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Hard to catch: Experimental evidence supports evasive mimicry

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ABSTRACT

Most research on aposematism has focused on chemically defended prey but signalling difficulty of capture remains poorly explored. Similar to classical Batesian and Müllerian mimicry related to distastefulness, such “evasive aposematism” may also lead to convergence in warning colours, known as evasive mimicry. A prime candidate group for evasive mimicry are *Adelpha* butterflies, which are agile insects and show remarkable colour pattern

convergence. We tested the ability of naïve blue tits to learn to avoid and generalise *Adelpha* wing patterns associated with difficulty of capture, and compared their response to that of birds that learned to associate the same wing patterns with distastefulness. Birds learned to avoid all wing patterns tested, and generalised their aversion to other prey to some extent, but learning was faster with evasive prey compared to distasteful prey. Our results on generalisation agree with longstanding observations of striking convergence in wing colour patterns among *Adelpha* species since, in our experiments, perfect mimics of evasive and distasteful models were always protected during generalisation and suffered the lowest attack rate. Moreover, generalisation on evasive prey was broader compared to that on distasteful prey. Our results suggest that being hard to catch may deter predators at least as effectively as distastefulness. This study provides empirical evidence for evasive mimicry, a potentially widespread but poorly understood form of morphological convergence driven by predator selection.

KEYWORDS

Adelpha - evasive aposematism - predator learning - distastefulness – convergence - prey defence

BACKGROUND

Many organisms with chemical, morphological or behavioural defences often display a conspicuous signal, such as a colour pattern, that warns predators of the potential cost of attacks [1]. Possession of such warning signals is known as aposematism [1,2]. In many cases, the effectiveness of aposematism in terms of

prey avoidance depends on the ability of predators to associate the signal with an unpleasant experience (i.e. learning and lasting memory), and to attribute signal properties to different prey individuals (i.e. generalisation, reviewed in [3]; [4–6]). Aposematic prey are under positive frequency-dependent selection, which can result in convergence of warning signals among co-occurring defended species, known as Müllerian mimicry [7]. Aposematism and Müllerian mimicry associated with distastefulness have been extensively studied in many taxa [8–11], and especially so in Lepidoptera [12–16]. However, there is increasing evidence that aposematism may also be associated with an alternative defence, namely evasiveness ([17,18]; reviewed in [19]). Theoretically, predators should avoid attacking evasive prey since unsuccessful attacks likely represent a significant cost in time and energy [19–21], similar to that described for prey that signal long handling times [22]. Selection exerted by predators is thus expected to drive convergence in signals that they associate with the evasiveness of their prey [18,23–27], in a process known as escape mimicry or evasive mimicry (hereafter we use the latter term).

Previous experiments have shown that bird predators can use visual cues to identify evasive prey [28–30], but more empirical work is needed to test whether outstanding potential examples of evasive mimicry could indeed be the result of selection for such signals related to evasiveness. One such example is the diverse Neotropical butterfly genus *Adelpha*, where repeated convergence of their apparently conspicuous and contrasting wing patterns among distantly related sympatric species has been interpreted as evidence for mimicry [31–33]. Putative aposematic displays occur in *Adelpha* butterflies when they are at rest (not perching) with the wings open, and during flight as well, which involves short

bursts of wing beats and longer periods of gliding i.e., with the wings open and horizontal).

Mimicry in *Adelpha* has been hypothesized to be at least partly driven by chemical defences in some species [34–36], but there is currently limited, conflicting evidence for distastefulness [25,35,37,38]. In contrast to most classic groups of chemically defended butterflies, *Adelpha* butterflies have short and stout thoraxes which are favourable traits for strong flight [37,39], and exhibit an irregular flight with sharp turns and powered dives when pursued by avian predators (K.W., personal observations, [21]), making the genus a prime candidate for evasive mimicry [40]. Moreover, species resembling *Adelpha* exist in distantly related lineages (e.g., females from the genus *Doxocopa*, belonging to a different subfamily [21,31]), whereas closely related, allopatric *Adelpha* species may harbour different wing colour patterns, suggesting convergence rather than inheritance from a shared ancestor [32,33,41].

In this study, we use artificial prey models based on common *Adelpha* wing patterns and wild blue tits as naïve bird predators to address the following questions: 1. Can birds learn to associate wing colours and patterns with evasiveness of prey? 2. Can such a signal be generalised across putative mimetic species? 3. What type of defence drives faster learning by predators, evasiveness or distastefulness?

MATERIALS AND METHODS

We used wild blue tits (*Cyanistes caeruleus*) to examine whether birds learn to avoid *Adelpha* colour patterns associated with evasive (escaping) behaviour, and whether birds generalise the learned avoidance across similar, naturally

100 occurring wing patterns. In addition, we conducted parallel experiments with
101 distasteful prey having the same colour pattern but not evasiveness. European
102 blue tits were chosen as predators because: 1) they have no previous experience
103 with wing colour patterns displayed by Neotropical *Adelpha* butterflies, 2) they
104 have been used extensively in learning experiments as naïve (and experienced)
105 predators with various types of prey [4,42–44], 3) they are visual foragers and
106 their visual capabilities are well understood [45,46], and 4) they eagerly catch
107 both stationary prey as well as moving prey (e.g., flying butterflies, JM personal
108 observations). Potential unwanted behaviours of naïve predators (e.g., startling,
109 fleeing prey sooner or no motivation to attack) were controlled first during the pre-
110 training phase in their home aviaries the day before the experiment, and then in
111 the experimental aviaries; an approximate 2–3-hour habituation period was
112 needed until startling, or no motivation to attack disappeared (See electronic
113 supplementary material S1).

