The predation costs of symmetrical cryptic coloration

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In psychological studies of visual perception, symmetry is accepted as a potent cue in visual search for cryptic objects, yet its importance for non-human animals has been assumed rather than tested. Furthermore, while the salience of bilateral symmetry has been established in laboratory-based search tasks using human subjects, its role in more natural settings, closer to those for which such perceptual mechanisms evolved, has not, to our knowledge, been investigated previously. That said, the salience of symmetry in visual search has a plausible adaptive rationale, because biologically important objects, such as prey, predators or conspecifics, usually have a plane of symmetry that is not present in their surroundings. We tested the conspicuousness to avian predators of cryptic artificial, moth-like targets, with or without bilateral symmetry in background-matching coloration, against oak trees in the field. In two independent experiments, symmetrical targets were predated at a higher rate than otherwise identical asymmetrical targets. There was a small, but significant, fitness cost to symmetry in camouflage patterns. Given that birds are the most commonly invoked predators shaping the evolution of defensive coloration in insects, this raises the question of why bilateral asymmetry is not more common in cryptic insects.

Keywords: defensive coloration; bilateral symmetry; bird vision; predation risk; visual search

1. INTRODUCTION

Predators relying on vision to detect cryptic prey must perceive subtle discontinuities in colour or texture between the target and its background, or recognize characteristic arrangements of pattern elements that are uncommon in the background (Lythgoe 1979; Endler 1978, 1981; Ruxton *et al.* 2005). One of the most potent cues of the latter type is a plane of symmetry, because most inorganic substrates lack the bilateral symmetry that is typical of most animals. Even when the resting place of the prey is itself organic (e.g. a leaf), the symmetry inherent in the prey item (e.g. a caterpillar) may not be at the same spatial scale as that of the substrate. The salience of symmetry in visual search for cryptic targets is well established in human perceptual psychology (e.g. Barlow & Reeves 1979; Gurnsey *et al.* 1998; Rainville & Kingdom 1999) and yet comparable data for non-human predators are lacking (Osorio 1994). Biological research has instead focused on the role of symmetry in conspicuous signals, whether in mate choice (reviewed by Møller & Pomiankowski 1993; Møller & Swaddle 1997; Swaddle 2000), flower detection by bees (Giurfa *et al.* 1996) or aposematic (warning) coloration (Forsman & Merilaita 1999). The goal of the present study was to assess whether bilateral symmetry, in otherwise highly cryptic prey, significantly increased detectability to avian predators in the field. The experiments, using artificial moth-like targets, can equally be thought of as an assessment of the fitness costs of symmetry in camouflage patterns.

2. MATERIAL AND METHODS

'Prey' were dead (frozen overnight at -80°C , then thawed) mealworms (*Tenebrio molitor* larvae, 20–30 mm long) pinned

onto triangular, coloured card. These artificial targets, with their coloured 'wings' and edible 'bodies', were not modelled on any real Lepidopteran and, although there is a strong tradition of studying crypsis in daytime-resting moths (e.g. Endler 1984), the fact that our targets were moth-like was irrelevant to our aims. Their general appearance may, however, have reduced any neophobia in the predators and, indeed, these prey were readily consumed. The targets were pinned onto oak trees (by Elly Hiby and Emily Lloyd in experiment 1; by Innes C. Cuthill in experiment 2) in the mixed deciduous Leigh Woods National Nature Reserve, N. Somerset UK ($2^{\circ}38.6' \text{W}$, $51^{\circ}27.8' \text{N}$) and Blaise Castle Estate, Bristol UK ($2^{\circ}38.3' \text{W}$, $51^{\circ}29.9' \text{N}$); their locations were carefully mapped. Young trees ($<1 \text{ m}$ circumference at *ca* 1.75 m above ground) were avoided, so all selected trees had ridged, complex bark patterns. To facilitate relocation by the experimenters, trees within 20 m either side of a footpath were selected and all targets were pinned at *ca* 1.75 m above ground on bark free of ivy, moss or lichen growth. To reduce the risk of interference by the public, only minor tracks and footpaths were used, and targets were pinned on the side of the tree facing away from the nearest footpath. Having selected a tree and location for attachment, the individual target was chosen blindly from a bag in which all targets for that experimental block had been previously thoroughly mixed (i.e. pseudo-random selection with respect to treatment and replicate).

