Title:
Stripes for warning and stripes for hiding: spatial frequency and detection distance

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Abstract:
Striped patterns are common in nature, and are used both as warning signals and camouflage. Their effectiveness in either role depends on their color and spatial frequency, and how these compare to the background. Although this general principle is well established, the specific detail of how visual texture influences defensive coloration remains untested in the field. For aposematic patterns, especially, little work has focused on how pattern components, as opposed to color, affect warning signal efficacy. By presenting artificial moth-like stimuli, pinned to tree bark, to wild avian predators and human observers, we examine how the spatial frequency and orientation of stripes affects the survival and detectability of yellow-and-black (aversive) and olive-and-black (cryptic) patterns. For the cryptic stripes, we find that matching the dominant spatial frequency and orientation of the background increases survival against bird predation and decreases the distance from which humans first detect the target. For aversive stripes, however, survival against birds peaked at spatial frequencies which neither matched the dominant background spatial frequency nor maximized the mismatch between target and background. This peak in survival at intermediate spatial frequencies did not match detectability by humans: there was no difference in the initial detection distance between stripes of different spatial frequencies, although the distance at which stripes could be resolved did differ. We suggest that, whereas the best cryptic strategy is to match the dominant components of the background as closely as possible, the optimal aposematic signal is one which balances signal distinctiveness and recognition at a distance.

Key words:
Aposematism, background matching, camouflage, coloration, detection distance, predation.
INTRODUCTION

Animals use color for a range of defensive functions, including to signal a warning (aposematism) or to conceal their presence (camouflage). The evolution of such colors is directly affected by both the visual systems of predators and the characteristics of the environment against which the pattern is viewed (Endler 1978; 1987; 2000). As a consequence, animal coloration has diversified into a huge range of different forms. It is, however, interesting to note that functions as different as aposematism and camouflage can both be expressed as striped patterns. It is well understood that camouflaged patterns are affected by the color, spatial frequency, relative phase, and orientation of the background; however, the relative importance of each aspect remains unknown (Stevens and Merilaita 2009). In contrast, the role of background on aposematic signals has not received as much attention (Gamberale-Stille and Guilford 2003; Aronsson and Gamberale-Stille 2009), especially in relation to pattern as opposed to color (Mappes et al. 2005; Stevens and Ruxton 2012).

Godfrey et al. (1987) used Fourier analysis to investigate the spatial frequency distributions of zebras and tigers and compared these to their respective natural backgrounds. They found that tigers matched the frequency distribution of their backgrounds, an indication that tigers are camouflaged; whereas they found that zebras mismatched their background, leading the authors to conclude their stripes evolved for signaling rather than concealment. The suggestion that stripes used for concealment should match the dominant spatial frequency of the background, whereas stripes used in signaling should mismatch the background has, however, not been tested under field conditions. We provide such a test. Aposematic patterns commonly consist of high contrast repetitive stripes, the function of which is still unresolved (Stevens and Ruxton 2012). In order to convey a warning signal
effectively, aposematic signals should maximize the likelihood they will be recognized as
aversive, and this has been suggested as the reason why aposematic organisms are often
highly conspicuous. Various authors have argued that conspicuous patterns benefit
aposematic animals as they are more easily learnt and remembered (Gittleman and Harvey
1980), are detected from further away, allowing a predator more time to remember the
association (Guilford 1986), or because defended species need to be distinct from palatable
species that are often camouflaged (Merilaita and Ruxton 2007). Alternatively, high contrast
stripes may evolve because they are a stable, salient signal regardless of occlusion or
variation across microhabitats (Kenward et al. 2004; Stevens and Ruxton 2012). None of
these mechanisms, of course, are mutually exclusive. There has, however, been a paucity
of research into the size and shape of aposematic pattern elements, with the only relevant
experiments focusing on the presence/absence of internal contrast boundaries (Aronsson

Recent work has highlighted the extent to which avian predators manage their intake of toxic
prey depending on the availability and detectability of alternative prey, as well as their own
current toxin burden, nutritional requirements, and energy expenditure (Barnett et al. 2007;
2012; Chatelain et al. 2013; Halpin et al. 2014). It may, therefore, be beneficial for
aposematic organisms to reduce their predator encounter rates by being camouflaged when
viewed from afar, while also retaining highly visible signals if encountered at close range
(Endler 1978; Tullberg et al. 2005; Bohlin et al. 2008; Caro et al. 2013; Barnett and Cuthill
2014). The design of an effective dual-function color pattern will depend on the predator’s
visual acuity, as this sets a limit on the salience of striped aposematic patterns (Mottram
1915; Campbell and Green 1965). With increasing distance, the size of the image projected
on to the retina decreases. Eventually the retinal spatial frequency increases beyond the
resolution limit, and stripes will be perceptually summed to an average color. It has long
been suggested that pattern blending, where stripes are summed to form an average color
that matches the background, could provide distance-dependent changes in defensive
strategy (Mottram 1915; Marshall 2000). Alternatively, where the spatial frequency of an
aposematic pattern matches the background, camouflage through background matching may
reduce detectability and increase survival (Stevens 2007; Dimitrova and Merilaita 2014).

Here we investigate how the spatial frequency of stripes affects the survival and detectability
of both camouflaged and aposematic patterns. Stripes may include aspects of background
matching, disruptive camouflage, and/or aversive signaling, depending on their dominant
coloration and spatial frequency, and that of the background. To assess the relative
contribution of each mechanism to different patterns, we conducted field experiments with
artificial moth-like stimuli, printed with either yellow-and-black or olive-and-black stripes at
nine different spatial frequencies and two orientations. Initially we measured relative
predation rates with wild avian predators (Experiment 1); then, using human participants
under the same conditions, we investigated the distances at which patterns could be
detected and identified (Experiment 2).