114 Experiments were conducted from January to March 2019 at Konnevesi
115 Research Station in Central Finland, which provided the infrastructure, wildlife
116 research and collection permits, and expertise needed to conduct experiments
117 with wild birds in captivity. Blue tits were captured from feeding sites around the
118 station and were maintained in captivity for a maximum of 10 days. During
119 captivity, they were kept singly in illuminated plywood cages (daily light period of
120 12 h 30 min) with food and fresh water available *ad libitum*. After experiments,
121 each bird's sex and age were determined, birds were ringed and released into
122 the site of capture. Our sample comprised individuals of both sexes (51 males
123 and 36 females) and was composed mainly by juveniles (65 out of 87), which
124 likely reflects natural variation in the composition of blue tit winter flocks (JM

personal observations, see [47]). We performed preliminary generalized linear mixed models (GLMMs) to test the effects of sex and age on learning. We did not find any significant effect of sex on learning ($Z=0.55$; p value= 0.58; CI= 0.82 – 1.42), therefore, we excluded the sex factor from further analyses. Although juveniles tended to learn slightly faster than adults (mean $\pm$ SD number of attacks until learning criterion is achieved: adults 53.1 $\pm$ 21.4; juveniles 49.4 $\pm$ 20.7), the effect of age on learning was not significant either ($Z=1.906$; p value= 0.06; CI 0.99 – 1.93), in agreement with studies that have not detected a strong age effect in blue tits' learning of novel prey (e.g., [48]). We therefore also excluded the age factor from further analyses.

Artificial prey

Artificial defended prey (4.1 x 2.5 cm) were constructed by printing images (HP Color Laserjet CP2025, regular printer paper) of different wing colour patterns displayed by the species *Adelpha salmoneus* (orange forewing band), *A. cocala* (orange and white transverse band), and *A. epione* (white forewing band; figure 1). These species represent three putatively distinct mimicry rings [31,33] and were chosen because they differ in colour and pattern. We used these to test if apparently distinct signals may provide protection from predation in evasive mimicry. An entirely dark brown model of a non-defended prey was constructed as a control. To make prey attractive for birds, a piece of almond (reward) was glued to the underside of prey. For distasteful models (see below), almonds were soaked in chloroquine phosphate solution (7%) to give them a bitter taste (following e.g., [49]).

Experimental procedures

The experiments took place in experimental aviaries of 49 x 48 x 67 cm. To mimic daylight conditions, aviaries were illuminated with a TRUE-LIGHT Daylight 6000 20W (960 lm) fluorescent light bulbs (these lamps do not emit enough amount of UV to be reflected by our paper models). Each aviary contained a perch and a water bowl. Birds were observed through a one-way glass situated on the front of the aviary. Two plastic prey holders gliding on aluminium profile rails (fixed on both sides of the aviary's floor) allowed simulation of the artificial prey's escaping (see electronic supplementary material, S1-figure 2 and a video is available in S4).

Avoidance learning

We used 87 birds, trained to attack artificial butterflies (see the electronic supplementary material, S1 for details of the training procedure), divided into 3 treatment groups (figure 1). The first two groups were trained to avoid evasive prey and a third group was trained to avoid distasteful prey with the same wing colour pattern as group 2. Before initiating the experiment, birds were habituated to the experimental aviary for at least an hour. In the treatment group where birds were trained to avoid escaping prey, the learning experiments consisted of presenting simultaneously two prey items to the bird, one control and one displaying an *Adelpha* wing colour pattern. Birds had one opportunity of attack per trial. If they approached the control prey, they were allowed to capture and eat the almond of that prey; if they approached the evasive prey, it was rapidly pulled out of reach (i.e., escaping) when the bird was less than 5 cm from the prey and displaying a clear intention to attack (see electronic supplementary material S1 and video S4). In the treatment group where birds were trained to avoid distasteful prey they were allowed to consume the attacked prey (i.e.,

distasteful prey and control prey). Training presentations continued for at maximum 80 trials or until the bird attacked an evasive or distasteful prey no more than twice over ten consecutive trials. This learning criterion was important for two reasons: 1) it allowed us to test if some treatments were associated with a faster learning than others; and 2) it ensured that all birds, i.e., “quick” and “slow” learners, reached the same level of knowledge despite encountering different numbers of preys (“quick” learners encountered fewer preys than “slow” learners) which is important for generalisation.

Birds that finished the experiment earlier and were able to continue with the generalisation experiment the same day received a break of at least 2 hours with 3 sunflower seeds offered every 30 minutes before starting the next experiment. Otherwise, those birds that finished the experiment late in the afternoon were placed back in their home cages and continued with the generalisation experiment the next day. Birds that finished the experiment but did not achieve the learning criterion were not included in the generalisation test.

Generalisation of learned avoidance to other prey (imperfect mimics)

We used only birds that achieved the learning criterion in the previous phase (group 1 n=23 out of 28, group 2 n=25 out of 31, group 3 n=18 out of 28) to test whether and to what extent the previously learned avoidance of warning colouration associated with evasiveness (group 1 and 2) or distastefulness (group 3) can be remembered and generalised to novel wing colour patterns that shared similar features (i.e., either colour or pattern, figure 1). Those novel colour patterns are referred to as imperfect mimics. This phase consisted of a single trial where birds encountered simultaneously four types of prey: a (i) control prey, (ii)

the model they have previously learned (which can be regarded as a perfect mimic) and (iii) two imperfect mimics (figure 1). The experiment was finished after the first choice of attack was registered (see electronic supplementary material, S1 for detailed description).

Before starting the experiment, each bird received, on average, a 15-minute habituation period to the new mechanism for presenting prey (see electronic supplemental material, S1 for details), during which three pre-training prey with one sunflower seed were offered. When the three pre-training prey and the sunflower seeds were consumed, the bird was considered ready to start the test.

