Multi-trait mimicry and the relative salience of individual traits

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Mimicry occurs when one species gains protection from predators by resembling an unprofitable model species. The degree of mimic–model similarity is variable in nature and is closely related to the number of traits that the mimic shares with its model. Here, we experimentally test the hypothesis that the relative salience of traits, as perceived by a predator, is an important determinant of the degree of mimic–model similarity required for successful mimicry. We manipulated the relative salience of the traits of a two-trait artificial model prey, and subsequently tested the survival of mimics of the different traits. The unrewarded model prey had two colour traits, black and blue, and the rewarded prey had two combinations of green, brown and grey shades. Blue tits were used as predators. We found that the birds perceived the black and blue traits to be similarly salient in one treatment, and mimic–model similarity in both traits was then required for high mimic success. In a second treatment, the blue trait was the most salient trait, and mimic–model similarity in this trait alone achieved high success. Our results thus support the idea that similar salience of model traits can explain the occurrence of multi-trait mimicry.

1. Introduction

Mimicry has long been recognized as a striking example of adaptation through natural selection [1–4]. For instance, Fisher [5, p. 156] stated that ‘Mimetic resemblances bear the hallmark of adaptation in the multiplicity of the simultaneous modifications to which they are due.’ The occurrence of mimicry is supported by observations of anatomical, behavioural and colour pattern changes acquired by mimics to better resemble their harmful assumed models [6]. However, the degree of mimetic similarity is quite variable in nature. There is a widespread (but usually not explicitly stated) conception that more perfect mimicry is achieved through similarity in a greater number of traits. Thus, similarity in a high number of traits is presumably good or perfect mimicry, and similarity in only few traits is regarded as imperfect or poor mimicry [7,8]. A closely related idea is that the more traits a mimic has in common with the model, the more avoided it is [9,10]. As a consequence, selection for an increased number of similar traits could be expected, yet there are many puzzling examples of sustained imperfect mimicry where only a few traits are similar [11–16].

One approach to the issue of the number of trait similarities in mimicry is to regard it simply as an aspect of the varying perfection of mimicry in nature, for which several explanations have been proposed [16]. There is, however, a related but distinct approach where certain traits play a specific role for predator discrimination. Recent research has incorporated the idea that predators might use only one or a few higher-ranked traits to categorize aposematic prey as unprofitable, and that these traits should be most important for mimic–model similarity [15,17–22]. Our aim is to extend this approach by testing the idea that the relative salience of traits, as perceived by a predator, determines their importance for mimicry.

The concept of salience, together with the related concept of associability, is used in learning psychology to indicate how a stimulus (e.g. a prey trait) facilitates associative learning, in the sense that learning proceeds faster with a higher-salience stimulus [23–26]. Salience typically corresponds to intrinsic properties of a stimulus, such as its intensity or its contrast in a context, whereas associability...
is used to denote any property of a stimulus, whether it is learned or intrinsic, that facilitates learning [25, 26].

Kazemi et al. [21] suggested an explanation of imperfect mimicry involving salience and overshadowing of stimulus components [23, 24]. They proposed that a high-salience trait (e.g., colour) could overshadow less salient traits (e.g., pattern and shape) in discrimination learning, and thus be important for mimicry. The idea was tested experimentally using wild blue tits as predators and artificial multi-trait prey stimuli. The study revealed that the birds only used the most salient (colour) of three equally reliable prey characteristics (colour, pattern and shape) when they discriminated between prey. Consequently, imperfect colour mimics were successful [21].

Thus, if one stimulus component is very salient compared with others, it can overshadow learning of the other components, and mimicry of only the most salient component can be successful. On the other hand, if two or more components have similar salience, the learned association will be shared between them. We therefore hypothesize that in scenarios where multiple traits have similar salience they will all contribute to successful mimicry.

The salience or associability of a stimulus in a discrimination task is dependent on several factors, including the learning situation at hand. A salient stimulus might stand out from the background in some way, for instance by being bright, loud or distinct from other stimuli present during learning [23]. The effective salience is also influenced, negatively or positively, by any previous experience with the stimulus component in question, as in latent inhibition (learned irrelevance) and learned predictiveness [27–29]. The factors influencing the salience of different prey traits could therefore affect the degree of mimic–model similarity and mimicry evolution in general.