The 'survival' of targets was then checked at 24 h intervals for a period of 5 days. Birds took all or most of the mealworm, leaving the 'wings' behind, whereas arthropods such as spiders sucked fluids out to leave a hollow exoskeleton, and slugs left slime trails. Non-avian predation in the latter two categories, complete disappearance of a target, or survival for the entire 5-day trial, were treated as 'censored' values in survival analysis. Censored data in a survival analysis are not

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missing data, but provide incomplete information (Lawless 2002; Klein & Moeschberger 2003). For example, if a mealworm was seen to have been consumed by a slug on day 3, we know that target had survived bird predation up until that time point, but do not know how much longer it would have avoided bird predation if a slug had not consumed it first. This is directly analogous to the use of survival analysis for biomedical studies where patients may 'leave' a randomized clinical trial for reasons unrelated to the treatments under test (e.g. refusal to continue participation or death from causes unconnected to the study). The mealworms of unpredated targets were replaced each day so that they remained fresh. Both experiments had randomized block designs: each block was a separate run of the experiment, with 30 targets of each treatment (symmetrical or asymmetrical) in experiment 1, or 25 of each treatment in experiment 2. Every individual replicate target within a treatment had a unique pattern; where a given tree's bark was used more than once as the source of a target pattern, a different area of the sample was used each time. The replicate blocks were run in different and non-adjacent areas of the woods on different dates. This, the low density of the targets within any one block (60 targets every *ca* 25 m along a *ca* 1500 m transect, 40 m wide, corresponds to <3% of available trees in each transect), and the polymorphic nature of the prey, were designed to minimize the influences of any search image formation by individual predators.

Experiment 1 used black patterns printed onto dark brown card (chosen subjectively such that the reflectance spectra of the card fell within the range of oak bark in the study site; figure 1). Although the brown card lacked any ultraviolet (UV) reflectance (birds can see UV; e.g. Cuthill *et al.* 2000), so too does lichen-free oak bark, as used in this study (own measurements and Majerus *et al.* 2000). Patterns were digital samples (using PAINTSHOP PRO; JASC Software, Minneapolis, USA) of scanned bark-rubbings from a sample of 60 oak trees in the study site. The rubbings were scanned as binary (black-and-white) bitmaps and colour-inverted (black to white and vice versa) so that, when printed onto brown card, they created brown-and-black patterns similar to those of deeply ridged mature oak bark. The targets were triangles 60 mm wide by 50 mm high. There were two treatments: unmanipulated patterns, which thus had no plane of symmetry, and patterns mirror-reflected about the midline to create perfect bilateral symmetry. The experiment was run during winter (November–December) over four blocks with 30 replicates per treatment per block for a total sample of 120 targets per treatment.

Experiment 2 was a refinement of experiment 1, designed to present a greater challenge to the predators because the targets were smaller (50 mm wide by 25 mm high) and so closer to the size of real Lepidopteran prey, and the colour patterns were samples of digital photos of 100 oak trees in the study area at 1 : 1 reproduction. These were converted using IMAGEJ (Rasband 2003) to greyscale and threshold at 50% to produce binary (black-and-white) images that, when printed onto brown card, produced bark-like brown/black spatial variation (figure 2*a,b*). The digital editing into triangular shapes was done using PAINTSHOP PRO. As in experiment 1, the two treatments were unmanipulated asymmetric patterns and mirror-reflected symmetrical patterns. As the targets were designed to be even more cryptic than in experiment 1 (without detailed maps, we would never have relocated them), the sample size was increased to a total of 500 targets

Figure 1. Reflectance spectra from the bark of a sample of 30 oak trees used in the main study site (Leigh Woods) and the brown card used for the experimental targets. Thin lines are oak bark spectra, the thick line is from the card. Each curve is the mean of five replicate measurements, repositioning the sample under the measuring probe each time. Protocol and spectrophotometry equipment are exactly as described in Cuthill *et al.* (1999).