Figure 1. Schematic illustration of the experimental design that consisted of 3 phases: pre-training, learning and generalisation. A forewing orange-banded prey (*A. salmoneus*) was presented as a model and perfect mimic for group 1, and as an imperfect mimic during generalisation for group 2 and 3. A transverse forewing orange/hindwing white-banded prey (*A. cocala*) was the model and perfect mimic for group 2 and 3, and an imperfect mimic during generalisation for group 1. The forewing white-banded prey (*A. epione*) was presented as an imperfect mimic during generalisation for all groups.

Statistical analyses

Avoidance learning

We examined whether wing colour pattern affected learning of birds from group 1 (n=28) and group 2 (n=31) by assessing the probability of prey survival within trials. Learning curves and their confidence intervals (figure 2A) were estimated as a function of the interaction between treatments (i.e., groups) and trial ("ggeffects" package version 4.0.3 in RStudio). We performed a generalized linear mixed model (GLMM) ("lme4" package v.1.1.23 in RStudio version v.4.0.2) with a logit link function and binomial distribution. Survival probability of prey within trial was explained by the wing colour pattern (explanatory variable). To account for repeated measurements, bird ID nested within trial was added as random factor. Additionally, we calculated the odds ratio (OR) based on GLMM estimates and its confidence interval (CI) to assess the strength of the effect of different wing patterns.

Comparison of avoidance learning between evasive and distasteful prey

To compare avoidance learning among birds facing aposematic prey signalling for evasiveness and birds facing aposematic prey signalling for distastefulness with the same colour pattern (group 2 and 3, respectively; figure 1), we performed another GLMM following the method above. Survival of prey within trial was explained by the type of prey defence (i.e. evasiveness or distastefulness). Bird ID nested within trial was defined as random factor. Odds ratio based on GLMM estimates and confidence intervals were calculated as well to assess the strength of the effect of type of defence.

238

239 **Figure 2.** Predicted probability of prey survival among trials. Each curve illustrates predicted
 240 values with their 95% confidence intervals (bands) for birds attacks on prey per group and
 241 among trials. Each plot shows the comparison between (A) group 1 versus group 2; and (B)
 242 group 2 (evasiveness) versus group 3 (distastefulness). The plotted data were derived from
 243 the generalized linear mixed models.

244 *Generalisation of learned avoidance to other prey (imperfect mimics)*

245 For each experimental group, to test for differences in attack probabilities
 246 between the different types of prey (the control, the model and the two imperfect
 247 mimics, figure 1), we calculated the log-likelihood of observing the number of

attacks that were recorded on each prey type compared to others in the group as follows (calculation details are in electronic supplementary material, S3.1).

$$\log_{10}(L) = \sum_i [a_i \log_{10}(P_i) + (N - a_i) \log_{10}(1 - P_i)] + K$$

Where i is one of the four prey types; N is the total number of trials; a_i is the number of times a butterfly of type i was attacked; P_i is the attack rate of butterflies of type i and K is a constant term that disappears in model comparisons. This maximum-likelihood method has been used in previous studies to estimate differences in probabilities of attack [16] and to investigate mate preference [50]. We explored several scenarios where attack rates of different types of prey could be equal or not (see figure 3 and electronic supplementary material S3.2 for a list of all those scenarios) and calculated the log-likelihood functions of those scenarios. As an example, a scenario where the attack rate on the control is equal to those on the imperfect mimics and higher than that on the perfect mimic means that birds only generalises the learned avoidance to the perfect mimic, and not to the imperfect mimics; a scenario where the attack rate on the perfect mimic is equal to those on the imperfect mimics and lower than that on the control means that birds have fully generalised the learned avoidance to the perfect and imperfect mimics; and a scenario where the attack rate on the imperfect mimics is lower than that on the control but higher than that on the perfect mimic means that birds have partially generalised the learned avoidance to the imperfect mimics, compared to the perfect mimic.

Models were selected on the basis of their AICc, which accounts for the number of parameters and the sample size. For each group, the model with the lowest

271 AICc was considered the best. We considered that models within a 2-unit AICc
 272 interval from the best model could not be rejected.

Here we present the case of group 1 where the model learned (and the perfect mimic) in the previous phase was the orange forewing band pattern P_o (orange forewing band). Bars illustrate the probability of attack of the control prey P_c (black coloured bar), imperfect mimics (grey coloured bars) such as the orange/white transverse band pattern P_{ow} for group 1 or orange forewing band pattern P_o for group 2 and group 3; and the white forewing band pattern P_w for all groups. For groups 2 and 3, the model (and the perfect mimic [orange coloured bar]) P_{ow} is the orange/white transverse band pattern.

273

274 **Figure 3.** Scenarios investigated based on attack rates from the generalisation experiment
 275 (group 1 is used as an example).

276

277 RESULTS

278 Avoidance learning

279 According to the learning criterion, most birds learned to avoid their evasive prey
 280 model: 23 out of 28 birds from group 1 (i.e., orange forewing band) and 29 out of
 281 31 birds from group 2 (i.e., orange/white transverse band). Additionally, 18 out of
 282 28 birds (group 3) learned to avoid the distasteful prey model.

283 The generalized linear mixed model detected no significant effect of treatments
 284 on predicted survival probabilities within trials ($Z=0.01$; $P=0.992$) ($OR=1.002$; CI

0.736 – 1.362) for group 1 and group 2, i.e., birds that learned to avoid different wing patterns of evasive prey. For group 2 and 3, (birds that learned to avoid evasive or distasteful prey that harboured the same pattern) a significant effect of treatment on predicted survival probabilities within trials was detected ($Z=3.60$; $P=0.0003$). Birds were 1.6 times more likely to attack distasteful prey than evasive prey ($OR=1.640$; $CI=1.248 - 2.159$) (figure 2B).