MATERIALS AND METHODS

Stimulus design
Our experiments were conducted in Leigh Woods National Nature Reserve (North Somerset,
UK) and followed an established paradigm using paper moth-like targets to record predation
rates and detectability under wild conditions (e.g. Cuthill et al. 2005; Barnett and Cuthill
2014). Our stimuli conceptually represented moths resting on tree bark, but were not
designed to mimic any particular species. Stimuli were triangular (50 × 25 mm) pieces of
waterproof paper (‘Rite-In-The-Rain’, JL Darling LLC, Tacoma, WA, USA), which were
printed with sine waves of different spatial frequencies, horizontally or vertically, and were
either olive-and-black (an inconspicuous color combination on this background) or yellow-
and-black (a common aposematic color pattern).

To test the effect of pattern blending and the potential for distance-dependent camouflage,
the mean color of the yellow-and-black stripes matched the mean color of the oak bark
background as viewed by an avian predator. To achieve this match we took the mean color
from photographs of oak bark (n = 100), taken in Leigh Woods and calibrated to represent
the relative photon capture rates of the blue tit (*Cyanistes caeruleus*, Paridae; Hart et al.
2000), a typical passerine predator in this environment. The particular colors used in the
yellow-and-black stripes were then designed such that each spatial frequency would blend to
the average color of the background, each at a different distance.

To produce the striped treatments (B, C, D, E, F, G, and H) we created a geometric series of
sine waves which, once printed, corresponded to 9.20 (B), 4.60 (C), 2.30 (D), 1.15 (E), 0.58
(F), 0.29 (G), and 0.14 (H) cycles per centimeter. Each of these striped treatments was
printed to be either horizontally (H) or vertically (V) orientated on the target, with phase
randomized between individual targets (i.e. the sequence dark-light-dark-light-etc. could start
at any point). These 14 treatments were printed to be colored either olive-and-black (G) or
yellow-and-black (Y). We also took the mean of each color combination to create two plain,
homogenously colored reference treatments (A). In total this created 30 different treatments,
15 of each color combination, based on olive-and-black (GA, GBH, GBV, GC, GCV, GDH, GDV,
GEH, GEV, GEFH, GEFV, GEGH, GEGV, GEGH, and GEGV) and yellow-and-black (YA, YBH, YBV, YCV,
YDH, YDV, YEH, YEY, YFV, YFV, YG, YGV, YGH, YGV, YGH, and YGV; Figure 1a; b).

In order to compare our stimuli to the background texture we extracted spatial frequency
information from calibrated photographs of oak bark, taken of a representative sample of the
trees to be used in the study. Photographs were taken with a Nikon D70 and 35 mm lens (Nikon Corporation, Tokyo, Japan) on a tripod, and included a ColorChecker Passport (X-Rite Inc., Grand Rapids, MI, USA) which allowed appropriate color calibration and scaling. The aperture of the camera was kept at f8 and the ISO at 100 while the shutter speed was on automatic. All 3008 x 2000 pixel images were subsequently converted to uncompressed 8-bit-per-color-channel TIFF files from the original RAW format data (Nikon NEF). Custom MATLAB code (The Mathworks Inc., Nattick, MA, USA) was used both to calibrate and select an area of bark from each image. The selected area was 768 x 768 pixels, equivalent to 61.4 x 61.4 cm.

Each photograph (n = 48) was calibrated and transformed to an 8-bit greyscale image that represented the relative photon catch of blue tit (C. caeruleus) double cones under standard D65 daylight (Wyszecki and Stiles 1982; Kelber et al. 2003; Stevens and Cuthill 2006). The experiments were done in winter, so D65 was a more appropriate open forest illuminant than woodland shade (Endler 1993). We then extracted spatial frequency information from each image using a Discrete Fast Fourier Transform (Image Processing Toolbox, MATLAB, The MathWorks Inc., Natick, MA, USA). A Gaussian Mixture Model from package mixtools (Benaglia et al. 2009) in R 3.1.3 (The R Foundation for Statistical Computing, Vienna, Austria) showed spatial frequency to be predominantly vertically orientated with a major peak at 8.26 cm (0.12 cycles per centimeter) and a minor peak at 19.05 cm (0.05 cycles per centimeter; Figure 2).

In Experiment 1B we further investigated the effect of background matching camouflage and focused on olive-and-black striped targets with patterns which matched the dominant orientation, and surrounded the dominant spatial frequency, of the background. We used five vertically orientated olive-and-black treatments (Figure 1c): \( \hat{O}_A \) (the mean color of olive-
and-black), $^G_{G V}$ (0.29 cycles/cm), $^G_{H V}$ (0.14 cycles/cm), and two extra designs $^G_{I V}$ (0.07 cycles/cm) and $^G_{J V}$ (0.04 cycles/cm). This meant that treatment $^G_{H V}$ (vertical stripes at 0.14 cycles/cm) closely matched the most common background spatial frequency (vertically orientated at 0.12 cycles/cm), and treatment $^G_{J V}$ (vertical stripes at 0.04 cycles/cm) closely matched the secondary peak in spatial frequency (vertically orientated at 0.05 cycles/cm). In order to accommodate the lower spatial frequency sinewaves we used larger paper-moth targets (100 x 50 mm). We made no comparisons between the treatments in experiment 1A and B, so the different size of the targets is not a confounding factor.