Figure 2. Example of the artificial moth targets used in experiment 2; (a) asymmetric treatment; (b) symmetric treatment. Actual targets were black printed on brown card, and were 50 × 25 mm.

per treatment (20 blocks with 25 targets per treatment per block). The experiment was run during summer (July–September).

Analysis was by Cox (1972) regression (SPSS Inc. 1998; Lawless 2002; Klein & Moeschberger 2003) with the factors treatment and block. Cox regression is a semi-parametric form of survival analysis that assumes all treatments have the same-shaped hazard functions, but makes no specific assumptions about the nature of the distribution. The proportional hazards assumption of the method was tested by visual inspection of plots of the partial residuals against the ranked survival time.

3. RESULTS

There were no block-by-treatment interactions in either experiment (experiment 1: Wald = 0.304, d.f. = 3, $p = 0.959$; experiment 2: Wald = 6.071, d.f. = 19, $p = 0.998$), so the interaction terms were removed from

Figure 3. 'Survival' curves of asymmetrically (solid lines) and symmetrically (broken lines) patterned targets in (a) experiment 1 and (b) experiment 2. Curves are the probability of surviving bird predation as a function of time, based on Kaplan–Meier estimates to account for censoring due to non-avian predation and survival to the end of the study period (SPSS Inc. 1998).

the models. Symmetrically patterned targets were predated at a higher rate than unmanipulated targets in both experiments (experiment 1, figure 3a: Wald=5.553, d.f.=1, $p=0.018$; experiment 2, figure 3b: Wald=4.471, d.f.=1, $p=0.034$). The effect sizes were such that in experiment 1 symmetrical targets had a 15% lower probability of survival over the 5-day experimental period compared to asymmetrical targets; for experiment 2 the probability of survival was 6% lower. There were significant main effects of block in both experiments (experiment 1: Wald=20.385, d.f.=3, $p<0.001$; experiment 2: Wald=55.898, d.f.=19, $p<0.001$), reflecting differences in average predation rates in different parts of the woods on different dates, but the specific causes of these were unknown and of no direct interest. Differences in average survival between blocks could have been due to different weather conditions on different dates, different predator densities in different parts of the woods, different light environments in different parts of the woods, different levels of human disturbance or subtle differences in the average bark pattern; differences between experiments could also have been due to different target design and size. Given the infinite number of possible causes, average block and between-experiment differences are not discussed further.

Species observed taking the prey included blue tits (*Parus caeruleus*), great tits (*Parus major*), European robins (*Erithacus rubecula*), chaffinches (*Fringilla coelebs*) and house sparrows (*Passer domesticus*). Other candidate species were observed in the area (e.g. woodpeckers), but were not actually seen taking prey. However, no

systematic program of observation was carried out, this being incompatible with the quantity of data required. The commonest non-avian predators (treated as censored data) were spiders, harvestmen (Opiliones), slugs and ants, taking 8% of prey in experiment 1 and 19% in experiment 2. When the data censored because of non-avian predation were analysed separately, there were no treatment differences, as one might expect for predators locating prey non-visually or side-on to the 'wing' patterns (experiment 1: Wald=2.633, d.f.=1, $p=0.105$; experiment 2: Wald=0.412, d.f.=1, $p=0.521$).

