



## Original Article

# Pattern edges improve predator learning of aposematic signals

Naomi F. Green,<sup>a</sup> Holly H. Urquhart,<sup>a</sup> Cedric P. van den Berg,<sup>a</sup> N. Justin Marshall,<sup>b</sup> and Karen L. Cheney<sup>a,b</sup>

<sup>a</sup>School of Biological Sciences, The University of Queensland, Brisbane, Queensland, 4072, Australia and <sup>b</sup>Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, 4072, Australia

Received 13 December 2017; revised 26 April 2018; editorial decision 30 April 2018; accepted 11 June 2018.

Edges are salient visual cues created by abrupt changes in luminance and color and are crucial in perceptual tasks such as motion detection and object recognition. Disruptively colored animals exploit edge detection mechanisms to obscure their body outline and/or to conceal themselves against their background. Conversely, aposematic species may use contrasting patterns with well-defined edges to create highly salient, memorable warning signals. In this study, we investigated how the amount of internal pattern edge, colored area, pattern type, or shape repetition of warning signals influenced avoidance learning in the triggerfish, *Rhinecanthus aculeatus*. Using 6 different warning signals, we found that fish learnt to avoid aposematic signals faster when they featured more internal pattern edge. We found little evidence that the amount of colored area or pattern type affected learning rates. An optimal amount of pattern edge within a warning signal may therefore improve how warning signals are learnt. These findings offer important insights into the evolution of prey warning signal evolution and predator psychology.

**Keywords:** color patterns, learning, body outlining, warning signals, aposematism, coral reef fish.

## INTRODUCTION

When viewing a scene, edges are perceived as abrupt changes in luminance and color and provide key information about object boundaries and the structure of the environment. In conjunction with additional cues such as color, symmetry, and shape, edges underpin crucial perceptual tasks such as navigation (Lau et al. 2006; Harris et al. 2007), object recognition (Webster et al. 2013), figure-ground organization (Driver and Baylis 1996), and depth perception (Palmer and Ghose 2008). Indeed, vertebrate visual systems are optimally configured for edge detection via retinal lateral inhibition, in which stimulated neurons inhibit the excitation of neighboring photoreceptors, increasing the contrast and sharpness of the visual response (Enroth-Cugell and Pinto 1972).

Many animals, such as the leopard (*Panthera pardus*) (Allen et al. 2011), the peach blossom moth (*Thyatira batis*) (Schaefer and Stobbe 2006), and the spotted grass frog (*Limnodynastes tasmaniensis*) (Osorio and Srinivasan 1991) display highly contrasting body patterns to inhibit detection of their body outline by predators or prey against complex visual scenery (Thayer 1909; Cott 1940; Cuthill et al. 2005; Endler 2006; Stevens et al. 2006). Disruptively colored animals may also exhibit nonmarginal, contrasting patterns that disguise telltale body parts such as eyes or limbs (Thayer 1909; Cott

1940; Cuthill and Székely 2009). Illusory contours may also be used to segment the body surface and prevent them from being recognized as a singular, cohesive figure (Stevens et al. 2009).

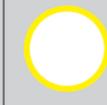
Conversely, contrasting patterns with well-defined edges may be used as warning signals by aposematic prey (Poulton 1890). Chromatically or achromatically contrasting edges within a pattern may increase the detectability of prey against the background (Troscianko et al. 2017), improve recognition of aposematic prey (Guilford 1986), and enhance predator learning (Osorio et al. 1999). Pattern edges may be provided by spots, stripes, or circles within the warning signal, or run parallel to the prey contour to enhance the body outline (Cott 1940; Hailman 1977). It is also proposed that many animals display eye-catching borders to accentuate their body profile and emphasize their characteristic shape (Cott 1940; Hailman 1977). Outlining is expected to be particularly important in flat animals such as butterflies (e.g. *Papilio ulysses*), and marine invertebrates such as flatworms (e.g. *Pseudobiceros gloriosus*) and nudibranch mollusks (e.g. *Chromodoris elisabethina*). Such animals lack the conspicuousness of a bulky form and have a single prominent contour that appears similar from most vantage points (Hailman 1977).