Here, we experimentally examine the importance of relative trait salience for multi-trait mimicry by manipulating the relative salience of two colour traits of artificial prey, both of which reliably indicate unprofitability. We hypothesize that when the traits are perceived as equally salient, both should be used to discriminate between profitable and unprofitable prey stimuli by the predators. However, if one trait has higher salience, it can overshadow the other trait and dominate the discrimination. Thus, in one situation a mimic–model similarity in both traits might be required to achieve high predator avoidance, and in another situation a mimic–model similarity in the single most salient trait should be sufficient for high avoidance. Our general hypothesis is that the relative salience of traits is an important determinant of required mimic–model similarity for effective protection from predators.

In our experiments, we took into account the fact that naturally occurring prey are variable in appearance, and that this may influence the distinctiveness of traits, and thereby their relative salience for discrimination between prey qualities. Consequently, the model prey traits that are easily distinguishable from the variety of available profitable prey traits might have higher salience to predators than traits that are closer in resemblance.

Based on this idea, we created two discrimination situations in which two-trait unrewarded model prey (combination of black and blue colours) were presented together with rewarded two-trait prey of either a single type (a combination of grey-green and grey-brown) or multiple types (combinations of green, brown and grey shades). In the single-type treatment, we expected both the black and the blue trait of the model to be equally salient, whereas in the multiple-type treatment we expected the black trait to be less salient due to the variation in brightness of the rewarded prey colours. We performed discrimination learning trials and generalization tests to investigate the influence of the rewarded prey treatments on the success of imperfect and perfect mimics. In order to establish the effect of the rewarded prey treatment on relative trait salience, we also experimentally measured the salience of the black and blue traits by measuring the rate of discrimination learning in both the single and multiple rewarded prey situations. We could thus determine the importance of each of the model prey traits for predator discrimination and mimicry, in two different situations, and measure how salient the birds perceived each of the traits to be depending on the situation.

2. Material and methods

The study involved two separate experiments. Experiment 1 consisted of a discrimination learning task followed by a generalization test. Experiment 2 consisted of a discrimination learning task which measured the salience of the traits used. The different prey types and treatments are illustrated in figure 1.

In the discrimination learning task in experiment 1, one group of birds was assigned to learn to discriminate between a black-blue unrewarded model prey and rewarded prey with a single type of appearance. The second group of birds was assigned to learn discrimination between the black–blue model prey and multiple types of rewarded prey (figure 1a). In the generalization test, the birds from these two treatment groups were presented with one of three mimics: a mimic of the black trait of the model prey; a mimic of the blue trait; and a mimic of both the black and blue traits, together with the rewarded prey appearances used in their discrimination learning (figure 1b; six treatment groups). In experiment 2, we tested the salience of the black and blue colour traits separately by measuring the discrimination learning rates of unrewarded prey that had either the black trait or the blue trait, in a set-up with either single or multiple types of rewarded prey (figure 1c; four treatment groups).

(a) Subjects and housing

We performed the experiments at Tovetorp Zoological Research Station (Stockholm University) in southeastern Sweden. Experiment 1 was performed in 2013 and experiment 2 in 2014. We used wild blue tits (Cyanistes caeruleus) as predators and paper items as artificial prey stimuli with pieces of mealworm (Tenebrio molitor) as reward. The birds were caught with trapping cages at feeding sites situated around Tovetorp. They were individually housed in 80 × 60 × 40 cm cages indoors with unlimited access to sunflower seeds, suet and water. The food was removed between 30 and 60 min before each trial to enhance the foraging motivation of the birds. The light in the holding room was set to approximate the natural daylight regime. After the experiments the birds were ringed and released near the feeding sites. We used all caught birds regardless of sex or age, and a total of 100 birds were used in the study.

(b) Artificial prey

The prey were small, cut-out paper items. During the trials they were placed on top of 30 wells in three untreated MDF boards (each 60 × 120 cm). The wells contained a piece of mealworm for the rewarded prey and were left empty for the unrewarded prey, and the birds could only see and access the rewards by lifting the paper items. In the pre-experimental training, the birds received white square paper items.

The colour patterns of the prey were created with R v. 2.15.2 software and printed (Epson Stylus Photo R3000) on white photo paper (Epson Photo Paper). We designed the prey with a
Table 1. Prey variants and treatments used in experiments 1 and 2. (a) The unrewarded (U) and rewarded (R) prey used in the discrimination learning in experiment 1, with the top and bottom rows illustrating the two treatments; a single type of rewarded prey (top) and multiple types of rewarded prey (bottom). (b) The three mimic types (mimicking the black trait, blue trait or both traits) used in the generalization test in experiment 1, with the top and bottom rows illustrating the single and multiple treatments. (c) The unrewarded prey (with either a black or blue trait) used in the salience test in experiment 2, with single and multiple rewarded prey type treatments in the top and bottom rows.

