale; defended: low pitch, falling scale). Prey items that were killed simply disappeared from the screen. Undefended prey were always killed on attack, but defended prey survived attack (Järvi et al. 1981; Wiklund and Järvi 1982) with a fixed probability s, in which case they temporarily changed color (to highlight the fact that a defended prey had been attacked) and then returned to their former appearance. We set s to 0 except where stated, since the evolution of aposematism is least likely to arise under these extreme conditions.

The foraging environment of our volunteers consisted of a series of five separate screens that could be scrolled through by pressing the keyboard space bar. Each screen displayed a random mosaic of 20% green and 80% white pixels (fig. 1 shows a sample screen). At the start of each generation, 10 individual prey items from each of the two prey species were randomly distributed on each screen (except where stated). Presenting predators with mixtures of prey species is the standard experimental practice in studies of this kind (e.g., Alatalo and Mappes 1996; Lindström et al. 1999), and we chose to distribute our artificial prey across several screens because natural predators cannot search in all areas of their environment simultaneously and because it is quite possible that some individual prey never come into contact with their key predators at all.

All prey items were square (20 × 20 pixels, except in our aggregation trials; see below) and symmetrical in pattern around a central vertical or horizontal line (a "backbone"), which was drawn in its nonwhite color. Other aspects of appearance of each individual prey were controlled by two genes. The first gene gave the percentage of pixels in that individual that were nonwhite (with the exception of its backbone), while the second gene coded for that nonwhite color. We appreciate that the genetics underlying animal coloration are much more complex than this, but aposematism is a taxonomically widespread phenomenon that is likely to be controlled by many different genetic systems, and we saw no need to invoke anything more sophisticated to test the intuitive ideas we have described.

At the outset, players were shown how to move between screens and attack prey. They were made aware that prey could "mutate" and were asked simply to maximize their personal scores by attempting to attack profitable (undefended) prey while avoiding unprofitable (defended) prey. Generations came to an end when 30% of the total

prey population had been killed or (in trial 5) after a short fixed time (30 s); no species extinctions occurred using these algorithms. Surviving individuals of each prey species at the end of each generation were allowed to reproduce back to their starting density by randomly selecting a parent for each of the new generation from the total pool of available survivors. These offspring were randomly distributed among screens (10/screen). During reproduction there was a 5% chance, analogous to a mutation, that an offspring would have a different appearance than that of its parent. In most cases, this meant a radical increase or decrease in conspicuousness, which was brought about by altering the offspring genotype for both the percentage of nonwhite pixels and the nonwhite color (cryptic forms were typically 15% or 25% green, while conspicuous forms were typically 60% blue, red, or yellow).

In trial 1, we formally tested whether morphs that we refer to as cryptic were indeed more difficult to detect than forms that we refer to as conspicuous by running 31 separate replicates with five of each of the four prey types listed in table 1 distributed on each of five screens. The human subjects were informed that all the prey types were profitable and asked to forage at their discretion. Each replicate ended after 25 prey items had been attacked. All other trials (2–15) included defended prey and were replicated a minimum of five times for a minimum of 30 generations each (trial 11 was conducted for 50 generations, while trial 12 was replicated 10 times for each of four parameter combinations).

In trials 2–8, members of defended and undefended species each could occur in one of two discrete morphs: a cryptic form or a conspicuous form. In trials 2–6, the cryptic morphs of the two prey species were similar in overall appearance, but the conspicuous forms were not. Conversely, in trials 7 and 8, the conspicuous forms of undefended and defended prey were similar in appearance (65% and 55% red, respectively), but the more cryptic forms were readily distinguishable (30% yellow and 30% green, respectively). In trials 9–11, we investigated what forms evolved in defended and undefended prey when complete mimicry was possible (trial 9), when there were two undefended prey species and only one could be mimetic (trial 10), and when undefended and defended prey had two conspicuous morphs, only one of which was identical (trial 11).

Modifications to the basic experimental design were necessary to test specific ideas. To quantify just how readily aposematism emerged when mutations were extremely rare and to simultaneously explore the effect of varying s ($=0$ or 0.5), we began trial 12 with cryptic undefended and defended prey and introduced x ($=1$ or 2) novel conspicuous mutants of each species into each generation over generations 2–4 (no other mutations were allowed,

thereby restricting the mutation rate to three or six individual mutations in 1,500 offspring over the course of 30 generations). The rare mutational forms were completely distinct in that their color differed not only between species but also between generations.

