Animal behaviour

Interference coloration as an anti-predator defence

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Interference coloration, in which the perceived colour varies predictably with the angle of illumination or observation, is extremely widespread across animal groups. However, despite considerable advances in our understanding of the mechanistic basis of interference coloration in animals, we still have a poor understanding of its function. Here, I show, using avian predators hunting dynamic virtual prey, that the presence of interference coloration can significantly reduce a predator's attack success. Predators required more pecks to successfully catch interference-coloured prey compared with otherwise identical prey items that lacked interference coloration, and attacks against prey with interference colours were less accurate, suggesting that changes in colour or brightness caused by prey movement hindered a predator's ability to pinpoint their exact location. The pronounced anti-predator benefits of interference coloration may explain why it has evolved independently so many times.

1. Introduction

Interference coloration, in which the perceived colour and brightness varies predictably with the angle of illumination or observation [1], is extremely widespread across animal groups, having evolved independently several times in insects such as beetles and butterflies, as well as in some birds, fish, reptiles and cephalopods, and at least one mammal [2–4]. However, despite widespread interest in the mechanisms underlying the production of interference colours in animals [5], its function is still unclear [3]. It has been suggested, for example, that because interference colours are often visually striking they may function in sex or species recognition, or have evolved as sexually selected signals [2,5–8]. Alternatively, the structures that produce interference coloration may also have non-communicative functions such as enhancing water repellence, friction reduction or thermoregulation, and may even alter light reaching the retina and so play a role in vision [2,9,10]. A further role of interference coloration may be as an anti-predator mechanism [2].

Although interference colours often exhibit conspicuous changes in colour and brightness when observed outside their natural context, some forms of interference colour have been suggested to have an anti-predator function by allowing animals to appear cryptic against their background [11–14]; for example, the most common interference colour in beetles is green [11,13]. However, animals with interference colours that contrast sharply with the background would seem poorly adapted for crypsis, leading to the suggestion that such colours may function as an aposematic signal [15,16] or as an active anti-predator mechanism [17,18]. In particular, interference colours can produce bright flashes of colour or sudden changes in brightness that might briefly startle a potential predator and thereby increase the prey's probability of escape [17], or because movement-induced changes in colour or brightness may hinder a predator's ability to pinpoint the prey's exact location in an attempted strike [18]. Such mechanisms have been proposed as a function of interference colour in beetles [15,19–21], butterflies [22], birds [2] and fish [23]. However, whether having interference

coloration decreases the chance of predation for mobile prey has never been empirically tested. Here, I experimentally tested this using Japanese quail (*Coturnix japonica*) predating virtual insects.

2. Material and methods

(a) Experimental design

The subjects were female Japanese quail ($n = 7$) from laboratory stock. They were aged between six and 18 months, and had all participated in a number of previous operant experiments involving visual search and detection of static objects (e.g. locating cryptic items against a heterogeneous greyscale background). They had not, however, participated in a task involving moving or coloured stimuli. All birds had the same prior experience.

Experiments took place in a wooden cage (160×65 cm and 25 cm high), at one end of which was a calibrated flat-screen CRT monitor (Iiyama VisonMaster 513) equipped with a 30×23 cm infrared touch screen bezel (CarrollTouch Smart Frame, Elo TouchSystems, Menlo Park, CA) to record pecks, and an automatic feeder. Stimuli, which were presented on the monitor, consisted of a circular moving 'prey', 20 mm in diameter (chosen because stimuli of this size were invariably attacked and caught, regardless of their appearance). Each prey item moved in a straight line at a constant speed of approximately 150 mm s^{-1} until it reached the edge of the screen, when it turned round and moved off at the angle of incidence. No attempt was made to replicate the locomotive behaviour of any given species, but rather to provide a target that was challenging for quail to catch and that did not introduce any 'behaviours' that may interact with colour in affecting its chances of predation, such as random movement patterns. When subjects made a 'successful' choice, defined as a peck within 10 mm of the centre of a prey item (i.e. within its body), they were rewarded with 10 s access to a feeder containing ad libitum whole dried mealworms (*Tenebrio molitor* larvae), a favourite food. There was no attempt to food-deprive the birds, as the mealworm reward provided sufficient motivation to complete the task.

Subjects were initially trained to peck at black moving target stimuli on a uniform grey background. Training continued until a subject caught six consecutive stimuli within three pecks, after which it progressed to the test phase of the experiment. The mean $\pm$ s.e. number of trials required to reach the training criterion was 23.9 ± 4.5.

After successfully completing training, each subject was presented sequentially with 20 stimuli, 10 with interference coloration (treatment) and 10 without (control), in a random order, against a uniform grey background. Stimuli were constructed as polygon models consisting of one half sphere, with the convex face pointing towards the observer. For treatment stimuli, all vertices on the polygon model were assigned a colour based on the angle between a virtual light source, the vertex and a fixed point in front of the centre of the screen representing the observer, and the faces of the polygon coloured by interpolation of these vertex colours. The colour assigned to a vertex was assumed to be produced by a simple planar multilayer composed of stacked chitin plates of equal thicknesses, separated by very thin air spaces. This alternating chitin/air multilayer structure is a common configuration in insects, producing colour through constructive interference [24]. Treatment stimuli were modelled on greenbottles, *Lucilia sericata* [24], which appear metallic green at normal incidence, turning to blue at large angles. As they moved around the screen, they, therefore, underwent angle-dependent changes in colour and brightness. The colour of each control stimulus was chosen at random from within the gamut of colours that could be produced by the treatment stimuli. These stimuli were

therefore uniformly coloured, and colour was independent of spatial position. Note that the colours displayed on the screen only loosely matched those produced by real flies. However, the aim was not to exactly replicate colours produced by real insects or replicate how real colours would be perceived by quail predators. Rather, the aim was to produce target stimuli that exhibited the salient characteristics of interference coloration, namely chromatic and achromatic shifts in response to changes in viewing angle as the target moved around the screen.