Generalisation of learned avoidance to other prey (perfect and imperfect mimics)

Bird attack frequencies on mimics differed within and among groups (figure 4, electronic supplementary material S2). For group 1 (prey with orange forewing band as evasive model, figure 4A, table 1), in the best scenario (s5) learned avoidance was fully generalised to the imperfect mimic that shared the orange colour with the model (orange/white transverse band), while the other imperfect mimic (white forewing band, which shares the pattern but no colour with the model) was attacked as much as the control. Two additional scenarios could be considered as similarly plausible based on their AICc. One scenario (s7) was similar to the previous, except that the orange/white imperfect mimic was attacked more often than the perfect mimic (but still less than the control), indicating partial generalisation. In the other scenario (s4), only the perfect mimic was attacked less than the control, implying generalisation only to the perfect mimic.

Regarding group 2, (orange/white as evasive model) (figure 4B, table 1), in the best scenario (s3) avoidance was fully generalised to both imperfect mimics, which both shared a colour with the model. Another scenario (s6), where generalisation to the mimics was partial, was within a 2-unit AICc interval with

that of the best scenario. In group 3 (orange/white as distasteful model) (figure 4C, table 1), a single scenario stood out as best, in which avoidance was fully generalised to the orange imperfect mimic (s5). See results from all the explored scenarios in electronic supplementary material S3.3.

Figure 4. Comparison among observed attack rates during generalisation tests. Bars illustrate proportion of attacks within groups on the control (black coloured bar), different putative mimics (grey coloured bars) and the perfect mimic (orange coloured bar) after birds learned to avoid the model pattern. Number of attacks are indicated inside the bars (at the top of the bars), as well as standard error bars. Birds from group 1 (A) learned evasive orange prey as the model; group 2 (B) learned orange/white as the model; and group 3 (C) learned distasteful orange/white as the model.

321 **Table 1.** Best scenarios from generalisation based on the AICc value. Scenarios within a 2-unit AICc interval with that of the best scenario are considered
 322 plausible as well.

group	best scenario	alternative scenarios	
(1) orange as evasive model	s5 [$P_c=P_w \neq P_o=P_{ow}$] Equal generalisation to the perfect mimic and to the orange/white imperfect mimic	s4 [$P_o \neq P_c=P_{ow}=P_w$] Generalisation only to the perfect mimic	s7 [$P_c=P_w \neq P_o \neq P_{ow}$] Generalisation to the perfect mimic and partial generalisation to the orange/white imperfect mimic
	ln(L) - K	-20.239	-20.545
	AICc	45.079	45.690
(2) orange/white as the evasive model	s3 [$P_c \neq P_{ow}=P_o=P_w$] Equal generalisation to the perfect mimic and both imperfect mimics	s6 [$P_c \neq P_{ow} \neq P_o=P_w$] Generalisation to the perfect mimic and partial generalisation equally to both imperfect mimics	
	ln(L) - K	-21.026	-20.710
	AICc	46.598	48.563
(3) orange/white as the distasteful model	S5 [$P_c=P_w \neq P_o=P_{ow}$] Equal generalisation to the perfect mimic and to the orange imperfect mimic		
	ln(L) - K	-14.095	
	AICc	32.990	

DISCUSSION

Learning and generalisation of signals associated with an effective escaping ability

The idea that some butterflies have evolved signalling of evasiveness as an anti-predator defence has a long history [21,24,51–53]. Still, surprisingly few experiments to date have tested the idea of evasive mimicry [25,28–30]. It is therefore unclear whether predators can learn, memorize and generalise naturally occurring signals associated with evasiveness, which is crucial for the evolution of evasive mimicry. Gibson [28,29] and Hancox & Allen [30] presented wild avian predators with artificial prey (i.e. dyed millet seeds, coloured mealworms or pastry models) that disappeared from sight when attacked. After extensive training (approx. 20 days), they observed that birds reduced their attacks on such hard-to-catch prey. We showed that wild birds, with no experience of *Adelpha* butterflies, were able to associate both orange and orange/white patterns with evasiveness within a day of training. Unlike previous experiments [28–30], our birds faced a “simpler” prey scenario [49], with a warningly coloured prey that could be easily discriminated from the non-defended prey, which may explain the reported faster avoidance learning compared to previous studies. In our experiment, birds were more likely to attack the control prey than the aposematic prey in the first trial of the learning phase. This is not surprising since wild birds often avoid colourful prey [54] and part of this bias seems to have a genetic basis [55]. However, our data provide evidence for additional learning on the top of this initial preference since our learning criterion (no more than two attacks in ten consecutive trials) implies that birds that fulfilled this criterion were at least four times more likely to attack control over aposematic

butterflies, which is much more than the initial bias (see electronic supplementary material S5).

Our results showed that birds were often able to generalise their learned avoidance to somewhat similar prey that shared either a colour or the pattern with the learned model. Perfect mimics were always strongly avoided but often at a level that could not be distinguished from those of the imperfect mimics. Previous work on distasteful prey found that learning and generalisation of aposematic visual signals by avian predators are primarily driven by colour rather than pattern [43,56–59] and wing shape [60]. Our findings are consistent with these studies for group 2 and partially for group 3 because birds generalised their avoidance to prey that presented a colour in common with the formerly learned model (orange imperfect mimic and white imperfect mimic for group 2; only to orange imperfect mimic for group 3), despite harbouring different wing patterns. Generalisation to imperfect mimics is supported with prey models from groups 2 and 3 (generalisation to only one imperfect mimic in the latter case). Generalisation to imperfect mimics was unclear for group 1 as results were mixed, possibly due to relatively low sample size and reduced statistical power. The different plausible scenarios for group 1 showed that avoidance was always generalised to the perfect mimic, was never generalised to the white imperfect mimic, and was sometimes generalised to the orange/white imperfect mimic, depending on the scenario. Overall, we showed that birds generalised their learnt association to evasive preys, although the cues used in generalisation remain unclear. Further experiments comparing models with different colours could shed light on whether some colours are better learned and/or generalised than others. The three *Adelpha* species we studied are not regarded as strongly co-mimetic, since a

