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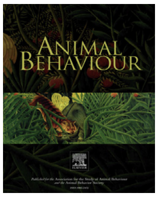

Hierarchical overshadowing of stimuli and its role in mimicry evolution

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An elegant study by Kazemi et al. (2014, *Current Biology*, *24*, 965–969) found that blue tits, *Cyanistes caeruleus*, that had been trained to distinguish between rewarding and nonrewarding prey subsequently avoided novel prey that had the same colour (but not the same shape or pattern) as the nonrewarding prey. Their work suggested that certain high-salience discriminatory traits can overshadow other informative traits, allowing imperfect mimics to establish. Here we began by replicating their ambitious experiment by evaluating the behaviour of 320 human subjects foraging on computer-generated prey. However, to fully understand how overshadowing might facilitate the establishment of incipient mimics, we significantly extended their protocol to a full factorial design involving mimics with colour, pattern and/or shape in common with their former models. As Kazemi et al. reported, participants placed more weight on colour similarity than on shape similarity when rejecting prey. Two-trait mimics with the same colour and pattern or the same colour and shape as their former models were avoided as frequently as perfect mimics, while colour-only mimics were avoided more frequently than shape-only mimics. Nevertheless, novel prey with no traits in common with nonrewarding models were avoided at high rates, possibly in part due to their dissimilarity to familiar rewarding prey. The implications of these findings for mimicry evolution are discussed.

Researchers have long debated how a palatable species can evolve to resemble an unpalatable species (‘Batesian mimicry’, Bates, 1862) when it involves changes in so many different appearance dimensions (Kikuchi & Pfennig, 2013). In the two-step model of mimicry evolution it is assumed that a mutation with a large effect on the phenotype first produces an approximate resemblance of the prey to the model, which may then be gradually improved by selection for resemblance-enhancing mutations of smaller phenotypic effect (Nicholson, 1927; Poulton, 1912; Turner, 1984). However, this argument implicitly assumes that predators would tend to overlook important discriminative traits in the incipient mimic and it is not entirely obvious why predators would do this (Gamberale-Stille, Balogh, Tullberg, & Leimar, 2012). Indeed in many well-known mimicry complexes the extent of mimicry is often far from perfect (see Kikuchi & Pfennig, 2013, for a recent review) and yet often predators do not appear to exploit these differences.

Predators ultimately determine the success of any defensive trait and so to fully understand how antipredator adaptations evolve and are maintained, we must evaluate how predators respond to rudimentary versions of these defences (Chittka & Osorio, 2007). In particular, if predators learn to discriminate between profitable and unprofitable prey by identifying the distinctive features of the two object types, then imperfect mimicry might be initially selected for and subsequently maintained if predators learn to associate prey defences with certain salient discriminative features and effectively overlook others, a phenomenon known as ‘overshadowing’ (Mackintosh, 1976; Shettleworth, 2010). Building on earlier studies on the degree of mimetic protection gained by new partial mimics (Ford, 1971; Schmidt, 1958, 1960; Terhune, 1977), there has recently been a surge of interest in understanding how objects are categorized and what traits are attended to when predators make their discriminative decisions (Aronsson & Gamberale-Stille, 2008, 2012; Bain, Rashed, Cowper, Gilbert, & Sherratt, 2007; Balogh, Gamberale-Stille, Tullberg, & Leimar, 2010). One of the most ambitious and systematic studies to date on this topic was recently conducted by Kazemi, Gamberale-Stille, Tullberg, and Leimar (2014), who trained wild-caught blue tits, *Cyanistes caeruleus*, to discriminate between rewarding prey (symbol-

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bearing laminated cards over wells containing pieces of mealworm) and nonrewarding prey (symbol-bearing cards over empty wells). The symbols over rewarding and nonrewarding wells differed from one another in their colour, pattern and shape, and the blue tits learned to feed almost exclusively on the rewarding wells over four separate trials. Naturally, this high level of discrimination could have been achieved by using any given combination of colour, pattern and/or shape in the symbols. To evaluate what traits were being used in the discrimination process, the authors then presented the same birds with an identical collection of rewarding prey, along with nonrewarding prey that only had one trait (colour, pattern or shape) in common with the earlier models that the birds had learned to avoid (the former models were also used as a control). These nonrewarding prey were essentially Müllerian mimics, but following Kazemi et al. (2014), we hereafter simply refer to them as ‘mimics’. The results of this test showed that rather than learning to avoid only prey with all three attributes, the birds generalized their avoidance to nonrewarding prey that had colour in common with the former models, to such an extent that they were not attacked any more frequently than the perfect controls. Collectively, this work suggests that high-salience discriminatory traits (in this case colour) can overshadow other informative traits, allowing incipient mimics a significant survival advantage even if they share only one trait with the model.