We also modified our approach to understand the evolution of other distinguishing traits in defended prey besides their conspicuousness. Thus, in trials 13 and 14 we kept defended and undefended prey 50% green but allowed selection on their degree of aggregation by introducing a "clustering" gene; all individuals on a given screen that had allele 1 for this gene were distributed near a randomly selected cluster-point on that screen for that species, while individuals with allele 0 were placed at a random position within the screen. In this set of trials we reduced prey size to 5 × 5 pixels, but all other default parameter values were the same ($s = 0$ and 5% probability of mutation of the aggregation allele during reproduction).

Student t-tests examined whether defended and undefended species or the same species in control and treatment differed significantly in their final mean compositions. All proportion data were arcsine transformed prior to testing. Means are expressed ± 1 SE.

Results

Any prey item with a similar proportion of green pixels as its background was difficult to detect. These subjective impressions were confirmed from our analysis of trial 1, where the 15% and 25% green morphs comprised 17.4% and 17.0% of the total diet, respectively, while the red and blue morphs comprised 32.6% and 32.9% (test for homogeneity $G = 76.25$, df $= 3$, $P < .001$). The two green morphs were approximately equally cryptic ($G = 0.034$, df $= 1$, $P > .05$), as were the two nongreen morphs ($G = 0.008$, df $= 1$, $P > .05$).

To assess how readily our two types of standard cryptic prey were discriminated, we compared the numbers of undefended (15% green) and defended (25% green) prey attacked in the first generation of trials 2, 9, and 11 before any conspicuous mutants had arisen. If predators could tell the difference between these two prey types, then one would expect more undefended prey to be attacked. As a control, we also compared the attack rates on the two prey types in the first generation of trial 4, where the cryptic morphs were switched for defended and undefended prey (fig. 2). In both cases, predators showed clear evidence of being able to discriminate between the two cryptic prey types (one-tailed tests; trials 2, 9, 11: $t_s = 3.983$, df $= 28$, $P < .001$; trial 4: $t_s = 2.127$, df $= 8$, $P < .05$), although their discrimination was by no means perfect. Analysis of

Table 1: A summary of the experimental trials and their rationale

Trial	Morphs of undefended prey	Morphs of defended prey	Generations of prey	Mutation rate (%)	Replication	Starting conditions	Question
1	15% green, 25% green, 60% red, 60% blue	None	1	Not applicable	31	Five of each prey type on each of five screens	Are the green morphs more cryptic than the nongreen morphs, and are the two green morphs equally cryptic?
2	15% green, 60% blue	25% green, 60% red	30	5	5	All prey cryptic	Do defended prey evolve the morph that reliably signals their defense, or do they remain cryptic?
3	15% green, 60% blue	25% green, 60% red	30	5	100	All prey cryptic	(Control for trial 2 with no predation)
4	25% green, 60% red	15% green, 60% blue	30	5	5	All prey cryptic	Do we get similar results to trial 2 when morphs are reversed?
5	15% green, 60% blue	25% green, 60% red	30	5	5	All prey cryptic	Do we get similar results to trial 2 when new generations occur after 30 s?
6	25% green, 60% red	15% green, 60% blue	30	5	5	All prey conspicuous	Do we get similar results to trial 2 with different starting conditions?
7	65% red, 30% yellow	55% red, 30% green	30	5	5	All prey in red conspicuous form	Do defended prey adopt the cryptic morph if it reliably signals defense?
8	65% red, 30% yellow	55% red, 30% green	30	5	100	All prey conspicuous	(Control for trial 7 with no predation)

9	15% green, 60% red	25% green, 60% red	30	5	5	All prey cryptic	Do defended prey evolve conspicuous morphs if they can be mimicked?
10	Species 1: 15% green, 60% red; species 2: 15% green, 60% blue	25% green, 60% red	30	5	5	All prey cryptic	Can additional nonmimetic prey allow defended prey and their undefended mimics to evolve conspicuousness?
11	15% green, 60% blue, 60% yellow	25% green, 60% red, 60% yellow	50	5	5	All prey cryptic	Do defended prey adopt the morph that most reliably signals defense?
12	15% green, 60% blue, (gen. 2), 60% magenta (gen. 3), 60% yellow (gen. 4)	25% green, 60% red, (gen. 2), 60% cyan (gen. 3), 60% light gray (gen. 4)	30	$x = 1$ or 2 mutations per species, gen. 2–4 only, no other mutations	10 replicates for each value of x ($=1$ or 2), and s ($= 0$ or .5)	All prey cryptic	Do defended prey evolve conspicuous morphs when mutations are extremely rare?
13	50% green, aggregative; 50% green, dispersed	50% green, aggregative; 50% green, dispersed	30	5	5	All prey aggregated	Do defended prey remain aggregated because it makes undefended prey vulnerable?
14	50% green, aggregative	50% green, aggregative; 50% green, dispersed	30	5% for defended prey only	5	All prey aggregated	Do defended prey become dispersed when undefended prey cannot?