number of other species show much more similar (practically identical) colour patterns, concordant geographic variation and broader sympatry [31]. Preliminary trials from a pilot test suggested that our predators were incapable of distinguishing among the most closely resembling co-mimics of *Adelpha cocala* (e.g., *Adelpha thesprotia*, see electronic supplementary material S1-figure 4), so we expanded our experiment to include more dissimilar species to examine the significance of mimetic accuracy. Our mixed generalisation results do not allow us to assess the extent of selection on mimetic fidelity in *Adelpha*. However, we show that perfect mimicry is at least as good as imperfect mimicry when providing protection to co-mimics since the model (perfect mimic) was always less attacked than the other mimics, although not always significantly so. Future work might include other aspects such as prey community structure, or the predator's level of hunger, that might affect prey mimetic fidelity, as has been studied in classical aposematism based on chemically defended prey [49,61–63].

In the case of *Adelpha*, it would be especially insightful to assess avoidance learning associated with evasiveness in the wild by their natural predators. Neotropical passerine birds tend to live longer than higher-latitude birds [64], which potentially means that they can learn more effectively and pay attention to finer signal details, and thus generalise less broadly to other similar prey. It is also important to keep in mind that not only the community structure of predators (see e.g., [65]), but also prey communities influence the outcome of avoidance learning and generalisation of distasteful prey [49].

Evasiveness versus distastefulness as deterrents to predators

Learning about distastefulness is thought to be generally quicker and easier than evasiveness because prey unprofitability can be determined, unambiguously, from a single experience when prey is ingested. By contrast, a prey individual might escape capture because of better escaping ability, or just because of chance [19]. There is thus some disagreement about the circumstances under which evasive aposematism and mimicry might occur and the extent to which its evolution might be different from that of aposematism and mimicry based on distastefulness [6].

In our experiments, in contrast to expectations [19], birds learned to avoid evasive prey faster than distasteful prey, and learning seemed to be easier as a higher proportion of birds achieved the learning criterion with evasive prey (94%) compared to distasteful prey (63%). It is well known that distastefulness of aposematic prey widely varies within and between populations [66,67], and that there is intra- and interspecific variation in predator's tolerance to distastefulness [63,68–71]. Signals associated with prey evasiveness may actually provide a more reliable message to birds about unprofitability than does aposematic signalling related to distastefulness. Moreover, catching a prey, even if distasteful, can be more rewarding for a predator than missing a prey completely. It is thus possible that the learning curve for avoiding evasive prey also depends on the physiological needs of a predator (e.g. its hungriness), the costs of pursuing and consuming a prey (i.e., the likelihood to catch, toxicity) and the nutritional benefits associated with a consumed prey. Although capturing an evasive butterfly prey might represent a rich source of nutrients compared to a distasteful prey (i.e., larger thoracic muscles that allow a powerful flight), there might also be a palatability spectrum, which likely affects the cost-benefit ratio.

Future experiments should simulate different scenarios to assess the importance of nutritional value of evasive versus a non-evasive prey in learning to avoid prey.

We also found a wider generalisation to imperfect mimics of the evasive prey when it was compared to that of the distasteful prey (i.e., group 2 versus group 3, where blue tits were trained on the same wing colour pattern). In group 2 (evasive treatment), in two out of three best scenarios birds generalised to some extent their learned avoidance toward the prey sharing any of the wing colours with the model, and both imperfect mimics were attacked less than the control. By contrast, in group 3 (distasteful treatment), birds only avoided the orange imperfect mimic, as the white imperfect mimic was highly attacked, despite the fact that the white colour was also present in the model. It has been suggested that selection for accurate mimicry can be affected by different factors [6] such as level of prey distastefulness or unpleasantness [72,73]. Although broad generalisation to imperfect mimics probably occurs when the model is highly distasteful or unpleasant (see in [74]), our results suggest that evasiveness is another powerful dimension of defence that affects a predator's decision whether to attack warningly coloured prey. Given that a wider generalisation was supported with some prey models, more experiments with different types of predators and signals are needed to examine whether generalisation tends to be broader across mimics where the model is defended by evasiveness rather than distastefulness or toxicity. In addition, a follow-up study to assess the memorability of naturally occurring signals of evasive prey (see [75]) and compare it to that associated to distasteful prey would be very insightful.

CONCLUSION

Although distastefulness has been considered a prime adaptive defence mechanism against predation in aposematic butterflies, evasiveness is also likely to be important in many groups. Our results give a strong experimental support for the hypothesis, mostly based on field observations, that predators can learn and generalise to some extent naturally occurring colour pattern signals that are associated with the escaping ability of prey. We therefore suggest that evasive mimicry could be a plausible explanation for colour pattern convergence in fast moving prey, such as *Adelpha* butterflies.

Ethics. The Southwest Finland Centre for Economic Development, Transport and Environment (VARELY/294/2015) and National Animal Experimental Board (ESAVI/9114/04.10.07/2014) provided permission to capture and keep wild blue tits (*Cyanistes caeruleus*) in captivity and to use them in behavioural studies.

Data accessibility The dataset and coding necessary to reproduce the results of this study are available in the Dryad Digital Repository (doi:10.5061/dryad.vq83bk3rj).