![Table 1](http://rspb.royalsocietypublishing.org/figure1.png)

(c) Experimental arena
We used two similar experimental rooms (230 × 240 × 195 cm), equipped with a bird stand with perches and a water bowl. In each room, we placed three experimental boards next to each other on the floor, and the rewarded and unrewarded prey items were randomly placed on top of the 30 wells. Before trials, we shuffled the prey items to avoid any spatial learning by the birds. The experiment started when a bird entered the room. The birds were released into the arena through a small hatch in the door and observed through a one-way mirror. We performed pre-experimental training both in the holding cages and in the experimental rooms to induce food searching under paper items and accustom the birds to the experimental arena (see the electronic supplementary material for details).

(d) Experimental procedures
The discrimination learning in experiment 1 consisted of five trials, and was followed by one generalization test trial. In the learning trials, the birds were presented with 30 prey stimuli, 15 unrewarded and 15 rewarded. Half of the birds (n = 30) received the treatment with a single rewarded prey appearance, referred to as ‘single’, and the other half (n = 30) received the treatment with multiple rewarded prey appearances, referred to as ‘multiple’ (figure la). As we used eight variants (×2) of rewarded prey in the multiple treatment, one was randomly left out in each trial to obtain a sum of 15.

All birds that successfully learned to discriminate between unrewarded and rewarded prey participated in the generalization test. Successful learning was defined as maximum two attacks out of seven on unrewarded prey (more than 70% rewarded prey accuracy) in the last learning trial; 59 of 60 birds reached this criterion. To study how the birds generalized their learned avoidance of the black–blue model, we replaced the model prey with mimic prey in the generalization trial. We randomly assigned the birds from the two discrimination learning treatments (single and multiple) to one of the three generalization treatments (black mimic, blue mimic and perfect mimic). Sample sizes were N = 10, 10, 9, 10, 10 and 10 for the treatment combinations single black, single blue, single perfect, multiple black, multiple blue and multiple perfect, respectively (figure 1b). All prey were unrewarded in the generalization test. In the salience test in experiment 2, 40 new birds performed six trials of discrimination learning of the treatments single black, single blue, multiple black and multiple blue (n = 10, 10, 10 and 10, respectively; figure 1c), with 30 prey of which half was rewarded and half unrewarded. For trial criteria, see [21].

(e) Statistics
We used generalized linear models in the package lme4 [30] in the statistical software R v. 3.2.0 [31]. The response variable was the number of attacks on rewarded prey (i.e. directed away from model and mimic prey) out of the first n attacks in a trial, where n = 7 for the discrimination learning and the generalization test in experiment 1, and n = 10 for the discrimination learning in experiment 2. The response was modelled as having a
binomial distribution with logit link function (i.e. logistic regression). We used the Akaike information criterion (AIC) to select the best-fitting models.

For the discrimination learning data, we used the glmer function in the lme4 package [30], and treatment (single and multiple) and trial number as fixed effects. The interaction between the two was not significant and thus excluded to obtain the best-fitting model (lowest AIC value). The random effects in the model represented variation between individual birds, and we allowed for variation in both the intercept and the slope of the regression in relation to trial number. For ease of interpretation, we centred the trial number variable to mid-trial.

The generalization test consisted of a single trial, meaning that there was one response per bird, so we used the glm function in R for model fitting. With the aim of performing planned comparisons, we used the treatments of the discrimination learning (single and multiple) and trial number as fixed effects. The interaction between the two was not significant and thus excluded to obtain the best-fitting model (lowest AIC value). The random effects in the model represented variation between individual birds, and we allowed for variation in both the intercept and the slope of the regression in relation to trial number. For ease of interpretation, we centred the trial number variable to mid-trial.

3. Results

(a) Experiment 1

In the discrimination learning, the birds increasingly avoided the model (figure 2), as shown by the positive common slope of the mixed-model logistic regression (slope ± s.e.: 0.813 ±

Table 1. Statistical analysis of the generalization test. The effects are derived from five treatment contrasts. The estimates and s.e. are on logit scale. Significant values are shown in bold.