**Experiment 1 – Survival**

In Experiment 1 we tested how effective each spatial frequency and color combination was at preventing predation by wild passerine birds which include blue tits (*Cyanistes caeruleus*, Paridae), European robins (*Erithacus rubecula*, Muscicapidae), and chaffinches (*Fringilla coelebs*, Fringillidae) (Cuthill et al. 2006). Our moth-like stimuli were pinned to the bark of mature oak trees along non-linear transects through Leigh Woods National Nature Reserve. Each moth was pinned at roughly head height (1.5 – 1.8 m), flush to the bark of mature (>1.5 m circumference) oak trees.

In order to assess survivability each moth was baited with a dead mealworm larva (*Tenebrion molitor*, Tenebrionidae) which was pinned so that ca. 5 mm protruded from beneath the target. The mealworm larva acted as an indicator of predation, and was mostly hidden by the target in order to minimize saliency. Any cue was, in any case, the same for all targets. The presence/absence of the mealworm larva was recorded at 24, 48, 72, and 96 h after the trial began. Relative mortality was analyzed with a Mixed Effects Cox Model from package *coxme* (Thernaeu 2015) and pairwise tests used the False Discovery Rate from package *multcomp* (Hothorn et al. 2008), to control for Type I error, in R 3.1.3. Evidence of avian
predation (a clean cut across or complete removal of a mealworm) was recorded as a full event, whereas non-avian predation (predominantly from slugs which left slime trails and spiders which left the hollow exoskeleton of the mealworm), missing targets, and those surviving to 96 h were included as censored values, and block was included as a random factor.

For Experiment 1A we used 30 treatment designs corresponding to seven spatial frequencies, in two orientations, and two color combinations, as well as two plain controls: $G_A, G_{B_H}, G_{B_V}, G_{C_H}, G_{C_V}, G_{D_H}, G_{D_V}, G_{E_H}, G_{E_V}, G_{F_H}, G_{F_V}, G_{G_H}, G_{G_V}, G_{H_H}, G_{H_V}, Y_A, Y_{B_H}, Y_{B_V}, Y_{C_H}, Y_{C_V}, Y_{D_H}, Y_{D_V}, Y_{E_H}, Y_{E_V}, Y_{F_H}, Y_{F_V}, Y_{G_H}, Y_{G_V}, Y_{H_H}, and Y_{H_V}$ (Figure 1a; b). In the winter months between October 2013 and March 2015 we placed out 24 independent blocks of moths comprising 12 blocks of olive-and-black and 12 blocks of yellow-and-black ($n = 1425$ per color). For each color, the first seven blocks contained 10 replicates of each treatment (run in 2013/2014) and, due to time constraints, the latter five blocks contained five replicates (run in 2014/2015). These minor differences in design between years were controlled and balanced and so were of no consequence to the outcome of the experiment. In order to prevent spatial confounds each block was located in a different part of the woodland and each pair of olive-and-black and yellow-and-black blocks was run concurrently in non-adjacent areas.

In Experiment 1B we focused on potential background matching effects, using olive-and-black stripes which matched the dominant spatial frequency and orientation of the background. For this we used five vertically striped treatments which centered on the mean spatial frequency distribution of the background ($G_{A}, G_{G_V}, G_{H_V}, G_{I_V}, and G_{J_V}$; Figure 1c). In November and December 2014 we set out three blocks of 15 of each treatment ($n = 225$) sequentially in non-adjacent areas of Leigh Woods.

**Experiment 2 – Detection**
Predation is a joint product of detection, recognition, and the decision to attack. In order to assess the detectability per se of our striped targets we modified a protocol used by Barnett and Cuthill (2014), using human participants as surrogate predators. Bird and human color vision (Cuthill 2006) and spatial acuity (Ghim and Hodos 2006) differ, but here it is the relative ranking of different spatial frequencies and orientations that is of interest, rather than absolute detection thresholds. This experiment used the same 30 target treatments, under the same conditions, in the same woodland, as in Experiment 1A. Treatments included all combinations of spatial frequency (A, B, C, D, E, F, G, and H), orientation (vertical (V) and horizontal (H)), and color (olive-and-black (G) and yellow-and-black (Y)) used in Experiment 1A: G_A, G_B, G_C, G_D, G_E, G_F, G_G, G_H, G_V, G_H, G_V, G_B, G_V, Y_A, Y_B, Y_C, Y_D, Y_E, Y_F, Y_G, Y_V, Y_H, and Y_V. For each of three independent blocks, targets were pinned along a non-linear transect through Leigh Woods National Nature Reserve. For each block, five replicates of each treatment (n = 150), were pinned in a random sequence, flush to the bark of mature oak trees, at head height (1.5 – 1.8 m). Each target was pinned within 10 m of the path and positioned to be unobscured by vegetation and potentially visible from at least 20 m along the trail. Between October 2013 and June 2014 18 participants (3 male and 3 female per block), with normal or corrected to normal vision, separately walked the transect route at a slow and steady pace followed by an experimenter. When a target was spotted the participant would record the initial detection distance (to within 0.01 m) with a Leica Disto™ A6 laser range finder (Leica Geosystems, Heerbrugg, Switzerland). The participants were then asked to identify the presence/absence and orientation of any stripes. If the participant answered incorrectly or was unsure, they were instructed to walk towards the target and recorded the distance at which the target could be correctly identified. We used General Linear Mixed
Effects Models, from package *lme4* (Bates et al. 2015), to analyze the normalized (log transformed) distributions of both the initial detection distance and the stripe identification distance, and pairwise tests used the False Discovery Rate from package *multcomp* (Hothorn et al. 2008) to control for Type I error, in R 3.1.3. The quoted p-values are after adjustment for multiple testing.