Author's contribution. JM, KRW, ME and PMM conceived the project. JM, EPV, JV, designed the experimental setup, with input from KRW and ME. EPV, JV, PMM and JM ran the experiments. EPV, JV and ME performed statistical analyses. All authors discussed the protocol and results throughout the study. EPV wrote the paper with contributions from all authors. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Footnotes. Electronic supplementary material is available online in Figshare:

REFERENCES

1. Poulton, E. B. 1890 *The colours of animals: their meaning and use. Especially considered in the case of insects*. The international scientific series, 2nd edn. London, UK: Kegan Paul, Trench Trübner, & Co
2. Cott HB. 1940 *Adaptive coloration in animals*. London, UK: Methuen

3. Mappes J, Marples N, Endler JA. 2005 The complex business of survival by aposematism. *Trends Ecol. Evol.* **20**, 598–603
4. Exnerová A, Štys P, Fučíková E, Veselá S, Svádová K, Prokopová M, Jarošík V, Fuchs R, Landová E. 2006 Avoidance of aposematic prey in European tits (Paridae): learned or innate? *Behav. Ecol.* **18**, 148–156. (doi:10.1093/beheco/arl061)
5. Skelhorn J, Halpin CG, Rowe C. 2016 Learning about aposematic prey. *Behav. Ecol.* **27**, 955–964. (doi:10.1093/beheco/arw009)
6. Ruxton GD, Allen WL, Sherratt TN, Speed MP. 2018 *Avoiding attack: the evolutionary ecology of crypsis, warning signals, and mimicry*. Oxford UK: Oxford University Press
7. Muller F. 1879 *Ituna* and *Thyridia*; a remarkable case of mimicry in butterflies. *Proc R Ent Soc Lond.* **1879**, xx–xxix
8. Symula R, Schulte R, Summers K. 2001 Molecular phylogenetic evidence for a mimetic radiation in Peruvian poison frogs supports a Müllerian mimicry hypothesis. *Proc. R. Soc. Lond. B* **268**, 2415–2421. (doi:10.1098/rspb.2001.1812)
9. Saporito RA, Zuercher R, Roberts M, Gerow KG, Donnelly MA. 2007 Experimental Evidence for Aposematism in the Dendrobatid Poison Frog *Oophaga pumilio*. *Copeia* **2007**, 1006–1011. (doi:10.1643/0045-8511(2007)7[1006:eefait]2.0.co;2)
10. Marek PE, Bond JE. 2009 A Mullerian mimicry ring in Appalachian millipedes. *Proc. Natl. Acad. Sci.* **106**, 9755–9760. (doi:10.1073/pnas.0810408106)

11. Wilson JS, Jahner JP, Forister ML, Sheehan ES, Williams KA, Pitts JP. 2015 North American velvet ants form one of the world's largest known Müllerian mimicry complexes. *Curr. Biol.* **25**, R704–R706. (doi:10.1016/j.cub.2015.06.053)
12. DeVries PJ. 1987 *The Butterflies of Costa Rica and Their Natural History. Vol. I: Papilionidae, Pieridae, Nymphalidae*. United Kingdom: Princeton University Press
13. Brown KS. 1988 Mimicry, aposematism and crypsis in neotropical Lepidoptera: the importance of dual signals. *Bull. Soc. Zool. Fr.* **113**, 83–101
14. Beccaloni GW. 1997 Ecology, natural history and behaviour of Ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiine). *Trop. Lepid.* **8**, 103–124
15. Mallet J, Mcmillan WO, Jiggins CD. 1988 Mimicry and warning colour at the boundary between races and species. 1998 in: *Endless Forms: Species and Speciation* (eds Howard, DJ. & Berlocher, SH), pp. 390-403. New York, Oxford Univ. Press
16. Willmott KR, Robinson Willmott JC, Elias M, Jiggins CD. 2017 Maintaining mimicry diversity: optimal warning colour patterns differ among microhabitats in Amazonian clearwing butterflies. *Proc. R. Soc. B* **284**: 20170744. (doi:10.1098/rspb.2017.0744)
17. Pinheiro CEG, Freitas AVL, Campos VC, DeVries PJ, Penz CM. 2016 Both Palatable and Unpalatable Butterflies Use Bright Colors to Signal Difficulty of Capture to Predators. *Neotrop. Entomol.* **45**, 107–113. (doi:10.1007/s13744-015-0359-5)

18. Guerra TJ. 2019 Evasive mimicry: too beetle, or not too beetle? *Ecology* **100**, e02773. (doi:10.1002/ecy.2773)
19. Ruxton GD, Speed M, Sherratt TN. 2004 Evasive mimicry: when (if ever) could mimicry based on difficulty of capture evolve? *Proc. R. Soc. Lond. B* **271**, 2135–2142. (doi:10.1098/rspb.2004.2816)
20. Hasson O. 1991 Pursuit-deterrent signals: communication between prey and predator. *Trends Ecol. Evol.* **6**, 325–329
21. Srygley RB. 1999 Incorporating motion into investigations of mimicry. *Evol. Ecol.* **13**, 691–708
22. Cyriac VP, Kodandaramaiah U. 2019 Don't waste your time: predators avoid prey with conspicuous colors that signal long handling time. *Evol. Ecol.* **33**, 625–636. (doi:10.1007/s10682-019-09998-9)
23. Brower JV. 1958 Experimental studies of mimicry in some North American butterflies. 1. The monarch, *Danaus plexippus*, and viceroy, *Limenitis achippus archippus*. *Evolution* **12**, 32–47. (doi:10.2307/2405902)
24. van Someren VGL, Jackson THE. 1959 Some comments on protective resemblance amongst African lepidoptera (Rhopalocera). *J. Lepidopterists' Soc.* **13**, 121–150
25. Pinheiro CEG. 1996 Palatability and escaping ability in Neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol. J. Linn. Soc.* **59**, 351–365. (doi:10.1111/j.1095-8312.1996.tb01471.x)
26. Pinheiro CEG, Freitas AVL. 2014 Some Possible Cases of Escape Mimicry in Neotropical Butterflies. *Neotrop. Entomol.* **43**, 393–398. (doi:10.1007/s13744-014-0240-y)