Note: In "Starting conditions," all green morphs were considered cryptic, while morphs with 60% nongreen color were considered conspicuous. In all trials except trial 1, there were initially 50 of each species in total, with 10 of each species distributed at random on each of five screens; gen. = generation.
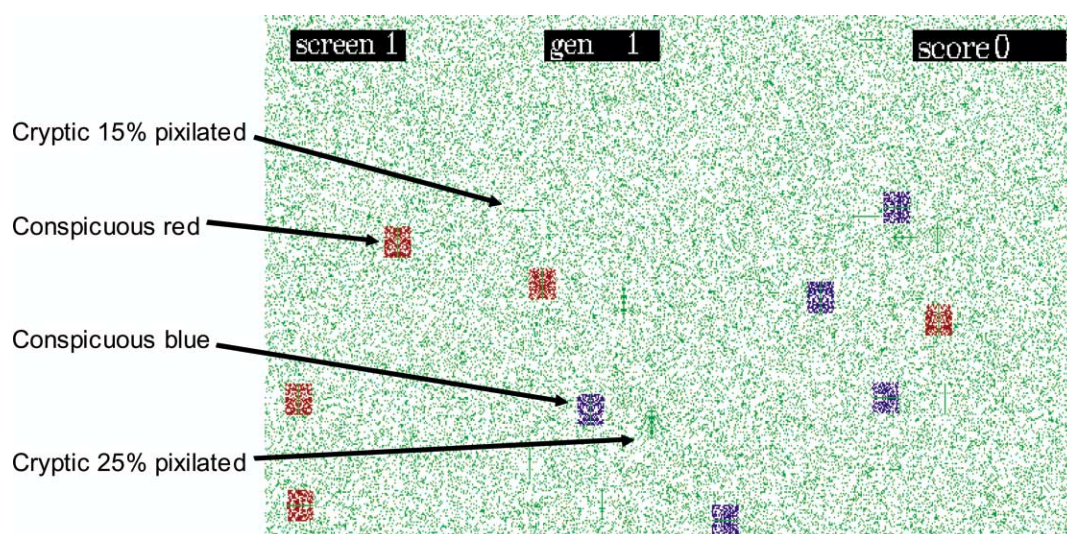
Figure 1: A typical screen shot showing randomly distributed conspicuous (60% red and 60% blue pixels) and cryptic prey (15% and 25% green pixels) on a background with 20% green pixels. The complementary pixel color for both the background and the prey was white.

the attack sequences show that, even toward the end of the first generation, mistakes were being made.

Starting with populations composed entirely of cryptic prey (trial 2), the undefended species tended to remain cryptic over 30 generations of selection while the defended species invariably became conspicuous (fig. 3*A* shows a typical replicate; the final mean proportions of conspicuous undefended and defended prey in all five replicates of this trial were 0.068 and 0.864, respectively; $t_s = 11.816$, df $= 8$, $P < .001$). This highly significant difference in conspicuousness between defended and undefended species cannot be explained by chance mutation and drift, which would act the same on the two species of prey. Indeed, control simulations with no predation (trial 3, 100 replicates) gave very different results (e.g., final mean proportion of undefended prey that were conspicuous was 0.480 ± 0.016; comparison of trials 2 and 3: $t_s = 7.166$, df $= 103$, $P < .001$). Most importantly, there was direct evidence of selection in all five replicates (and, indeed, in replicates of related trials): while predators were more likely than their cryptic conspecifics to attack conspicuous defended prey when they first appeared, they eventually avoided such prey types almost entirely (fig. 3*B*). The final mean score of predators in the first generation of prey in this trial was low (1.2) and not significantly different from 0 ($t_s = 0.514$, df $= 4$, $P > .05$). By contrast, the mean score of predators in the 30th prey generation (25.2) was significantly higher than zero ($t_s = 31.50$, df $= 4$, $P < .001$).

