

Automimicry destabilizes aposematism: predator sample-and-reject behaviour may provide a solution

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Aposematism, the use of conspicuous colours to advertise unpalatability to predators, is perhaps the most studied signalling system in nature. However, its evolutionary stability remains paradoxical. The paradox is illustrated by the problem of automimicry. Automimics are palatable individuals within a population of unpalatable aposematics. Automimics benefit from predators avoiding warning coloration without carrying the models' cost of unpalatability, and should increase in the population, destabilizing the signalling system, unless selected against in some way. Cautious sampling, instead of avoidance, by predators may offer a solution to this problem. Here, we investigate the effect of automimic frequency on predator sampling behaviour, and whether predator sampling behaviour may provide a selection pressure against mimics. Domestic chicks (*Gallus gallus domesticus*) were subjected to the task of discriminating between green (signalling) and untreated brown chick crumbs. Some of the green crumbs were quinine treated and thus unpalatable. The frequency of palatable signalling prey items varied in four treatments; all unpalatable, low automimic frequency, high automimic frequency and all palatable. The results show that predator sampling behaviour is sensitive to automimic frequency and that predators may discriminate between models and mimics through sampling, and thereby benefit unprofitable prey. The results suggest somewhat surprisingly that aposematic signalling is stable only because of the actions of those predators not actually deterred by warning signals.

Keywords: animal signalling; cheating; frequency dependence; mimicry; warning coloration

1. INTRODUCTION

Aposematism, the use of conspicuous warning colours to advertise unpalatability to predators, has been studied and discussed for over a century (Bates 1862; Darwin 1871). Accordingly, much is known about the circumstances under which an aposematic strategy may evolve, and how warning coloration works as an effective signal of unprofitability to potential predators (reviewed in Guilford 1990). Nevertheless, there are several factors that may destabilize an aposematic strategy, of which perhaps intraspecific Batesian mimicry, or automimicry (Brower *et al.* 1967), has been the least discussed. An automimic is a palatable individual within a species where typically individuals are unpalatable and warningly coloured. The degree of unpalatability may vary between individuals within a species (Brower *et al.* 1972; Gibson 1984). This variation may be a result of the fact that many unprofitable insects depend on their host plants for their anti-predatory defences (Brower 1969; Peterson *et al.* 1987), and that the concentrations of these defensive compounds often vary between plant individuals, both between and within host plant species. Nevertheless, many defences are specific adaptations, toxin sequestration can have a negative effect on biomass (Bowers & Collinge 1992) and growth (Camara 1997), and feeding on hosts containing high levels of toxins can reduce larval survival (Adler *et al.* 1994) and digestive and metabolic efficiency (Kelley *et al.* 2002), demonstrating that it too is costly.

Because automimics are visually indistinguishable from their models, then where unpalatability is costly, automimicry renders aposematism unstable (Guilford 1994). This means that when automimics are rare they benefit from predator avoidance of the warning signal, without bearing the cost of unpalatability, and so increase in the population: they are perfect cheats. Subsequently, as mimic numbers increase, predator attacks increase as a result of reduced avoidance learning but as much towards unpalatable as palatable individuals, according to their frequencies. Eventually, the warning coloration, which in turn carries the cost of a high risk of detection, ceases to be sufficiently associated with unpalatability, and aposematism destabilizes. Without any specific cost to the automimics, to balance the cost of unpalatability carried by the models, there is no reason to believe that the frequency of automimics would stop increasing in the system. Theoretical models support the importance of automimicry effects, and suggest that warning coloration, when producing a lower attack rate, may work antagonistically to unprofitability, and that a physiological cost of unprofitability should be balanced by anti-predation benefits to the prey (Leimar *et al.* 1986).