<table>
<thead>
<tr>
<th>effect</th>
<th>estimate</th>
<th>s.e.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>perfect versus imperfect</td>
<td>0.720</td>
<td>0.191</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>single perfect versus multiple perfect</td>
<td>0.781</td>
<td>0.555</td>
<td>0.159</td>
</tr>
<tr>
<td>single black – blue versus multiple black – blue</td>
<td>–0.005</td>
<td>0.146</td>
<td>0.973</td>
</tr>
<tr>
<td>single black versus single blue</td>
<td>0.119</td>
<td>0.200</td>
<td>0.551</td>
</tr>
<tr>
<td>multiple black versus multiple blue</td>
<td>–0.602</td>
<td>0.211</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 2. Statistical analysis of the salience test. The effects are derived from three treatment contrasts with trial number centred at mid-trial. The table shows mid-trial intercepts, overall centred trial slope and the interaction between treatment contrasts and centred trial. The estimates and s.e. are on logit scale. Significant values are shown in bold.

<table>
<thead>
<tr>
<th>effect</th>
<th>estimate</th>
<th>s.e.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>single black versus multiple black</td>
<td>0.227</td>
<td>0.085</td>
<td>0.008</td>
</tr>
<tr>
<td>single blue versus multiple blue</td>
<td>–0.004</td>
<td>0.085</td>
<td>0.961</td>
</tr>
<tr>
<td>black versus blue</td>
<td>0.028</td>
<td>0.060</td>
<td>0.641</td>
</tr>
<tr>
<td>centred trial</td>
<td>0.402</td>
<td>0.032</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>single black versus multiple black × centred trial</td>
<td>0.130</td>
<td>0.045</td>
<td>0.004</td>
</tr>
<tr>
<td>single blue versus multiple blue × centred trial</td>
<td>0.056</td>
<td>0.045</td>
<td>0.217</td>
</tr>
<tr>
<td>black versus blue × centred trial</td>
<td>–0.049</td>
<td>0.032</td>
<td>0.123</td>
</tr>
</tbody>
</table>

For the salience test in experiment 2, we also used the glmer function and the treatment, trial number and their interaction as fixed effects. The treatment factor had four levels describing the rewarded and unrewarded prey stimulus combination: single black, single blue, multiple black and multiple blue. For the analysis, we used three orthogonal contrasts (listed in table 2) corresponding to the following comparisons: (1) single black versus multiple black; (2) single blue versus multiple blue; and (3) black versus blue. The random effects in the model represented variation between individual birds, and we allowed for variation in both the intercept and the slope of the regression on trial number. For ease of interpretation, we centred the trial number variable to mid-trial.
A comparison between the single and multiple treatment (intercept at mid-trial) showed no significant difference, although there was a tendency towards a lower intercept in the multiple treatment (effect $\pm$ s.e.: $-0.208 \pm 0.115$, $p = 0.07$).

We performed the analysis of the generalization test by way of planned comparisons, involving five orthogonal contrasts (table 1; figure 3). Comparison (1) showed that perfect mimics were more avoided than imperfect mimics, (2) showed that there was no significant difference in avoidance of the perfect mimics between the two discrimination learning treatments (single versus multiple) and (3) showed that there was no significant difference in avoidance of the imperfect mimics between the two discrimination learning treatments. The last two comparisons (4, 5) were between the black and blue mimics in the two discrimination learning treatments, respectively. They showed that there was no significant difference in avoidance between the black and blue mimics in the single treatment, whereas in the multiple treatment the blue mimics were significantly more avoided than the black mimics (table 1; figure 3).

**4. Discussion**

The results confirm our general hypothesis that the relative salience of prey traits can have an important influence on mimetic success. By using single or multiple rewarded prey appearances, we successfully manipulated the relative salience of the black and blue traits of the unrewarded prey, as perceived by the birds. We found that when the two traits had similar salience, both were used in the discrimination between prey, and mimic–model similarity in just one trait resulted in moderate protection, whereas similarity in both traits (perfect mimics) produced highly successful mimicry (figure 3, ‘single’). In contrast with this, when the blue prey trait was perceived to be the most salient, a mimic–model similarity in the black trait was not very effective against attacks, whereas mimicry of the blue trait was more effective, approaching the success of perfect mimics (figure 3, ‘multiple’).