It is of course possible that the above results arose from a tendency of the human predators to avoid red or as some unexpected consequence of the criterion for starting a new generation. To control for these possibilities, we ran further replicated trials in which the two cryptic and two conspicuous mutational forms were switched for defended and undefended prey species (trial 4) and in which new generations arose after a fixed time rather than after consuming a fixed proportion of prey (trial 5). In both cases, the final mean proportion of conspicuous defended prey was significantly higher than the final mean proportion of conspicuous undefended prey (when colors were reversed, conspicuous defended prey was 0.744, and conspicuous undefended prey was 0.072 [$t_s = 8.365$, df $= 8$, $P < .001$]; when a time-based algorithm was employed, conspicuous defended prey was 0.728, and conspicuous undefended prey was 0.252 [$t_s = 3.706$, df $= 8$, $P < .01$]). As a final control, we noted that if the green morphs were cryptic, then one would expect undefended prey to evolve their green morphs even if they started out in their conspicuous form (trial 6). As anticipated, undefended prey rapidly became cryptic while defended prey remained conspicuous (final mean proportions of conspicuous undefended and defended prey were 0.316 and 0.900, respectively [$t_s = 13.106$, df $= 8$, $P < .001$]).

In the above trials, the cryptic forms of prey were readily confused, but the conspicuous forms were not. In trial 7, we examined how prey evolved when undefended and defended cryptic morphs were distinct in appearance while their more conspicuous forms were not. Despite the potential for predators to more rapidly learn to associate noxious qualities with conspicuous traits (Guilford 1990; Speed 2000), defended species always evolved their distinct cryptic form (final mean proportion of defended prey that
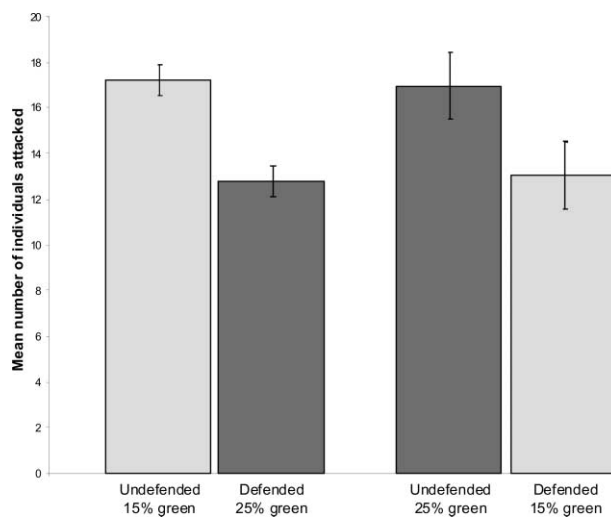
Figure 2: The mean numbers (± 1 SE) of the cryptic morphs of undefended and defended prey attacked per replicate in the first generation of trials 2, 9, and 11 and in the first generation of trial 4, where cryptic forms were reversed. No other morphs were present. In both cases, significantly more undefended prey than defended prey were attacked per replicate, suggesting that humans could tell these two equally cryptic morphs apart but not perfectly. (Color version is online.)

were cryptic was 0.784 ± 0.06, when compared to control trial 8 with no predation [effectively the same as trial 3; $t_s = 4.474$, df $= 103$, $P < .001$]).

In the preceding experimental trials (2, 4–7) we found that defended prey consistently adopted traits that allowed them to be distinguished from undefended prey. It was therefore of interest to determine what traits tended to evolve in defended prey when many of their characteristics could be mimicked by undefended prey. In trial 9, we ran five replicates in which defended and undefended prey had similar but distinguishable cryptic forms and an identical conspicuous form. Aposematism did not evolve under these conditions (final mean proportions of undefended prey and defended conspicuous prey were 0.40 and 0.252 respectively; $t = 0.14$, df $= 8$, $P > .05$). Interestingly, when we introduced a third, nonmimetic undefended prey species at the same density (trial 10), predators eventually foraged mainly on this species, and both the defended species and its potential mimic gained selective advantage by being conspicuous (final mean proportions of conspicuous forms in defended and mimetic species were 0.796 ± 0.135 and 0.788 ± 0.139). Similarly, when we allowed two conspicuous color morphs for each species, one of which was identical between species (trial 11), defended species eventually evolved their unique conspicuous form while undefended species remained cryptic (fig. 4). The final mean proportions of undefended and defended prey

that were conspicuous following five such replicates were 0.388 (65.6% of which were yellow) and 0.956 (2.9% of which were yellow), respectively ($t_s = 5.816$, df $= 8$, $P < .001$).