Previous research investigating the role of pattern in warning signals has produced conflicting results. Black internal patterns have been shown to reduce avian attacks toward both spotted ladybirds (Dolenská et al. 2009) and striped, caterpillar-like models (Barnett et al. 2016), compared with unpatterned prey. Similarly, avoidance of striped prey against complex backgrounds was learned faster by

Address correspondence to N.F. Green. E-mail: naomi.green@uq.net.au.



**Table 1**  
Aposematic (S-) signals used indicating group name, amount of pattern edge (mm), and colored area (mm<sup>2</sup>) that each stimulus contained

	Spots		Stripes		Circles	
S-						
Group	A	B	C	D	E	F
Edge (mm)	170	50	178	45	176	176
Colored Area (mm <sup>2</sup> )	254	202	198	50	176	176

Gray shading indicate the groups that were compared to determine whether pattern type (rather than the amount of edge) influenced learning rate (Figure 1d). Gray outlines around stimuli in the table only show edges of stimuli area, rather than the presence of a colored edge.

feeding board. Crosses were required to provide a target for the fish while learning to peck the feeding board for food; however, the crosses differed significantly in size, color, and pattern from all experimental stimuli, to prevent this impacting learning during the experiment. Once fish learnt to peck at the crosses, squid was removed from the feeding board and, instead, fish were fed immediately after they had pecked either cross, with squid held with forceps from above. Fish took from 1 to 3 weeks to be trained to this behavior and were required to demonstrate this behavior confidently, by immediately approaching the board and pecking on either cross for 5 sessions before testing commenced. During testing, the crosses were then replaced with the white, nonaposematic (S+) and colored, aposematic (S-) stimuli and experimental trials commenced.

Stimuli were designed to disentangle whether differences in learning rates were due to the amount of pattern edge, the amount of colored area, shape repetition, or pattern type. Here, we use the term “pattern” to refer to any colored element within a signal (not necessarily repetitive). We use the term “pattern edge” to refer to edges created by the yellow colored areas in the signal, which were internal patterns in stimuli A–E, but included the internal and external edge in stimulus F (single circle). Stimuli were designed in Adobe Illustrator, and pattern edge and colored area were determined using simple geometric equations.

Eight fish could not be trained to a satisfactory level and therefore did not progress to the testing phase. The remaining fish ( $n = 50$ ) were randomly allocated to 6 groups, which were each trained to avoid a different aposematic stimulus (S-, see Table 1). There was no significant difference in fish size between groups (one-way Anova:  $F = 0.59$ ,  $df = 5$ ,  $P = 0.70$ ).

Learning rates for each group were compared with another group that had a similar pattern type, but differed in the amount of internal pattern edge, colored area, and/or shape repetition (Table 1). “Spots” consisted of Group A ( $n = 9$ ; 9 yellow spots) and Group B ( $n = 7$ ; single yellow spot) both of which featured internal spots; however, Group A had more internal pattern edge and more shape repetition than Group B, but a similar amount of colored area (Table 1). “Stripes” consisted of Group C ( $n = 8$ ; 4

vertical stripes) and Group D ( $n = 10$ ; single yellow vertical stripe) that both featured vertical stripes; however, Group C had more pattern edge, more shape repetition and more colored area than Group D. Finally, “Circles,” comprised Group E ( $n = 7$ ; 4 open circles) and F ( $n = 9$ ; single circle that outlines the stimulus) which both featured open circles; however, Group E, had more shape repetition than Group F but a similar amount of pattern edge and colored area. Data from Group F have been presented previously (Winters et al. 2017).