**RESULTS**

**Survival**

In Experiment 1A we found a significant interaction between color and treatment ($\chi^2 = 100.99$, df = 14, $P < 0.001$), and so olive-and-black and yellow-and-black stripes were split for subsequent analysis.

Of the 1425 olive-and-black paper moths, 937 (66%) showed evidence of avian predation and were included in the analysis as full events (Figure 3a). We found a significant overall effect of treatment ($\chi^2 = 101.48$, df = 14, $P < 0.001$) so, to investigate this in more detail, we dropped treatment A (no stripes) and analyzed the remaining treatments as a balanced 7 x 2 factorial design. This showed a significant interaction between spatial frequency and orientation ($\chi^2 = 24.75$, df = 6, $P < 0.001$). We therefore analyzed vertically and horizontally orientated stripes separately.

We found no significant effect of spatial frequency on the survival of horizontally striped olive-and-black moths ($\chi^2 = 6.10$, df = 6, $P = 0.412$). The survival of vertically orientated striped moths, however, did differ between different spatial frequencies ($\chi^2 = 57.48$, df = 6, $P < 0.001$). Pairwise tests showed no significant stepwise differences ($^{GBv}_{GCv} z = 0.62$, $P = 0.996$; $^{GCv}_{GDv} z = 0.89$, $P = 0.975$; $^{GDv}_{GEv} z = 1.86$, $P = 0.504$; $^{GEv}_{GFv} z = -0.04$, $P = 1.00$; $^{GFv}_{Gv} z = 2.29$, $P = 0.247$; $^{Gv}_{GHv} z = -0.19$, $P = 1.00$), however, survival was
significantly higher at lower spatial frequencies than higher spatial frequencies ($^G_{Bv} - ^G_{Ev}$: $z = 3.28, P = 0.018$; $^G_{Bv} - ^G_{Fv}$: $z = 3.25, P = 0.019$; $^G_{Bv} - ^G_{Gv}$: $z = 5.40, P < 0.001$; $^G_{Bv} - ^G_{Hv}$: $z = 5.17, P < 0.001$; $^G_{Cv} - ^G_{Ev}$: $z = 4.90, P < 0.001$; $^G_{Cv} - ^G_{Fv}$: $z = 4.68, P < 0.001$; $^G_{Dv} - ^G_{Gv}$: $z = 4.06, P < 0.001$; $^G_{Dv} - ^G_{Hv}$: $z = 3.85, P = 0.003$). Although there was no significant difference between $^G_{Cv}$ and the lower spatial frequency $^G_{Ev}$ and $^G_{Fv}$ patterns p-values are marginal ($^G_{Cv} - ^G_{Ev}$: $z = 2.72, P = 0.092$; $^G_{Cv} - ^G_{Fv}$: $z = 2.70, P = 0.099$). There were no other significant pairwise comparisons ($z < 2.29, P > 0.247$).

In a separate experiment (1B) we tested the survival of vertically orientated patterns with spatial frequencies equal to, or lower than, the dominant spatial frequency of the background. Overall, 143 out of 225 targets (63%) were eaten by birds (Figure 3b). We found a significant effect of spatial frequency ($\chi^2 = 27.53, df = 4, P < 0.001$) and pairwise tests showed significantly lower survival for spatial frequencies lower than that of the background ($^Cv - ^G_{Hv}$: $z = 0.42, P = 0.994$; $^G_{Gv} - ^G_{Iv}$: $z = -3.24, P = 0.010$; $^G_{Gv} - ^G_{Jv}$: $z = -3.24, P = 0.004$). Our data therefore suggest that for olive-and-black stripes the greatest survival comes from matching the most common spatial frequency and orientation of the background.

For the yellow-and-black striped moths (Experiment 1A), 1070 of 1425 (75%) stimuli were predated by birds and included as full events in the analysis (Figure 4). There was a significant overall effect of treatment on survival ($\chi^2 = 73.09, df = 14, P < 0.001$) so, as with the olive-and-black targets, we dropped treatment A and analyzed the remaining treatments as a balanced 7 x 2 factorial design. This showed no significant interaction between orientation and spatial frequency ($\chi^2 = 3.58, df = 6, P = 0.73$) although, after dropping the interaction term, the main effect of Orientation was marginal (vertical stripes tending to survive longer; $\chi^2 = 3.80, df = 1, P = 0.051$) and Frequency was highly significant ($\chi^2 = 65.73, df = 6, P < 0.001$). Being so close to significance, we retained Orientation in the model in
order to improve the precision with which Frequency effects can be estimated. However, dropping Orientation from the model does not affect the following conclusions. Pairwise tests generally showed a decrease in survival at the lowest spatial frequencies (\( \gamma_B - \gamma_G: z = -4.07, P = 0.001; \gamma_B - \gamma_H: z = -3.80, P = 0.003; \gamma_E - \gamma_G: z = -4.44, P < 0.001; \gamma_E - \gamma_H: z = -4.17, P < 0.001 \)), although comparisons between \( \gamma_C \) and \( \gamma_E \) were marginal (\( \gamma_C - \gamma_E: z = 2.93, P = 0.052 \)). However, rather than survival increasing with spatial frequency there was a peak in survival at 2.30 cycles/cm (treatment D – \( \gamma_D - \gamma_C: z = -4.68, P < 0.001; \gamma_D - \gamma_F: z = -4.57, P < 0.001; \gamma_D - \gamma_G: z = -6.15, P < 0.001; \gamma_D - \gamma_H: z = -5.88, P < 0.001 \); Fig. 4.6). There were no other significant pairwise comparisons (\( z < 2.60, P > 0.127 \)).