While conspicuous traits readily arose as reliable warning signals in many of the above trials, it is clear that this may have been facilitated by the continued reoccurrence of identical mutational forms in a given species. To quantify just how frequently aposematism might arise when mutations were extremely rare, we allowed only x ($= 1$ or 2) distinct conspicuous mutants to appear in each species over generations 2–4 (trial 12). The conspicuous forms of undefended prey never spread. By contrast, the final population of defended prey consisted of more than 50% of the conspicuous form s by generation 30 on three-tenths of replicates for $s = 0$, $x = 1$; one-tenth of replicates for $s = 0.5$, $x = 1$; six-tenths of replicates for $s = 0$, $x = 2$; and five-tenths of replicates for $s = 0.5$, $x = 2$. Here, conspicuous defended mutants managed to spread initially from extreme rarity, primarily by drift (the same chance mechanism occasionally reduced small but growing populations of conspicuous defended mutants to extinction), with aposematism more likely to arise when the original mutations first appeared in screens that were unvisited.

In our final trials 13 and 14, we allowed selection on the tendency of prey to aggregate. Starting with aggregated defended and undefended prey, undefended prey tended to lose their aggregation (although not entirely), while defended prey retained theirs (fig. 5). The overall final mean proportions of undefended and defended prey that were aggregated following five such replicates were 0.516 and 0.908, respectively ($t_s = 4.315$, df $= 8$, $P < .004$). Yet when undefended prey were forced to remain aggregated by preventing their mutation, then defended prey became disaggregated (mean proportion for defended prey with cluster gene was 0.432; difference in proportions of aggregated defended prey between the trials: $t = 9.746$, df $= 8$, $P < .001$).

Discussion

Although there appears to be a generally accepted hypothesis for the widespread occurrence of aposematism (Lindström et al. 2001, p. 9181: "Aposematic species are conspicuously colored because predators learn faster to avoid conspicuous patterns"), we do not believe that there is enough empirical evidence to accept this theory without reservation, particularly when there are plausible alternatives. Aposematism frequently arose in our study, and we believe that its emergence can be understood without the need to invoke any special educational properties of conspicuous signals. Indeed, our study strongly suggests
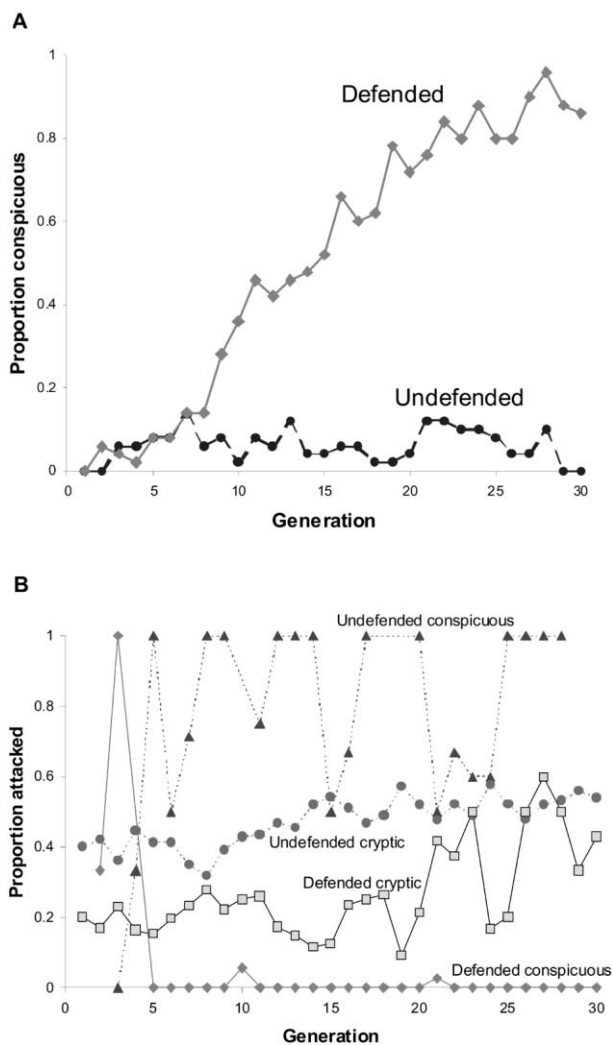
Figure 3: Results of a single replicate of trial 2. Here, cryptic morphs of undefended and defended prey were similar in appearance, but their conspicuous morphs were not. *A*, Changes in frequencies of the conspicuous forms of undefended (*dotted line*, *circles*) and defended (*continuous line*, *diamonds*) when subject to continued selection by a human volunteer. *B*, Proportions of particular morphs (*circles*, cryptic undefended; *triangles*, conspicuous undefended; *squares*, cryptic defended; *diamonds*, conspicuous defended) attacked each generation in the same replicate (only calculated when there were at least two such morphs initially present). Note that proportionately more undefended cryptic prey tended to be attacked than defended cryptic prey.