4. DISCUSSION

Our two experiments clearly show that bilateral symmetry in body coloration significantly increases the mortality risk of an otherwise highly cryptic target. The effect was not large in absolute terms (both experiments, particularly the second, had very high power through large sample sizes), but was significant on a timescale of a few days, and thus likely to be a relevant selection pressure on the small insects that are the prey of woodland birds. One obvious question arises from these results: why do so many apparently cryptically coloured animals have symmetrical colour patterns? The same question was raised by Forsman & Merilaita (2003) after their analysis of the relative symmetry of cryptic forewings and conspicuous hindwings of a sample of butterflies revealed no marked differences. They had predicted, based on previous work showing that symmetry enhances the effectiveness of aposematic signals (Forsman & Merilaita 1999), that pattern symmetry should be far greater in the conspicuous aposematic wing patterns, but the differences were small or absent.

A developmental or genetic constraint on producing asymmetrical integumentary patterns is possible. For example, butterfly wing colours can be understood in terms of threshold responses to global and local morphogens diffusing across the epithelium (Nijhout 1991; Carroll *et al.* 1994; Brakefield *et al.* 1996; Dilão & Sainhas 2004). Monteiro *et al.* (1997) showed, through artificial selection, that wing shape and colour patterns are developmentally coupled in a nymphalid butterfly *Bicyclus anynana*. If the adaptive value of wing symmetry for flight favours wing shape symmetry, then decoupling the control of wing coloration from that of wing morphology may require many mutations or may be developmentally costly. That said, at least one insect with a marked colour asymmetry in wing coloration has been described: a mantid in which many individuals have one red and one green wing (Barabas & Hancock 1999). However, it is certainly possible that the developmental costs of producing an asymmetrical colour pattern on a fundamentally bilaterally symmetrical body often outweigh the predation costs of higher detectability. So, the most relevant question is to what extent do the results of our experiments over- or under-estimate these costs? For animals that are most likely to be viewed from the side, because of the medium they live in and the likely direction of observation, bilateral symmetry will rarely increase conspicuousness. This would be true, for example, of butterflies resting with their wings folded. However, what of moths and the many other animals which potential predators will view from above? The costs of being symmetrical may vary with the

type of camouflage employed and the nature of the background. At a trivial level, on a homogeneous background which favours a unicoloured camouflage (e.g. a yellow animal on sand), the marginal benefits of asymmetrical coloration will be low as there is little 'pattern' on the animal anyway.

Two interrelated, but logically distinct, means of camouflage are background pattern matching and disruptive coloration (Cott 1940; Merilaita 1998; Cuthill *et al.* 2005). In the former, the colours are a random sample of the background and the function is to minimize any disparity in hue, brightness or texture that may reveal the organism (Endler 1981, 1984). However, as first advanced by Thayer (1896, 1909), and soon recognized by the pioneers of the scientific approach to animal and military camouflage (see Behrens 2002), shadow and pattern discontinuities at the body's periphery render an object with even perfect background pattern matching open to detection. Disruptive coloration, therefore, uses bold contrasting colours on the body's periphery to break up its outline, and across prominent body parts (head, limbs) to reduce perceptual 'grouping' of adjacent features into a recognizable object (Cott 1940; Thayer 1909). Our artificial targets had elements of both background pattern matching (brown and black colours, and textural variation as present on the bark) and disruptive coloration (only two colours, with pattern elements overlapping the 'wing' margins). Disruptive pattern elements can, it is proposed (Thayer 1909; see Cott 1940, p. 50), function even if they are more conspicuous than the colour patches used in simple background pattern matching. Their effect relies on the predator's failure to perceive the conspicuous pattern elements as part of a single object. Symmetry in high-contrast disruptive patterns would therefore seem to be particularly costly, whereas symmetry of simple background matching colours may incur a lower marginal cost of increased detectability. In either case, animals can employ behavioural strategies, such as folding one wing over another or asymmetrical placement of appendages, to reduce the costs of symmetry. However, why complete asymmetry in cryptic coloration is not ubiquitous, as it is with military camouflage (Behrens 2002), remains to be satisfactorily explained.

Many thanks to Geoff Hancock of the Hunterian Museum, University of Glasgow, for bringing his mantid asymmetry paper to our attention. Thank you to Lars Chittka and two anonymous referees for their suggested improvements to the manuscript.

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