27. Pinheiro CEG, Campos VC. 2019 The responses of wild jacamars (*Galbula ruficauda*, Galbulidae) to aposematic, aposematic and cryptic, and cryptic butterflies in central Brazil. *Ecol. Entomol.* **44**, 441–450. (doi:10.1111/een.12723)
28. Gibson DO. 1974 Batesian mimicry without distastefulness? *Nature* **250**, 77–79
29. Gibson, DO. 1980 The role of escape in mimicry and polymorphism: I. The response of captive birds to artificial prey. *Biol. J. Linn. Soc.* **14**, 201–214
30. Hancox AP, Allen JA. 1991 A simulation of evasive mimicry in the wild. *J Zool.* **223**, 9–13
31. Willmott KR. 2003 *The Genus Adelpha: Its Systematics, Biology and Biogeography (Lepidoptera: Nymphalidae: Limenitidini)*. Gainesville, FL: Scientific Publishers
32. Willmott KR. 2003 Cladistic analysis of the Neotropical butterfly genus *Adelpha* (Lepidoptera: Nymphalidae), with comments on the subtribal classification of Limenitidini. *Syst Entomol.* **28**, 279–322
33. Ebel ER, DaCosta JM, Sorenson MD, Hill RI, Briscoe AD, Willmott KR, Mullen SP. 2015 Rapid diversification associated with ecological specialization in Neotropical *Adelpha* butterflies. *Mol. Ecol.* **24**, 2392–2405. (doi:10.1111/mec. 13168)
34. Aiello A. 1984 *Adelpha* (Nymphalidae): deception on the wing. *Psyche* **91**, 1–45. (doi:10.1155/1984/ 87930)
35. Finkbeiner SD, Salazar PA, Nogales S, Rush CE, Briscoe AD, Hill RI, Kronforst MR, Willmott KR, Mullen SP. 2018 Frequency dependence

shapes the adaptive landscape of imperfect Batesian mimicry. *Proc. R. Soc. B.* **285**, 20172786. (doi:10.1098/rspb.2017.2786)

36. Hill RI, Mullen SP. 2019 Adult Feeding as a Potential Mechanism for Unprofitability in Neotropical *Adelpha* (Limenitidini, Limenitidinae, Nymphalidae). *J. Lepid. Soc.* **73**, 66. (doi:10.18473/lepi.73i1.a11)

37. Chai P, Srygley RB. 1990 Predation and the Flight, Morphology, and Temperature of Neotropical Rain-Forest Butterflies. *Am. Nat.* **135**, 748–65

38. Finkbeiner SD, Briscoe AD, Mullen SP. 2017 Complex dynamics underlie the evolution of imperfect wing pattern convergence in butterflies. *Evolution* **71**, 949–959. (doi:10.1111/evo.13165)

39. Srygley RB, Dudley R. 1993 Correlations of the Position of Center of Body Mass With Butterfly Escape Tactics. *J. Exp. Biol.* **174**, 155–66

40. Mallet J, Singer MC. 1987 Individual selection, kin selection, and the shifting balance in the evolution of warning colours: the evidence from butterflies. *Biol. J. Linn. Soc.* **32**, 337–350. (doi:10.1111/j. 1095-8312.1987.tb00435.x)

41. Mullen SP, Savage WK, Wahlberg N, Willmott KR. 2011 Rapid diversification and not clade age explains high diversity in neotropical *Adelpha* butterflies. *Proc. R. Soc. B* **278**, 1777–1785. (doi:10.1098/rspb.2010.2140)

42. Kikuchi DW, Mappes J, Sherratt TN, Valkonen JK. 2016 Selection for multicomponent mimicry: equal feature salience and variation in preferred traits. *Behav. Ecol.* **27**, 1515–1521. (doi:10.1093/beheco/arw072)

43. Rönkä K, De Pasqual C, Mappes J, Gordon S, Rojas B. 2018 Colour alone matters: no predator generalization among morphs of an aposematic moth. *Anim. Behav.* **135**, 153–163. (doi:10.1016/j.anbehav.2017.11.015)
44. Rojas B, Mappes J, Burdfield-Steel E. 2019 Multiple modalities in insect warning displays have additive effects against wild avian predators. *Behav. Ecol. Sociobiol.* **73**. (doi:10.1007/s00265-019-2643-6)
45. Hart NS, Partridge JC, Cuthill IC, Bennett ATD. 2000 Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: The blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *J. Comp. Physiol. A* **186**, 375–87
46. Hart NS, Vorobyev M. 2005 Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *J. Comp. Physiol. A*. **191**, 381–92
47. Smith HG. 1987 Intraspecific Variation in Migratory Pattern of a Partial Migrant, the Blue Tit (*Parus caeruleus*): An Evaluation of Different Hypotheses. *The Auk* **104**, 109–115. (doi:10.2307/4087239)
48. Hämäläinen L, Mappes J, Rowland HM, Teichmann M, Thorogood R. 2020 Social learning within and across predator species reduces attacks on novel aposematic prey. *J. Anim. Ecol.* **89**, 1153–1164. (doi:10.1111/1365-2656.13180)
49. Ihalainen E, Rowland HM, Speed MP, Ruxton GD, Mappes J. 2012 Prey community structure affects how predators select for Müllerian mimicry. *Proc. R. Soc. B* **279**, 2099–2105. (doi:10.1098/rspb. 2011.2360)