Automimicry therefore presents a problem for the theory of aposematism as it is conventionally understood. A possible solution lies in understanding the subtleties of predator behaviour. Specifically, if predators can somehow discriminate between automimics and their models, they could provide a selection pressure against automimics that has a stabilizing effect on aposematism. As models and mimics in this case are visually indistinguishable from each other, predators cannot use the warning coloration as an avoidance signal, and are left with directly sampling the prey. So, if at least some predators adopted a sample-and-reject

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strategy of carefully sampling signalling prey to distinguish cheats from honest signallers, automimics would suffer more from predation than models, unpalatability would remain beneficial, and an aposematic strategy could be stabilized (Guilford (1994); see also Augner & Bernays (1998) for similar reasoning about mimicry in plant–herbivore systems). All previous approaches to this problem have assumed that predators cannot distinguish between models and mimics, and that predator sampling always leads to death, independently of prey profitability (Huheey 1964; Brower *et al.* 1970; Pough *et al.* 1973). However, if predator sampling behaviour is to affect automimic and model frequencies, then truly aposematic individuals would need to survive predator attacks better than do mimics. Several experiments using real aposematic species as prey are consistent with this, because they show a surprisingly high survival of distasteful prey after predator attacks (Boyden 1976; Järvi *et al.* 1981; Wiklund & Järvi 1982, and references therein; Tullberg *et al.* 2000; Nylin *et al.* 2001).

The critical question, though, is whether predators can respond to the frequency of automimics in a way that reduces automimic viability selectively with their increasing frequency, when both they and their unpalatable models display the same visual signal. Here we present an experimental test of the sample-and-reject hypothesis by investigating predator sampling behaviour in a discrimination between signalling and non-signalling prey. We investigate how attack behaviour is affected by the frequency of automimics in a signalling prey population, and whether this in turn affects the relative survival of models and automimics.

2. METHODS

Domestic chicks (*Gallus gallus domesticus*) of mixed sexes were used as model predators in the experiment. Newly hatched birds were obtained from a commercial hatchery, and were housed in groups of *ca.* 20 individuals in metal cages (80 cm × 45 cm × 35 cm) that were heated with ceramic heat lamps, and had the floor covered with wood chips. We kept the birds in a room temperature of 20 °C and a 12 L : 12 D regime, and supplied them *ad libitum* with chick starter crumbs and water.

Both brown (untreated) and green chick crumbs were used as prey in the experiment. The green crumbs had been dyed by spraying them twice with 1 : 5 parts of green food colouring (Langdale's Green, E.F. Langdale Ltd, Suffolk, UK): water and allowed to dry in-between. Some of the green crumbs were also made distasteful by spraying them with a 3% solution of quinine hydrochloride. Quinine hydrochloride is water soluble and chicks do not produce much saliva, so to ensure that the chicks would immediately be able to taste the noxiousness of the quinine flavoured crumbs when pecking at them, the unpalatable crumbs were kept moist during the experiment by spraying them with distilled water. To moisten the crumbs did not seem to hinder the birds from picking them up.

The experiment took place in a circular runway made of wood that was *ca.* 20 cm in width and with 25 cm high walls on either side. The inner diameter of the runway was *ca.* 60 cm. The arena was lit with three 60 W desk lamps, as well as with the general fluorescent strip lights of the experimental room, none of which emit any ultraviolet (UV) light (quinine can fluoresce under UV). There were 24 circular wells of 4 cm in diameter, sunk *ca.* 1 cm into the floor, and spaced uniformly around the runway. The prey

were presented in these wells on a background of white office paper.

The experiment was designed as a discrimination task between green (signalling) and brown (palatable) crumbs. There were four treatment groups with different proportions of automimics present among the signalling green crumbs: (i) all unpalatable (0%); (ii) low automimic frequency (50%); (iii) high automimic frequency (75%); and (iv) all palatable (100%). Before the start of the experiment, we trained the birds to forage alone for single crumbs placed in the wells of the runway. This training started with birds in groups of three or four individuals being allowed to forage from piles of both green and brown palatable crumbs placed in the wells. By gradually reducing the number of companion chicks, and the number of crumbs presented in the wells, most chicks eventually habituated to feeding alone for short periods in the arena. Birds that repeatedly showed signs of distress from this treatment were excluded from the experiment. At the end of training and during the discrimination learning experiment, every second well contained a green crumb and every other well contained a brown crumb. Thus, the stimuli were familiar to the birds at the start of the experiment. We chose this procedure to avoid the possibility that neophobic responses might affect the birds' behaviour and confuse the results.