The effectiveness of the treatment as an anti-predator strategy was assessed using (i) the number of pecks needed to successfully catch a prey item, (ii) the mean distance of all the unsuccessful pecks from the centre of the prey item prior to capture (a measure of peck accuracy) and (iii) the latency to make the first attack. Full details of the experimental set-up, stimulus design, training and testing procedures are given in the electronic supplementary material.

(b) Statistical analyses

To test whether the number of pecks needed to successfully catch a stimulus differed between treatment and control stimuli, I used a generalized linear mixed-effects model with a negative binomial error distribution to account for overdispersion [25]. Stimulus type (treatment or control) was included as a fixed factor, and there was a random effects term of subject identity. I also included trial number, and the interaction between trial number and stimulus type, in order to test for improvements in predator performance over successive trials (i.e. learning). To test whether peck accuracy and latency to peck differed between stimuli, I used linear mixed-effects models, parametrized as described above. Both peck latency and accuracy were normalized using a log-transformation prior to analysis. For all models, *p*-values were calculated by comparing the full model to a reduced model lacking the fixed effect term using likelihood ratio tests [25], and models simplified using backwards stepwise elimination of non-significant terms. Minimum adequate models are presented. All analyses were conducted in R (R Core Development Team, v. 2.15.2) using either the 'lmer' function in the lme4 package [26] or the 'glmmadmb' function in the glmmADMB package [27], and models validated following [28].

3. Results

The number of pecks needed to catch a treatment (interference-coloured) stimulus was significantly greater than the number needed to catch a control (non-interference-coloured) stimulus ($\chi_1^2 = 7.89$, $p = 0.005$; figure 1*a*) and peck accuracy was significantly poorer for unsuccessful pecks at treatment than control stimuli ($\chi_1^2 = 10.99$, $p < 0.001$; figure 1*b*). There was no difference between stimulus types in the latency to attack (mean $\pm$ s.d., treatment: 24.15 ± 10.62 s; control: 23.06 ± 9.78 s; $\chi_1^2 = 0.29$, $p = 0.592$). In all models, trial number and the interaction between trial number and stimulus type were non-significant (all $p > 0.3$), suggesting that performance did not improve with learning.

4. Discussion

The results of this experiment show that predators attacking treatment prey items with interference-like colours require significantly more pecks, and unsuccessful pecks are significantly less accurate, than when attacking otherwise identical control prey. These findings suggest that significant anti-predator benefits may be gained by having interference
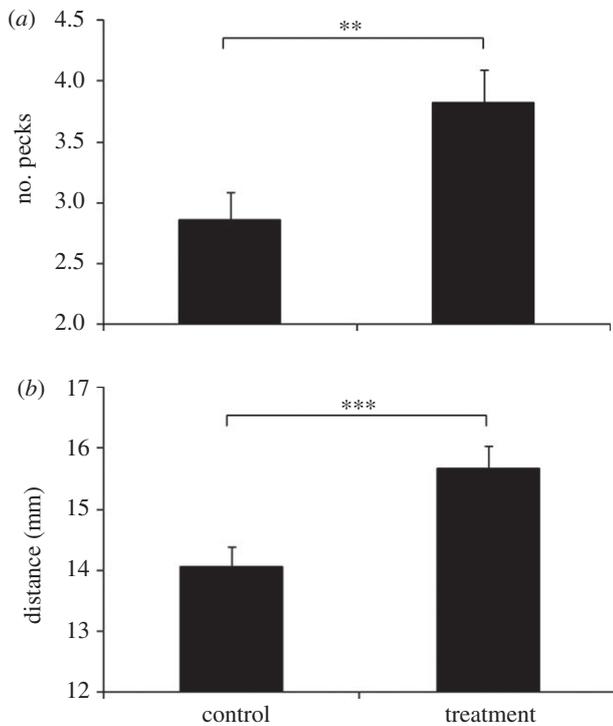
Figure 1. (a) Mean ± s.e. number of pecks needed to successfully catch interference-coloured (treatment) and non-interference-coloured (control) stimuli. (b) Mean ± s.e. distance of unsuccessful pecks at treatment and control stimuli, measured in mm from the centre of the prey item at the time of pecking. $**p < 0.01$; $***p < 0.001$.

coloration, and may explain why interference colours are so widespread [15,16,19–23]. Because the accuracy of prey-directed pecks, but not the latency to peck, was significantly reduced when attacking treatment prey items, it is likely that perceived changes in colour or brightness caused by the movement of animals with interference coloration hinders a predator's ability to pinpoint the prey's exact location in an attempted strike [18], rather than startling them through sudden chromatic or achromatic changes [17,29]. This is consistent with recent work on motion dazzle, in which high-contrast colour patterns can impair a predator's ability to judge the speed and direction of moving prey, making them harder to catch [29–32]. The sudden changes in brightness perceived by predators viewing rapidly moving prey with interference colours may therefore act as a form of dazzle coloration. More generally, this finding raises the intriguing possibility that changing appearance per se may be important in predator avoidance (see [33,34]), and that interference colours are a special case of this phenomenon.

While this study presents evidence that, at least in some cases, interference coloration has evolved as an anti-predator defence, whether this mechanism acts to protect prey in natural situations and how this relates to its other putative roles [2,3,5–10] remains to be tested.

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