**Initial detection distance**

In Experiment 2A we tested for any differences in the initial distance at which targets were detected. We found a significant interaction between color and treatment, and so we analyzed olive-and-black and yellow-and-black treatments separately (\( \chi^2 = 112.28, df = 14, P < 0.001 \)). Dropping treatment A (no stripes), as in the previous analyses, we also found a significant interaction between orientation and spatial frequency for both olive-and-black (\( \chi^2 = 29.75, df = 6, P < 0.001 \)) and yellow-and-black (\( \chi^2 = 15.19, df = 6, P = 0.019 \)) striped treatments. We therefore split both color combinations by orientation.

For the horizontally orientated olive-and-black stripes there was a significant effect of spatial frequency (\( \chi^2 = 22.09, df = 6, P = 0.001 \)) and pairwise tests showed a significantly greater detection distance for treatment \( ^G\text{H}_H \) when compared to higher spatial frequency patterns (\( ^G\text{D}_H - ^G\text{H}_H: z = -3.12, P = 0.030; ^G\text{E}_H - ^G\text{H}_H: z = -3.13, P = 0.029; ^G\text{F}_H - ^G\text{H}_H: z = -4.29, P < 0.001; ^G\text{G}_H - ^G\text{H}_H: z = -3.64, P = 0.005 \)). Although differences between the highest and lowest spatial frequencies were marginal (\( ^G\text{B}_H - ^G\text{H}_H: z = -2.87, P = 0.061 \)). There was no difference between other treatments \( z < 2.36, P > 0.215 \); Figure 5a).
We also found a significant effect of spatial frequency for the vertical olive-and-black stripes \((\chi^2 = 24.31, \text{df} = 6, P < 0.001)\) but, conversely, pairwise tests found a significantly lower detection distance for the lowest spatial frequencies \((^\text{G}_\text{V}, \text{G}_\text{H})\) when compared to higher spatial frequencies \((^\text{C}_\text{V}, \text{G}_\text{V}), z = 3.14, P = 0.028; \text{G}_\text{V}, \text{G}_\text{H}: z = 3.90, P < 0.001; \text{D}_\text{V}, \text{G}_\text{H}: z = 3.42, P = 0.011)\). No further difference between treatments could be distinguished by pairwise tests \((z < 2.87, P > 0.062; \text{Figure 5b}).\)

The initial detection distance of the horizontally orientated yellow-and-black stripes differed significantly according to spatial frequency \((\chi^2 = 19.30, \text{df} = 6, P = 0.004)\). Pairwise tests showed a significantly greater detection distance for \(^\text{Y}_\text{G}, \text{H}\) \((\text{Y}_\text{C}, \text{H} - \text{Y}_\text{G}, \text{H}): z = -3.13, P = 0.029; \text{Y}_\text{G}, \text{H}: z = 3.87, P = 0.002)\), but no other significant differences \((z < 2.70, p > 0.097; \text{Figure 5c}).\)

For the vertically orientated yellow-and-black stripes, however, although we did find a significant effect of treatment \((\chi^2 = 14.42, \text{df} = 6, P = 0.025)\), but no significant effect could be distinguished from pairwise comparisons, although \(^\text{Y}_\text{E}, \text{V}\) and \(^\text{Y}_\text{H}, \text{V}\) were marginal \((\text{Y}_\text{E}, \text{V} - \text{Y}_\text{H}, \text{V}: z = 2.92, P = 0.053; \text{all other pairwise comparisons: } z < 2.53, P > 0.149; \text{Figure 5d}).\)

**Stripe identification distance**

For the distance at which stripes could be fully resolved and identified, we found a significant interaction between color and treatment \((\chi^2 = 112.28, \text{df} = 14, P < 0.001)\) and, after splitting the two colors, a significant interaction between orientation and spatial frequency for both olive-and-black \((\chi^2 = 16.07, \text{df} = 6, P = 0.013)\) and yellow-and-black \((\chi^2 = 16.45, \text{df} = 6, P < 0.012)\) stripes. We, therefore, analyzed each color-orientation combination separately.

We found a significant effect of spatial frequency for the horizontal olive-and-black striped targets \((\chi^2 = 436.58, \text{df} = 6, P < 0.001)\), and from treatment \(^\text{G}_\text{H}\) to \(^\text{G}_\text{E}\) lower spatial frequencies were identified at greater distances \((^\text{G}_\text{H}, ^\text{C}_\text{H}: z = -7.31, P < 0.001; ^\text{G}_\text{H}, ^\text{D}_\text{H}: z =\)
-14.08, $P < 0.001$; $G_{B_H}G_{E_H}$: $z = -18.60, P < 0.001$; $G_{B_H}G_{F_H}$: $z = -21.51, P < 0.001$; $G_{B_H}G_{G_H}$: $z = -20.89, P < 0.001$; $G_{B_H}G_{H_H}$: $z = -14.87, P < 0.001$; $G_{C_H}G_{D_H}$: $z = -7.76, P < 0.001$; $G_{C_H}G_{E_H}$: $z = -13.01, P < 0.001$; $G_{C_H}G_{F_H}$: $z = -16.15, P < 0.001$; $G_{C_H}G_{G_H}$: $z = -15.45, P < 0.001$; $G_{C_H}G_{H_H}$: $z = -9.04, P < 0.001$; $G_{D_H}G_{E_H}$: $z = -5.48, P < 0.001$; $G_{D_H}G_{F_H}$: $z = -8.41, P < 0.001$; $G_{D_H}G_{G_H}$: $z = -7.73, P < 0.001$), but this reached a plateau between treatments $G_{E_H}$ and $G_{G_H}$ ($z = 18.76, P < 0.001$; $G_{E_H}G_{G_H}$: $z = 2.08, P = 0.0365$; $G_{E_H}G_{G_H}$: $z = 0.66, P = 0.995$), and then declined with treatment $G_{H_H}$ which ended up equal to $G_{D_H}$ ($z = 14.08, P < 0.001$; $G_{H_H}G_{F_H}$: $z = -5.84, P < 0.001$; $G_{H_H}G_{E_H}$: $z = -3.19, P = 0.239$; $G_{H_H}G_{D_H}$: $z = 1.93, P = 0.461$; Figure 5a).