We also tested the salience of the black and blue traits separately by measuring the discrimination learning rate [23,25,26] in trials with either single or multiple types of rewarded prey appearances. The salience test provided a justification for our interpretation of multi-trait mimicry in terms of relative salience. The results confirmed that the black trait was less salient in trials with multiple types of rewarded prey appearances (figure 4a), whereas the blue trait had similar salience in both treatments (figure 4b). Based on our analyses of brightness and colours (electronic supplementary material, figures S2 and S3), the lower salience of the black trait was probably due to a lower discriminability [32] of the black colour trait when it was presented together with the multiple rewarded prey because there was a greater similarity in brightness between the black and the darker shades of the rewarded prey (electronic supplementary material, figure S3 and table S1). It is of course possible that the learning rate was influenced by other properties of the palatable prey we used than variation in brightness, for instance variation in hue. In any case, our results show that the relative salience of prey traits can depend on the context in which the prey occur.

The evolution of mimicry has often been described and modelled as a mimic appearance that achieves a general similarity to a model appearance, either as a one-dimensional trait, or as a multidimensional trait in a space where general similarity is applied (see e.g. [13]). Based on experimental evidence of predator generalization behaviour in mimicry studies, it might, however, be more fruitful to consider a spectrum of mimicry systems. In some systems, predators distinguish aposematic prey based on one or a few features

![Figure 4](http://rspb.royalsocietypublishing.org/)
of appearance, and in other systems predators use more traits for discrimination. The former would allow imperfect mimics to succeed, whereas in the latter some of the predators might distinguish almost perfect mimics from their models [6,9,10,21,22,33].

Starting from our general hypothesis—that the relative salience of traits is an important determinant of their contribution to mimicry—it is of interest to examine the ways in which traits can acquire higher or lower salience. One possibility is that certain stimulus components are very readily perceived by predators and are relevant in different discrimination tasks. The high salience of colour signal components for bird predators would be an example [21,34,35]. Another partially overlapping possibility is that certain stimulus components have higher distinctiveness or discriminability in a comparison between unprofitable model prey and profitable non-mimicking prey. Accordingly, many explanations for the evolution of conspicuous warning colours in nature emphasize discriminability from background and profitable prey, both of which can affect salience and increase the rate of avoidance learning (see table 7 of [13] for a review). Warning colours are therefore expected to be high-salience traits also in mimicry, whereas other more general prey traits that are shared with profitable prey, such as a variegated pattern or body shape, are expected to have lower salience. An example from our experiments would be the higher salience of the blue colour component compared with the black in the treatment with multiple rewarded prey appearances, some of which approached the low brightness of the black component of the unrewarded prey. It is known from studies of discrimination learning that higher distinctiveness of stimulus components can increase their salience [32].

A different and important possibility is that the salience of stimulus components changes over a predator’s time of interaction with a prey community. Consider a prey community with an unprofitable model prey together with high enough frequencies of profitable prey that share a high-salience trait with the model (for instance Batesian mimics). The high-salience trait might be a colour or colour pattern.

For a predator that gains sufficient experience of the prey community, the original high-salience trait will lose salience because it fails to reliably predict unprofitability. The expectation from animal psychology is then that stimulus components that previously had lower salience can gain in salience if they can contribute to reliable discrimination [36]. We suggest that this is an important process behind the evolution of more perfect mimicry, through the successive evolutionary incorporation of traits that originally have had lower salience, until model–mimic difference is only present in traits of very low salience for the predators. However, this process of incorporating traits with lower salience into the discrimination process requires an extensive interaction between predators, models and mimics. Such a scenario would perhaps require long-lived predators, a low speed–accuracy trade-off [15] and a low cost of errors (e.g. a less poisonous model).

With these different possibilities of how components of a multi-trait prey appearance can acquire salience, our general hypothesis about the importance of relative salience for mimicry has the potential to explain much of the observed variation in mimetic resemblance in nature, ranging from mimicry of a single or a few traits to essentially perfect mimicry. Although the appearance of an aposematic model consists of a multitude of traits, their relative salience strongly affects the role of each specific trait in recognition and classification. The relative salience of traits is in turn influenced by the context in which the discrimination is performed.

Ethics. These experiments were done under permit from Linköpings djurförsöksstäta nämnd (Dnr 91–12) and carried out in accordance with the regulations regarding wild birds.

Data accessibility. Data from the experiments are available at Dryad Data Repository: http://dx.doi.org/10.5061/dryad.ng78s.

Authors’ contributions. B.K. carried out all the experimental work. O.L. and B.K. were responsible for the statistical analysis. All authors participated in the design of the study and in manuscript preparation.

Competing interests. We have no competing interests to declare.

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