50. Mérot C, Frérot B, Leppik E, Joron M. 2015 Beyond magic traits: Multimodal mating cues in *Heliconius* butterflies. *Evolution* **69**, 2891–904. (doi:10.1111/evo.12789)
51. Lindroth CH. 1971 Disappearance as a Protective Factor. A supposed case of Batesian mimicry among beetles (Coleoptera: Carabidae and Chrysomelidae). *Ent. scand.* **2**, 41–48
52. Holm E, Kirsten JF. 1979 Pre-adaptation and speed mimicry among Namib Desert scarabaeids with orange elytra. *J. Arid. Environ.* **2**, 263–271.
53. Srygley RB. Locomotor mimicry in butterflies? 1994 The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Philos Trans. R. Soc. London B* **343**, 145–55
54. Ham AD, Ihalainen E, Lindström L, Mappes J. 2006 Does colour matter? The importance of colour in avoidance learning, memorability and generalisation. *Behav. Ecol. Sociobiol.* **60**, 482–91
55. Lindstrom L, Alatalo R V, Mappes J. 1999 Reactions of hand-reared and wild-caught predators toward warningly colored, gregarious, and conspicuous prey. *Behav. Ecol.* **10** 317–322
56. Aronsson M, Gamberale-Stille G. 2008 Domestic chicks primarily attend to colour, not pattern, when learning an aposematic coloration. *Anim. Behav.* **75**, 417–423. (doi:10.1016/j.anbehav.2007.05.006)
57. Chittka L, Osorio D. 2007 Cognitive dimensions of predator responses to imperfect mimicry. *PLoS Biol.* **5**, e339. (doi:10.1371/journal.pbio.0050339)
58. Arias M, le Poul Y, Chouteau M, Boisseau R, Rosser N, Théry M, Llaurens V. 2016 Crossing fitness valleys: empirical estimation of a fitness

landscape associated with polymorphic mimicry. *Proc. R. Soc. B* **283**:
20160391. (doi:10.1098/rspb.2016.0391)

59. Finkbeiner SD, Briscoe AD, Reed RD. 2014 Warning signals are
seductive: relative contributions of color and pattern to predator avoidance
and mate attraction in *Heliconius* butterflies. *Evolution* **68**, 3410–3420.
(doi:10.1111/evo.12524)

60. Kazemi B, Gamberale-Stille G, Tullberg BS, Leimar O. 2014 Stimulus
Salience as an Explanation for Imperfect Mimicry. *Curr. Biol.* **24**, 965–969.
(doi:10.1016/j.cub.2014.02.061)

61. Hetz M, Slobodchikoff CN. 1988 Predation pressure on an imperfect
Batesian mimicry complex in the presence of alternative prey. *Oecologia*.
76, 570–573

62. Beatty CD, Beirinckx K, Sherratt TN. 2004 The evolution of müllerian
mimicry in multispecies communities. *Nature* **431**, 63–66.
(doi:10.1038/nature02818)

63. Arias M, Mappes J, Théry M, Llaurens V. 2015 Inter-species variation in
unpalatability does not explain polymorphism in a mimetic species. *Evol.*
Ecol. **30**, 419–433. (doi:10.1007/s10682-015-9815-2)

64. Muñoz AP, Kéry M, Martins PV, Ferraz G. 2018 Age effects on survival of
Amazon forest birds and the latitudinal gradient in bird survival. *The Auk*
135, 299–313. (doi:10.1642/auk-17-91.1)

65. Rönkä K, Valkonen JK, Nokelainen O, Rojas B, Gordon S, Burdfield-Steel
E, Mappes J. 2020 Geographic mosaic of selection by avian predators on
hindwing warning colour in a polymorphic aposematic moth. *Ecol. Lett.* **23**,
1654–1663. (doi:10.1111/ele.13597)

66. Ritland DB. 1995 Comparative unpalatability of mimetic viceroy butterflies (*Limenitis archippus*) from four south-eastern United States populations. *Oecologia* **103**, 327–336. (doi:10.1007/ BF00328621)
67. Brower LP, Ryerson W, Coppinger L, Glazier S. 1968 Ecological Chemistry and the Palatability Spectrum. *Science* **161**, 1349–1351
68. Pinheiro CEG. 2003 Does Müllerian Mimicry Work in Nature? Experiments with Butterflies and Birds (Tyrannidae). *Biotropica*. **35**, 356–364
69. Terhune, E. C. 1977. Components of a visual stimulus used by scrub jays to discriminate a batesian model. *Am. Nat.* **111**, 435–451
70. Endler JA, Mappes J. 2004 Predator Mixes and the Conspicuousness of Aposematic Signals. *Am. Nat.* **163**, 532–547
71. Hämäläinen L, Mappes J, Rowland HM, Thorogood R. 2019 Social information use about novel aposematic prey is not influenced by a predator's previous experience with toxins. *Funct. Ecol.* **33**, 1982–1992. (doi:10.1111/1365-2435.13395)
72. Duncan CJ, Sheppard PM. 1965 Sensory discrimination and its role in the evolution of batesian mimicry. *Behaviour* **24**, 269–282.
73. Goodale MA, Sneddon I. 1977 The effect of distastefulness of the model on the predation of artificial batesian mimics. *Anim. Behav.* **25**, 660–665
74. Lindstrom L, Alatalo RV., Mappes J. 1997 Imperfect Batesian mimicry - the effects of the frequency and the distastefulness of the model. *Proc. R. Soc. Lond. B* **264**, 149–153
75. Brower AV. Locomotor Mimicry in butterflies? A critical review of the evidence. 1995 *Phil. Trans. R. Soc. Lond. B* **347**, 413–25