that even cryptic defended prey will be avoided if such prey are distinct and reliably defended (trials 7, 8), and this intuitive result is supported by a number of earlier experiments on warning signals (Papageorgis 1975; Gittleman and Harvey 1980; Gittleman et al. 1980). As Mallet and Singer (1987) report, many ithomiine butterflies are highly unpalatable, but to human eyes they appear more distinctive in appearance than conspicuous.

Clearly, we have not unequivocally proved that aposematism has arisen as a consequence of selection on defended prey to avoid being confused with undefended prey. However, our results go some way toward confirming Fisher's (1930) intuition that if defended and undefended prey are readily confused, then there will be strong selection on defended prey to exhibit characteristics that reliably distinguish them from undefended prey. Although novel conspicuous mutants of defended prey were always at an early selective disadvantage, compared to cryptic conspecifics (fig. 3A), informal student feedback suggested that they rapidly learned to avoid these prey types (with occasional reevaluations) because they were consistently unprofitable. By contrast, cryptic prey were frequently profitable, giving rise to selection in defended prey to evolve a form that could more reliably reflect their unprofitability.

Fisher's perspective is very different from the contemporary approach that has tended to focus on the evolution of effective educational aids in defended prey alone rather than on selection to maximize the phenotypic difference between defended and undefended prey. Nevertheless, there is some overlap between Fisher's theory and certain elements of the "receiver psychology" literature as it applies to aposematism. In particular, as Mallet and Joron (1999) noted, Fisher's view (1930) shares many features with the more recent application of the concept of "peak shift" (Hanson 1959; Leimar et al. 1986; Gamberale and Tullberg 1996; Gamberale-Stille and Tullberg 1999) whereby mutants of unpalatable prey are thought to gain selective advantage by being even further apart in appearance from palatable prey than current unpalatable prey. One key distinction, however, is that the peak shift mechanism is typically understood to work by a gradual increase of the conspicuousness of defended prey (mutations that are only marginally more conspicuous than the current unpalatable prey are at the greatest selective advantage), whereas Fisher's theory can explain selection for any mutant form that reduces the likelihood of its carrier from being confused with palatable prey.