Training took place twice a day, and each training period was preceded by a maximum of 2 h of food deprivation. From the second day of training and on throughout the experiment, each training period consisted of two sessions of a maximum of 2 min each (four sessions per day). We started a bird's discrimination training when it had learned to traverse the runway readily and consecutively eat all the crumbs, one from each of the 24 wells. This usually happened after the third training day. The final sample sizes were: (i) all unpalatable: $n = 7$; (ii) low automimics: $n = 7$; (iii) high automimics: $n = 6$; or (iv) all palatable: $n = 5$.

The chicks were subjected to 10 sessions of discrimination training. The unpalatable crumbs were evenly distributed among the wells containing green crumbs and their locations were known. In each session we recorded the number of palatable and unpalatable crumbs attacked, how many of each type of prey were eaten or rejected after tasting, and the time it took for birds to traverse the runway.

3. RESULTS

The proportion of automimics among the signalling prey affected the birds' behaviour (mean total attack probability on green crumbs, Kruskal–Wallis ANOVA by ranks; $H_{3,25} = 20.692$, $p = 0.001$; figure 1). With no automimics present, birds learned to avoid attacking green crumbs (mean attack probability in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 2.366$, $p = 0.0180$). When the proportion of automimics was relatively low, birds still learned to avoid signalling prey to some degree (mean attack probability in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 2.201$, $p = 0.0277$), but when the proportion of automimics was higher, birds did not avoid the green crumbs at all (mean attack probability in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 0.135$, $p = 0.8927$; figure 1).

When looking more closely at the birds' sampling behaviour of the signalling crumbs, we see that they generally seem to taste and reject most unpalatable crumbs attacked (mean proportion eaten of attacked crumbs \pm s.e.m. = 0.022 ± 0.0093) and eat most of the palatable

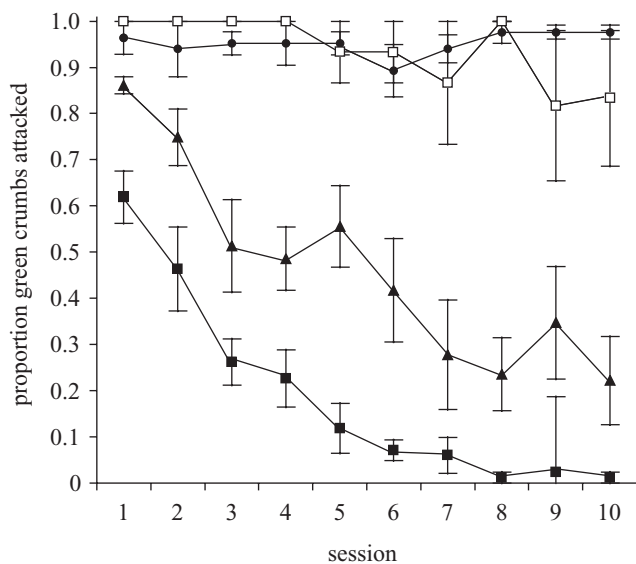


Figure 1. The proportion (mean \pm 1 s.e.m.) of all the green crumbs presented that were attacked in sessions 1–10 by birds in the four treatments: all unpalatable (closed squares); low automimic frequency (triangles); high automimic frequency (circles); and all palatable (open squares).

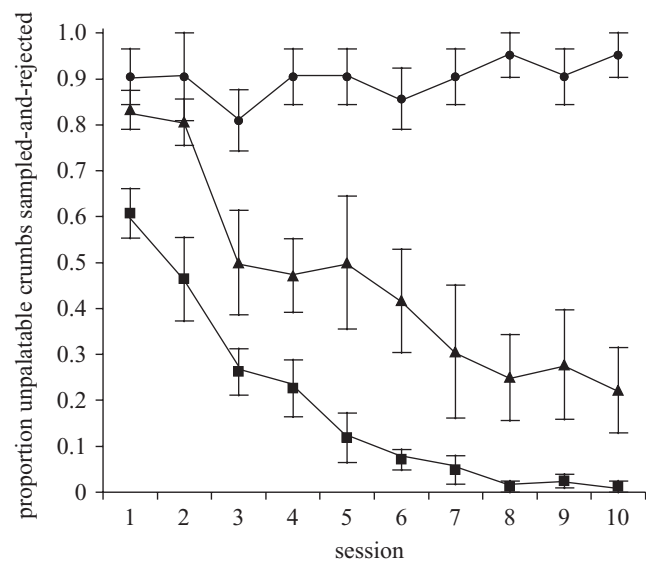


Figure 2. The proportion (mean \pm 1 s.e.m.) of the unpalatable green crumbs presented that were tasted and rejected in sessions 1–10 by birds in the treatments: all unpalatable (squares); low automimic frequency (triangles); high automimic frequency (circles).