Similarly, for the vertical olive-and-black stripes, we found a significant effect of spatial frequency ($\chi^2 = 552.14$, df = 6, $P < 0.001$), and pairwise tests showed stripe identification distance increased from treatment $G_{B_V}$ to $G_{E_V}$ ($G_{B_V}G_{C_V}$: $z = -9.52, P < 0.001$; $G_{B_V}G_{D_V}$: $z = -18.76, P < 0.001$; $G_{B_V}G_{E_V}$: $z = -23.47, P < 0.001$; $G_{B_V}G_{F_V}$: $z = -27.33, P < 0.001$; $G_{B_V}G_{G_V}$: $z = -24.91, P < 0.001$; $G_{B_V}G_{H_V}$: $z = -24.73, P < 0.001$; $G_{C_V}G_{D_V}$: $z = -9.33, P < 0.001$; $G_{C_V}G_{E_V}$: $z = -14.92, P < 0.001$; $G_{C_V}G_{F_V}$: $z = -18.67, P < 0.001$; $G_{C_V}G_{G_V}$: $z = -16.02, P < 0.001$; $G_{C_V}G_{H_V}$: $z = 15.63, P < 0.001$; $G_{D_V}G_{E_V}$: $z = -6.41, P < 0.001$; $G_{D_V}G_{F_V}$: $z = -10.02, P < 0.001$; $G_{D_V}G_{G_V}$: $z = -7.17, P < 0.001$; $G_{D_V}G_{H_V}$: $z = -6.58, P < 0.001$; $G_{E_V}G_{F_V}$: $z = -3.16, P = 0.026$), before declining at the lowest spatial frequencies $G_{F_V}$ to $G_{H_V}$ ($G_{F_V}G_{G_V}$: $z = 2.89, P = 0.059$; $G_{F_V}G_{H_V}$: $z = 3.65, P = 0.005$), such that $G_{G_V}$ and $G_{H_V}$ are indistinguishable from $G_{E_V}$ ($G_{G_V}$: $z = 0.40, P < 0.999$; $G_{H_V}G_{E_V}$: $z = -0.28, P = 1.00$; $G_{H_V}G_{G_V}$: $z = -0.71, P = 0.992$; Figure 5b).

The yellow-and-black striped moths show a similar pattern. There is a significant effect of treatment for the horizontal stripes ($\chi^2 = 892.78$, df = 6, $P < 0.001$), and pairwise tests show a significant increase in identification distance as spatial frequency decreases from treatment
\[ Y_B \rightarrow Y_E \ (Y_B \rightarrow Y_C; \ z = -8.85, P < 0.001; Y_B \rightarrow Y_D; \ z = -21.04, P < 0.001; Y_B \rightarrow Y_E; \ z = -30.22, P < 0.001; Y_B \rightarrow F_H; \ z = -35.81, P < 0.001; Y_B \rightarrow G_H; \ z = -37.85, P < 0.001; Y_B \rightarrow H_H; \ z = 29.82, P < 0.001; Y_C \rightarrow Y_D; \ z = -12.21, P < 0.001; Y_C \rightarrow Y_E; \ z = -21.32, P < 0.001; Y_C \rightarrow F_H; \ z = -26.67, P < 0.001; Y_C \rightarrow G_H; \ z = -28.93, P < 0.001; Y_C \rightarrow H_H; \ z = -21.08, P < 0.001; Y_D \rightarrow Y_E; \ z = -9.02, P < 0.001; Y_D \rightarrow F_H; \ z = -13.98, P < 0.001; Y_D \rightarrow G_H; \ z = -16.53, P < 0.001; Y_D \rightarrow H_H; \ z = -9.00, P < 0.001; Y_E \rightarrow Y_F; \ z = -4.72, P < 0.001; Y_E \rightarrow Y_G; \ z = -7.49, P < 0.001), \]

which then plateaus (\( Y_F \rightarrow Y_G; \ z = -2.94, P = 0.051) and declines at the lowest spatial frequencies such that \( Y_H \) and \( Y_E \) are indistinguishable (\( Y_H \rightarrow Y_G; \ z = -7.25, P < 0.001; Y_H \rightarrow Y_F; \ z = -4.52, P < 0.001; Y_H \rightarrow Y_E; \ z = -0.12, P = 1.00; \) Figure 5c).