We have argued that conspicuousness may be particularly effective and stable as a warning trait because it increases prey detectability, a burden that undefended prey items are unlikely to be able to adopt. Indeed in the absence of mimicry, undefended prey consistently evolved their more cryptic morph (trials 2, 4–6) because conspicuous mutants of undefended prey were rapidly exterminated. Our experiments have also shown that even a single conspicuous mutant of defended prey, which is easily detected and at first not as readily recognized as defended, may occasionally spread from rarity by chance alone (trial 12). The frequency at which aposematism arose in this particular trial was impressive (up to 50% of replicates), and it is possible to argue that even had aposematism
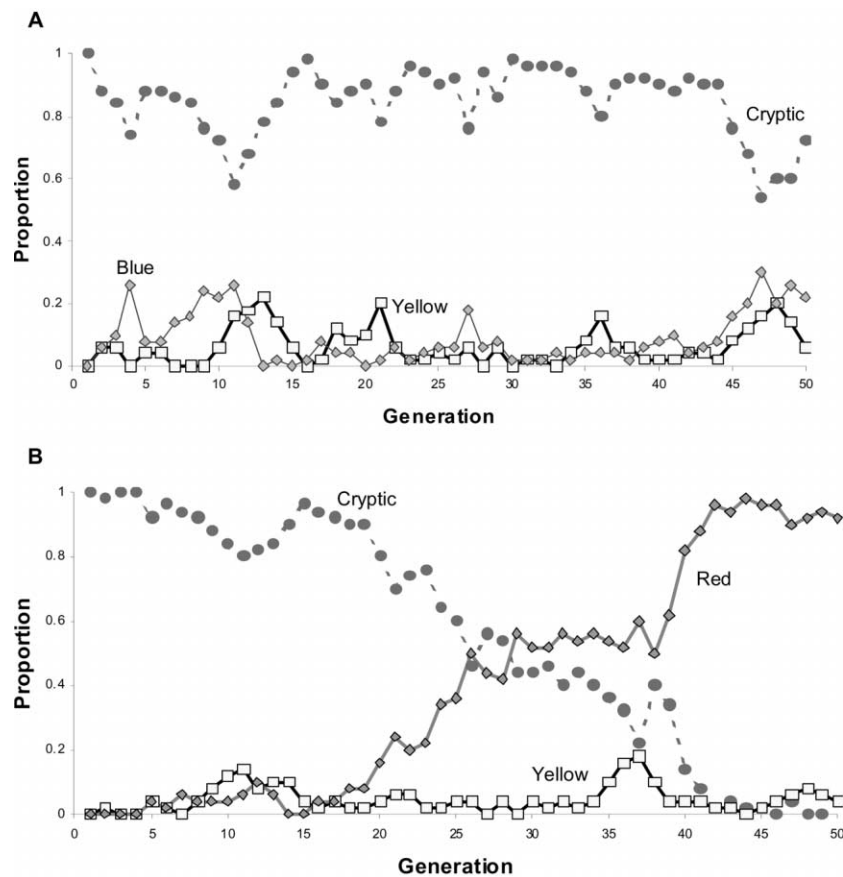
Figure 4: Results of a single replicate of trial 11. Undefended prey could occur in a 15% green (cryptic), a 60% blue, or a 60% yellow form (both conspicuous). Defended prey could occur in a 25% green (cryptic), a 60% red, or a 60% yellow form (both conspicuous). The graphs show the changes in frequencies of the forms of (*A*) undefended (*circles*, cryptic; *squares*, conspicuous yellow; *diamonds*, conspicuous blue) and (*B*) defended prey (*circles*, cryptic; *squares*, conspicuous yellow; *diamonds*, conspicuous red). (Color version in electronic edition.)

arisen in only 1% of trials, then its evolution would be likely, given sufficient time and sufficient unique mutational forms. Although there is an understandable tendency to seek deterministic solutions to evolutionary problems, our results provide some of the first experimental support for the contention that chance alone can allow conspicuous mutants to spread from extreme rarity (Mallet and Singer 1987; see also Mallet and Joron 1999 for further discussion of the application of "shifting balance" theory). It is clear that, once established in a local area, aposematic forms could resist dilution by cryptic immigrant conspecifics since the former would be at a strong selective advantage. Conversely, the continual emigration of aposematic forms may eventually allow sufficient education of neighboring predators for aposematic forms to spread in these areas.

Many traits are capable of being exploited by others, and there is often selection to reduce the extent to which

this occurs. For example, it is now widely recognized that the occurrence of nonaltruists may play an important role in determining the nature of cooperation that evolves between nonrelatives (see Dugatkin 1997). Likewise, it has long been appreciated that warning signals become less of a deterrent to predators when there is a high mimetic load (e.g., Sheppard 1959; Brower 1960; Pilecki and O'Donald 1971; Nonacs 1985; Lindström et al. 1997) and that models and mimics may therefore be engaged in an evolutionary "race" (Fisher 1930; Gavrilets and Hastings 1998). Given such observations, it is somewhat surprising that there has been so little discussion of the role of mimicry in shaping the initial evolution of warning signals. The fact that aposematism did not evolve when extensive amounts of mimicry was possible (trial 9) strongly supports the view that the exploitability of a signaling system is an important determinant of its long-term evolutionary success. Note that when not all prey can become mimetic (e.g., they lack
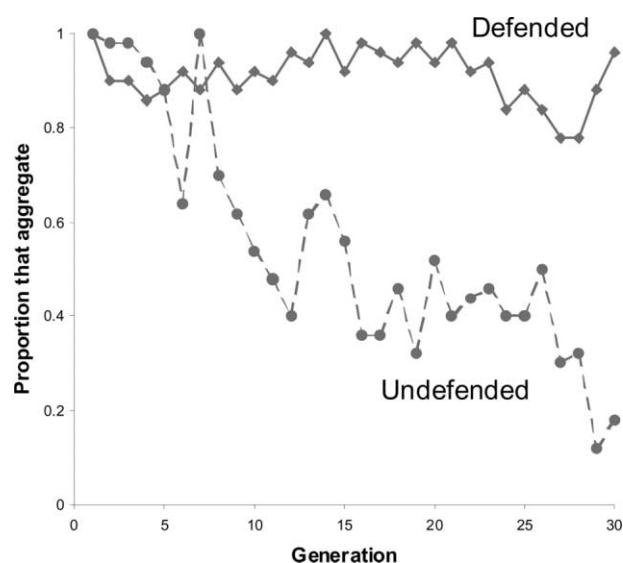
Figure 5: Results of a single replicate of trial 13. All prey items could occur in an aggregated or a dispersed form. The graph shows the changes in the proportion of individuals carrying the aggregation allele in undefended (*dotted line, circles*) and defended (*continuous line, diamonds*) prey when subject to selection by a human volunteer.