crumbs attacked (mean proportion eaten of attacked crumbs \pm s.e.m. = 0.963 ± 0.017). Thus, they seem to differentiate between automimics and models on the basis of sampling. Also, the fact that the chicks did not show any difference in attack probability between palatable and unpalatable green crumbs (mean attack probability in sessions 6–10, Mann–Whitney U -tests; low proportion of automimics: $Z = 0.447$, $p = 0.6547$; high proportion of automimics: $Z = 0.16$, $p = 0.8728$) suggests that they could not visually detect the quinine treatment.

Critically, the frequency of automimics in the system also seems to have an effect on the proportion of the unpalatable crumbs present that were sampled and rejected (Kruskal–Wallis ANOVA by ranks; $H_{2,20} = 16.9382$, $p = 0.0002$; figure 2). Birds' sampling of the unpalatable crumbs when there are no automimics present decreases with avoidance learning and eventually almost ceases (mean proportion unpalatable crumbs sampled in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 2.366$, $p = 0.018$). Also, when there is a low frequency of mimics in the system, the sampling of unpalatable crumbs decreases with experience (mean proportion unpalatable crumbs sampled in sessions 1–2 versus 3–10, Wilcoxon signed rank test: $Z = 2.201$, $p = 0.0277$), but not to the same degree as when there are no mimics present (mean proportion unpalatable crumbs sampled in session 6–10, Mann–Whitney U -test: $Z = 2.571$, $p = 0.0101$). However, when there is a high frequency of automimics in the system, birds continuously sample and reject the unpalatable green crumbs (mean proportion green crumbs sampled in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 0.734$, $p = 0.4631$; figure 2).

The relative 'survival' of models and automimics is also affected by the frequency of automimics in the system (figure 3), if we assume that both unattacked crumbs and crumbs that have been sampled and rejected 'survive' the encounter. Throughout the experiment, 'survival' is

greater for the unpalatable prey than for palatable mimics, both when there is a high (Mann–Whitney U -test: $Z = 3.1304$, $p = 0.0017$), and a low frequency of automimics present (Mann–Whitney U -test: $Z = 2.8823$, $p = 0.00395$). Whereas the 'survival' of unpalatable prey, the models, does not change with the frequency of automimics (Kruskal–Wallis ANOVA by ranks; $H_{2,20} = 1.8571$, $p = 0.395$), the 'survival' of automimics differs between treatments, and is greater when there are fewer automimics present (Mann–Whitney U -test: $Z = 3.000$, $p = 0.0027$). In the situation with a low frequency of automimics, the 'survival' of the automimics increases as the birds start to learn to avoid green crumbs (mean proportion survived prey in sessions 1–5 versus 6–10, Wilcoxon signed rank test: $Z = 2.201$, $p = 0.0277$), but never during the experiment does it reach the same survival rate as the models (figure 3).

The presence of automimics did not, however, affect the time it took the birds to traverse the runway and sample the prey. There was no significant difference in the mean duration of sessions between treatments (mean \pm s.e.m.: (i) 32.58 ± 4.86 ; (ii) 33.93 ± 3.98 ; (iii) 43.07 ± 6.03 ; (iv) 39.30 ± 3.01 ; Kruskal–Wallis ANOVA by ranks: $H_{3,25} = 4.158$, $p = 0.2449$).