The work of Kazemi et al. (2014) was impressive (see review in Cuthill, 2014), even more so because the authors used three different combinations of model and mimic phenotypes (model variants A–C, see Fig. 1) to test whether their conclusions were robust to variation in the nature of the specific colours, shapes and patterns used in discrimination. However, as with all experiments aimed at delivering general insights, it is of interest to know whether similar results would also be generated by other species, including our own. The first aim of our study was therefore to repeat the ambitious experiments of Kazemi et al. (2014), this time using humans, which share with natural predators a finite capacity to process information (e.g. see Beatty, Beirinckx, & Sherratt, 2004). However, there was an additional aim, namely to extend the authors' experiment to a full factorial design. Note that Kazemi and colleagues presented perfect ‘mimics’ and prey that had only one trait in common with the former models (colour, shape or pattern), yielding four different treatments in total per model variant (A–C). To broaden the scope and relevance of the approach to mimicry evolution, here we implemented the complete design, which allowed us to evaluate the success of prey types that had two traits in common (colour and pattern, colour and shape, or pattern and shape), as well as prey with nothing in common with the former models, that is, eight treatments in total ($=2^3$).

A ‘nothing in common’ treatment is particularly informative for two reasons. First, it enables us to compare the success of nonmimics and incipient mimics with one, two or three traits in common, which is clearly an informative comparison when attempting to elucidate evolutionary pathways (Kazemi et al. were only able to compare the success of a perfect mimic with a one-trait mimic). More importantly, under the Kazemi et al. design, the nonrewarding prey presented in the generalization trials not only generally shared one feature with the former nonrewarding prey, but it also shared two features in common with the rewarding prey. Given that the same phenotypes of rewarding prey were presented throughout both stages of the experiment, it is quite possible that at least some of the birds learned to identify the unique features of rewarding prey, rather than unrewarding prey. In this case, any new variant phenotype might have been avoided not because it looked like the unrewarding prey, but because it looked less like the rewarding prey that the subjects had learned to exploit. In other words, it is possible that some subjects might have succeeded in identifying the attributes of rewarding prey and simply carried this information through to the next stage of the experiment.

METHODS

Our design closely followed that of Kazemi et al., this time using human volunteers as surrogate predators (see Fig. 1). No human subject participated more than once. In a pilot study following only the Kazemi et al. treatment conditions (eight replicates of three model variants (A–C) and four generalization tests (perfect, colour, pattern, shape); i.e. 96 human volunteers), we observed an unexpected response with model variant B, in which the stripes on the nonrewarding model subsequently appeared to be generalized (and hence avoided) particularly strongly in the generalization test. To explore this further, we included an additional model variant (D) in our full factorial design involving a striped yellow square as the nonrewarding prey (this extended complete design involved 10 replicates of four model variants and eight different generalization tests based on all possible permutations of model–mimic similarity; i.e. 320 human volunteers).

Images of the various forms of prey were extracted directly from Kazemi et al.'s pdf and saved as a jpegs for display in our computer program. No attempt was made to calibrate the targets' luminance or colour for human vision, as we were interested in the responses of humans to images similar to those used by the authors. The images of the nothing in common prey (those with no colour, pattern or shape in common with the nonrewarding model) were not part of Kazemi et al.'s experiment and so these images were our own design (see last column of Fig. 1). All of our human subjects were first presented with a short video explaining the nature of the experiment (neither the purpose, nor the specific prey types were introduced in this presentation). They were then presented with artificial prey and invited to forage.

The computer program presenting the artificial prey was developed in Microsoft® Visual Basic 6 and involved taking subjects through a series of discrimination learning trials and a series of generalization trials. In each discrimination learning trial (1–4), 16 artificial prey were presented in a 4 × 4 grid, eight of them nonrewarding prey (all identical in appearance) and eight of them rewarding prey (eight different phenotypes), with nonrewarding and rewarding prey distributed at random across the grid (see Supplementary Fig. S1). To enhance motivation, subjects clicking their mouse on a rewarding prey item heard a pleasant cash register sound, their points tally was increased by 1 and the prey item was replaced by a green tick. Conversely, subjects clicking a nonrewarding prey item heard an unpleasant electric shock sound, their points were decreased by 1 and the prey item was replaced by a red cross. Following the general approach of Kazemi et al., subjects continued clicking on prey until all eight rewarding prey were attacked or 20 s had elapsed, whichever came first. At the end of a given trial, the remaining prey were rendered unavailable and subjects were allowed to move to the next screen. After four such discriminative learning trials, the program then seamlessly switched into the generalization test, presenting one each of the same eight rewarding prey types and two (or four) each of four (or two) different nonrewarding prey that differed from the former nonrewarding models in none, one, two or all three traits (colour, pattern and/or shape). Four screens (trials 5–8) containing the same mixtures of phenotypes were presented in this generalization test, with passage to the next screen governed by the same rules as above. At the end of our study, participants were evaluated for colour blindness using Ishihara plates (numbers 5 (plate 6), 15 (plate 8), 45 (plate 13), 42 (plate 23)). Three subjects were assessed to be colour blind on the basis of this test, and their data were