Likewise, the identification distance of vertical yellow-and-black striped moths is significantly affected by spatial frequency (\( \chi^2 = 859.64, df = 6, P < 0.001 \)). Again, from pairwise tests we find a stepwise function where, as spatial frequency decreases, identification distance increases from \( Y_B \rightarrow Y_F \ (Y_B \rightarrow Y_C; \ z = -7.51, P < 0.001; Y_B \rightarrow Y_D; \ z = -19.33, P < 0.001; Y_B \rightarrow Y_E; \ z = -28.46, P < 0.001; Y_B \rightarrow Y_F; \ z = -34.36, P < 0.001; Y_B \rightarrow Y_G; \ z = -33.05, P < 0.001; Y_B \rightarrow Y_H; \ z = -29.59, P < 0.001; Y_C \rightarrow Y_D; \ z = -12.20, P < 0.001; Y_C \rightarrow Y_E; \ z = -22.00, P < 0.001; Y_C \rightarrow Y_F; \ z = -27.91, P < 0.001; Y_C \rightarrow Y_G; \ z = -26.58, P < 0.001; Y_C \rightarrow Y_H; \ z = -23.15, P < 0.001; Y_D \rightarrow Y_E; \ z = -10.44, P < 0.001; Y_D \rightarrow Y_F; \ z = -16.08, P < 0.001; Y_D \rightarrow Y_G; \ z = -14.87, P < 0.001; Y_D \rightarrow Y_H; \ z = -11.83, P < 0.001; Y_E \rightarrow Y_F; \ z = -5.03, P < 0.001; Y_E \rightarrow Y_G; \ z = -4.00, P = 0.001), \) but then plateaued between treatment \( Y_F \rightarrow Y_G \) (\( Y_F \rightarrow Y_G; \ z = 1.01, P = 0.952), \) and then declined at the lowest spatial frequency such that \( Y_H \) was equal to \( Y_E \ (Y_H \rightarrow Y_G; \ z = -2.41, P = 0.194; Y_H \rightarrow Y_F; \ z = -3.41, P = 0.012; Y_H \rightarrow Y_E; \ z = 1.49, P = 0.752; \) Figure 5d).

**DISCUSSION**

We found that the effect of spatial frequency differs radically depending on whether the dominant color is olive (cryptic) or yellow (conspicuous). For olive-and-black stripes, in
accordance with Godfrey et al.’s (1987) prediction for camouflage, we find that survival is
highest and detectability lowest when the stripes match the dominant spatial frequency and
orientation of the background. In contrast, yellow-and-black stripes are not affected by the
background to such an extent, and the greatest survival is found at higher spatial frequencies
than those dominant in the background.

Effective camouflage reduces the detectability of a target by minimizing the mismatch
between background characteristics and the target’s pattern. For the olive-and-black stripes,
our results show survival (against birds) and detectability (by humans) to be inversely related
(i.e. survival is greatest when detectability is lowest). Therefore, our olive-and-black striped
patterns do provide effective camouflage against both taxon’s visual systems on the oak bark
background. This is most likely a result of background matching of spatial frequency and
orientation. We cannot rule out a role for disruptive coloration, although the smooth sine
wave transition between colors is unlike the sharp boundary contrasts we would expect for a
disruptive effect (Osorio and Srinivasan 1991; Merilaita 1998; Cuthill et al. 2005).

The yellow-and-black striped patterns were also predated differentially according to spatial
frequency although, in contrast to the olive-and-black stripes, survival appears to peak at
higher (but not the highest) spatial frequencies. Furthermore, we found no effect of stripe
orientation on survival, and very little difference in detection distance between treatments.
We therefore find no evidence to suggest that the yellow-and-black pattern provided
camouflage. Instead, the observed differences in survival are more likely the result of
differential aversive responses to the striped pattern. Indeed, we have shown an aversive
effect of yellow-and-black stripes in a previous experiment where placement on non-
matching backgrounds in the open precluded any influence of camouflage (Barnett & Cuthill
2014). Our results are, therefore, broadly in accordance with Godfrey et al.’s (1987)
prediction that patterns involved in salient signals should mismatch the background spatial
frequency distribution.

It has been suggested that aposematic patterns can incorporate aspects of camouflage in
order to reduce their predator encounter rates (Endler 1978; Tullberg et al. 2005; Bohlin et al.
2008; Caro et al. 2013; Barnett and Cuthill 2014). One proposed mechanism for reducing
detection distance is disruptive coloration through high internal contrast combined with
differential blending (Cott 1940), where a salient color, not found in the background, is
combined with a background color in order to break up the target’s outline into
unrecognizable features (Schaefer and Stobbe 2006; Stevens et al. 2006; Fraser et al. 2007;
Stevens, 2007). We find no evidence for background matching or differential blending in our
yellow-and-black patterns. That said, the more complex patterns frequently associated with
disruptive camouflage have yet to be tested in combination with aposematism.

An alternative means of combining warning signals with camouflage, suggested by Mottram
(1915) is that, as striped patterns cannot be resolved when viewed from a distance, adjacent
high contrast patches will blend together to produce a camouflaged color to distant
observers. As the average color of our yellow-and-black striped targets was equal to the
mean color of the background, we were able to test whether pattern-blending can produce
effective camouflage in situ. We find that, although there were differences in the distance at
which stripes could be resolved, there was no convincing trend in the initial detection
distance of the yellow-and-black targets. Interestingly, however, this means that many of the
aversive targets were detected before they could be identified as striped.

The average color of the background has been used as a control treatment in numerous
experiments into the optimal design of camouflage patterns (e.g. Cuthill et al. 2005; Fraser et
al. 2007; Barnett and Cuthill 2014). These previous studies show that a single homogenous
color is poor at preventing detection, even if it is the average color of the background, as the pattern mismatches the background texture and leaves the target’s edge intact (Stevens and Cuthill 2006). As the majority of our stimuli were of a higher spatial frequency than the dominant background spatial frequency, targets would have been initially detected as a plain homogenously colored object resting on a coarse grained background. This disparity between detection and recognition may have evolutionary consequences, as an aposematic organism could at first be identified as a poorly camouflaged palatable prey.