4. DISCUSSION

The results suggest that an aposematic system may suffer quite a large frequency of automimics, here at least 50% (cf. the 'low automimic frequency' treatment), and birds may still learn to avoid the warning signal, albeit to a lesser degree than when there are no automimics present. However, in the presence of too many automimics (cf. the 'high automimic frequency' treatment), the warning signal ceases to work as an avoidance signal, because birds do not reduce attacks on signalling prey at all. Thus, if signalling prey were discriminated on the basis of their signals alone,

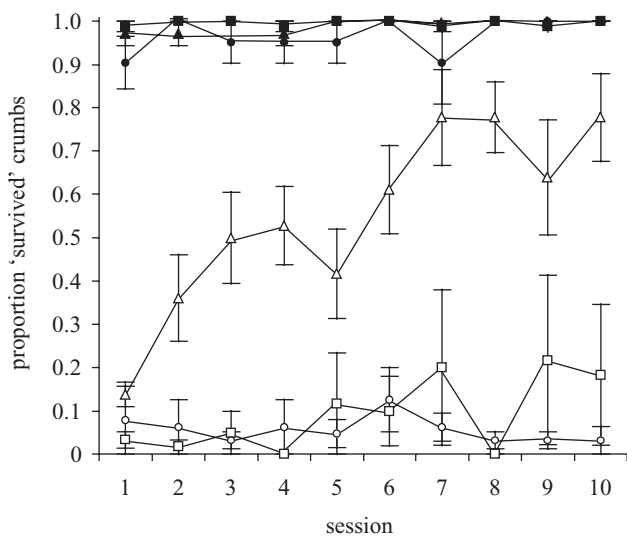


Figure 3. The proportion (mean \pm 1 s.e.m.) of palatable (open symbols) and unpalatable (filled symbols) green crumbs presented that survived in sessions 1–10 by birds in the four treatments: all unpalatable (squares); low automimic frequency (triangles); high automimic frequency (circles); and all palatable (open squares).

an aposematic system might well be sensitive to the existence of automimics, if there is no cost to cheating.

However, this experiment suggests such a cost, as predator sampling may affect models and automimics differently, and benefit unpalatability within an aposematic species. As the birds were able to sense prey palatability immediately on attack, they could sample and reject most unpalatable crumbs and eat most palatable crumbs, showing that they discriminate visually identical automimics and models by sampling. In both treatments containing automimics in our experiment, the unpalatable prey items potentially ‘survived’ sampling better than did the automimics, as they were not consumed by predators. As we used artificial prey in this experiment, we could not ascertain the cost of being sampled and rejected by the chicks. Then again, as mentioned above, distasteful prey often survive sampling by predators, at least better than do palatable prey. Also, the existence of species possessing a chemical or mechanical defence but lacking warning coloration suggests a direct individual advantage of unprofitability, as predators supposedly reject these prey on post-attack characteristics alone.

This critical role of sampling behaviour suggests that the traditional view of aposematic signalling needs refining. Sampling, in which unpalatable prey survive attacks, is essential for the continued favourability of anti-predator defences, and may have led to characters such as tough bodies, active chemical and/or mechanical defences that directly can avert predators, either before or during capture (e.g. Whitman *et al.* 1990), and disagreeable substances secreted on attack (Kay *et al.* 1969; Holloway *et al.* 1991) or located in the outer parts of the body (Brower & Glazier 1975). However, sampling is also essential for stabilizing the signals that advertise these defences against invasion by cheats (automimics), and our results demonstrate that sampling behaviour may be used actively to distinguish palatable from unpalatable signalling prey in a way responsive to the

frequency of cheats. Hence, in stable aposematic systems, signalling is only stable because of those predators that do not regard the signals as warning signals in the traditional sense of avoidance signals. This is a situation similar to a signalling system of intraspecific aggressive displays that are stable because receivers often test them by counter attacks (Maynard Smith 1979; Andersson 1980; Enquist *et al.* 1985; but see Lange & Leimar (2003) and references therein).

Thus, aposematic systems are stabilized by the actions of naive or erroneously sampling predators, which associate no aversion with the signal. Or they are stabilized by the actions of experienced predators, which associate the signals with ambiguity about prey palatability, and sample adaptively in response. In neither case is the critical advantage to aposematic signalling to be found in its warning or avoidance function. Future work will be needed to distinguish which of these two mechanisms is most important, because both may be operating in our experiment.

We acknowledge Julian Howe for his help, and thank two anonymous referees for their comments. This study was financed by STINT (The Swedish Foundation for International Cooperation in Research and Higher Education) and VR to G.G.S.

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