the size or shape), then predators may concentrate on prey that are reliably undefended (trial 10), allowing warning signals to evolve. Similarly, we have shown that signaling may still evolve if defended prey can adopt conspicuous phenotypes that for some reason cannot be mimicked (trial 11).

When defended prey and undefended prey could occur either in an aggregated or in a dispersed form, undefended prey rapidly became more dispersed. This arose simply because predators tended to concentrate on the clumps of prey and would pick off all undefended prey in a cluster if the first prey item was found to be undefended. Yet when undefended prey were forced to remain aggregated, it was defended prey that became dispersed because all dispersed prey in this system were reliably defended. Of course, there are other costs and benefits to living in groups besides signaling to predators, and groups may also be more conspicuous (Gagliardo and Guilford 1993; Riipi et al. 2001), but our results once again indicate that defended prey will tend to evolve characteristics that undefended prey cannot evolve, and/or would render undefended prey highly vulnerable.

We have primarily explored the possibility that defended prey are conspicuous because such traits set them apart from undefended prey, but it is worth noting that a similar set of arguments might also be applied to understand the evolution of other potential signals of defence, such as locomotory behavior. Slow and predictable movement is

common in defended species (Pasteels et al. 1983; Chai and Srygley 1990; Srygley and Chai 1990*a*, 1990*b*; Marden and Chai 1991; Hatle and Faragher 1998; Hatle et al. 2002), so much so that it is typically included in the syndrome of traits associated with chemical defense (Whitman et al. 1985). In a recent study Chai (1996, p. 63) commented on the rarity of Batesian mimics in Neotropical butterflies, noting that "the low frequency of cheaters is probably due to the fact that unpalatable butterflies with their associated morphological and behavioral adaptations facilitate detection and capture." We do not believe that this is coincidental. If defended prey were selected to adopt characteristics that reduced the rate at which they are confused with undefended prey, then one such set of characteristics that might be actively selected for is slow and predictable movement, since it would render any undefended species that possessed these characteristics highly vulnerable. Of course, this theory is untested, but it serves to show that the simple idea of honest signaling can explain the evolution of several very different traits regularly associated with defended prey.

It has been argued that warning signals are not handicaps (Zehavi and Zahavi 1997) in the sense of costly extravagances that only defended prey can afford (Guilford and Dawkins 1993). Our data are consistent with this view because once aposematic forms established, then signal reliability was maintained more by the costs of dishonesty in undefended prey than by the costs of honesty in all prey types (see also Viljugrein 1997; Lachmann et al. 2001). Here, all our similar aposematic prey were protected by virtue of sharing a common trait: benefits were transferred among individuals of like phenotype rather than specifically through relatives (see also Guilford 1985; Mallet and Joron 1999). We appreciate that once aposematism arises, then it may generate behavioral responses in predators that make its evolution even more likely in other species (Turner 1984; Sherratt 2002; Speed and Ruxton 2002). However, our experiments suggest that the most fundamental reason why defended prey tend to be conspicuous is because it sets them apart from undefended prey. For this reason, mathematical models of the evolution of aposematism that have concentrated exclusively on the evolution of traits in defended prey (e.g., Harvey et al. 1982; Sillén-Tullberg and Bryant 1983; Yachi and Higashi 1998; Speed 2001) may have missed something important. Indeed, if the reliability of a warning signal is an important determinant of its evolutionary success and conspicuousness facilitates honesty, then the predisposition of predators to learn more rapidly from conspicuous signals may be more of a symptom than a primary cause of aposematism.

Acknowledgments

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