As aposematism depends on reliable recognition in order to be effective, it would be expected that survival would be highest where the pattern minimizes the disparity between initial detection and recognition distance. This is not, however, what we find: instead, survival is greatest at intermediate spatial frequencies. One possible explanation is that the spatial frequency that maximized survival, 2.30 cycles/cm, may match the spatial frequency of one or more aposematic species that avian predators have already acquired an, or have an innate, aversion to. There are no butterflies or moths in the area that have yellow-and-black striped patterns similar to our artificial targets (Barnett et al. 2003; 2008). However, that birds generalize the pattern from a wasp or cinnabar moth (Tyria jacobaeae, Erebidae) caterpillar is not impossible, if color and pattern dominate shape cues (see e.g. Kazemi et al. 2014). That said, the spatial frequency of the stripes of a cinnabar moth caterpillar or the most common wasps in the area (Vespula vulgaris and V. germanica, Vespidae), are about twice that of the best-surviving treatment in our experiment (Barnett JB. unpubl. data).

A second explanation could be that 2.30 cycles/cm is indeed a ‘special’ frequency, not because it directly mimics the patterning of particular species, but because at a particular, ecologically relevant, viewing distance this frequency projects a pattern on the retina that is itself aversive. The fact that light of particular temporal or spatial frequencies evoke ‘visual
discomfort' and can be (often subconsciously) aversive, is well known for humans (Wilkins et al. 1984; Wilkins 2016). However, more recently it has been suggested that this arises when patterns depart from the spatial statistics of the natural scenes the brain has evolved to encode efficiently (Penacchio and Wilkins 2015), and that warning colors could exploit this (Cole and Wilkins 2013). This intriguing possibility remains untested.

The final possible explanation is that there is a trade-off between factors favoring larger stripes versus more stripes. Certainly the lowest spatial frequencies tested (F, G, and H; Figure 1) allowed few stripes per target, and this may have compromised the distinctiveness of the signal. At the high spatial frequency end, a simple constraint might be that any frequencies higher than the 2.3 cycles/cm of the best-surviving treatment, are not resolvable by the predators at the distance they make the decision to attack. We feel that this is unlikely. Although birds seem to have much lower contrast sensitivity than humans (Ghim and Hodos 2006), the yellow-and-black stripes were high contrast and the highest spatial frequency we used (9.20 cycles/cm) would correspond to a visual angle of about 1° from a distance of 6 cm, the height of the eye of a small passerine like a blue tit when perched next to the target and inspecting prey. This visual angle is far larger than the acuity limit of those species measured (Ghim and Hodos 2006). However, even if detectability is not an issue, lower contrast sensitivity to high frequency stripes may reduce their salience and thus effectiveness. The optimization of aposematic patterning, even when that pattern consists of simple stripes, appears to be a complex affair.

A final caveat to the generality of our results is that the avian predation was carried out at a particular time of year (winter) in a particular woodland. There have been shown to be seasonal differences in the response to novel aposematically colored prey, for example due to completely naïve fledglings dominating the community in spring and early summer.
There may also be local regional differences, presumably due to different communities of predators with different prior experience of aposematic prey (Carroll and Sherratt, 2013; Exnerová et al., 2015). There may also be differences in the detectability of different patterns as a result of changes in the foliage canopy, height of the sun, weather and other factors affecting the light environment (Endler 1993; Rojas et al., 2014). These are all important factors to investigate in the future.

In conclusion, by altering color we provide support for Godfrey et al.’s (1987) thesis for how stripes may function as camouflage or signals dependent upon background characteristics. Our data demonstrate the importance of spatial frequency and orientation matching in background matching camouflage, and also that the optimization of aposematic patterns is complex and cannot be explained simply by maximizing dissimilarly to the background or detection distance: rather, a range of factors interact to produce an effective signal.

Aposematism, or more generally signaling, is not necessarily incompatible with concealment at a distance (Endler 1978; Tullberg et al. 2005; Bohlin et al. 2008; Caro et al. 2013; Barnett and Cuthill 2014; Barnett et al. 2016). Furthermore, although the lack of a lower-frequency textural match could compromise the effectiveness of high-frequency pattern blending for producing camouflage on complex textured backgrounds, we would stress that observer distance is an important, and often underappreciated, component of signal design.
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**Figure 1**  Treatment designs. In Experiment 1A and Experiment 2 we used a wide range of yellow-and-black, (a), and olive-and-black, (b), moths of both vertical and horizontal orientations. For Experiment 1B we used larger sized vertically orientated olive-and-black moths, (c), which surrounded the dominant spatial frequency and (vertical) orientation of the oak bark background.
Figure 2  Oak bark background spatial frequency distribution. Wavelength density peaks at 8.26 cm (0.12 cycles per centimeter), with a small secondary peak at 19.05 cm (0.05 cycles per centimeter).
Figure 3  Relative survival (odds ratios with 95% CI from the model) of the olive-and-black moths. For both Experiments 1A, (a), and 1B, (b), survival is highest where spatial frequency and orientation match the background (\(^G_{GV}\) and \(^G_{HV}\)).
Figure 4: Relative survival (odds ratios with 95% CI from the model) of the yellow-and-black striped moths (Experiment 1A). There is no effect of orientation on survival and no evidence of camouflage; survival is highest at midlevel spatial frequencies (\( \gamma_{DH} \) and \( \gamma_{DV} \)).
Figure 5  Initial detection distance (black) and stripe identification distance (orange) of each color-orientation combination (mean log$_{10}$ of distance (m) with standard deviations from the model). Top: Olive-and-black moths: for the horizontally orientated stripes, (left), lower spatial frequencies are detected from further away, whereas for the vertically orientated stripes, (right), detection distance is lower for lower spatial frequencies. Bottom: Yellow-and-black moths: for horizontal stripes, (left), detection distance peaks at $YG_H$, and no significant differences between vertically orientated stripes, (right). For all treatments identification distance increases as spatial frequency declines, but this plateaus and declines where it is constrained by the initial detection distance.