We predicted 3 possible scenarios: first, if an increased amount of pattern edge improved predator learning, we would expect a difference in the learning rate for “Spots” (Group A would learn more quickly than B) and “Stripes” (Group C would learn more quickly than D), but not for “Circles” (Group E would learn at the same rate as Group F) (Table 1). Second, if an increased amount of colored area improved learning, we would expect Group B to learn more quickly than Group D and also predict a difference in learning rate in “Stripes” (Group C would learn more quickly than D), but not between paired groups for “Spots” or “Circles.” Third, if differences were due to increased repetition of shape alone, we anticipated that there would be a difference for all patterns (“Spots”: Group A would be learnt more quickly than B; “Stripes”: Group C learnt more quickly than D; and “Circles”: Group E learnt more quickly than F).

We then compared results from groups A, C, E, and F to determine whether pattern type affected predator learning of aposematic stimuli. Stimuli from these groups featured a similar amount of pattern edge and colored area, but differed in pattern type (spots, stripes, circles) (Table 1). The amount of pattern edge and colored area varied slightly between these stimuli due to differences in geometry; however, the stimuli we used were designed to ensure that both of these features were as similar as possible.

### Statistical analyses

We analyzed the data using a Cox proportional hazards survival analysis and the function `coxph`, in the survival package (Therneau 2015) in R v.3.1.3 (R Core Team 2015). In our data, the “survival



Edges may enhance predator learning of aposematic signals as highly contrasting transitions evoke a stronger response in the retina compared to gradual changes in luminance and color (Bruce et al. 1996). As the eyes scan an image, the amount of edge transitions will determine the amount of stimulation across the retina and increase the overall salience of a signal (Endler 2012). Aposematic patterns use edges that are highly contrasting and combine abrupt, simultaneous changes in both luminance and color, which distinguish them from the many false edges produced by variations in texture and illumination in natural scenes (Troscianko et al. 2009). Although we found that the amount of edge within a pattern improved predator learning, an optimal level may be set by the visual acuity of the predator and the complexity of the background. Beyond this limit, highly complex patterns with a profuse amount of pattern edges may appear blurred and inconspicuous, due to the decreased spatial frequency. Indeed, highly complex body patterns, such as reticulated patterns that feature a high amount of internal pattern edge, may become harder to learn as edges become less detectable.

We found no difference in learning rates for different pattern types when we controlled for the amount of edge within the stimuli; however, the pattern displayed in a warning signal may be determined by a range of other factors. Pattern type will be important to detection in natural environments, where conspicuousness may be enhanced through pattern edges that contrast with the orientation (Webster et al. 2009) or spatial frequency of common lines within their habitat (Godfrey et al. 1987; Phillips et al. 2017). Although spots and stripes may evolve because they are developmentally simple to produce (Turing 1952), or because spots are well suited to stimulate the circular receptive fields of vertebrate retinal ganglion cells (Lythgoe 1979; Stevens 2005), stripes may provide camouflage from a distance (Barnett et al. 2016). Concentric rings such as eye spots are abundant in animal signals and may function to confuse or startle predators, by mimicking the pupil and iris of vertebrate eyes (e.g. Blest 1957; De Bona et al. 2015). However, we propose that in some cases concentric rings may evolve to increase the amount of salient edges within a pattern. Indeed, this is consistent with recent research suggesting that some eyespots deter predators through increased conspicuousness, not through eye mimicry (Stevens 2009).

Shape is an important component of predator search images (Troscianko et al. 2009) and so patterns which accentuate this, such as body outlining, are expected to aid predator detection and recognition (Cott 1940; Hailman 1977). We did not find evidence to support this hypothesis in our study, with stimuli featuring a yellow outline (Group F), learnt at the same rate as signals featuring spots and stripes (Figure 1d). However, body outlining may be more important to increase prey conspicuousness in natural scenes, as opposed to learning speed. In a previous study (Winters et al. 2017), it was demonstrated that triggerfish only learnt avoidance of one element of a multicomponent warning signal, rather than the entire signal. Fish learnt to avoid the yellow outline of a multicomponent warning signal, but not internal red spots. However, it was previously unclear whether this was because the yellow outline highlighted the shape of the stimulus, or whether it was a shape or edge effect. The present study untangles these different hypotheses and suggests that the yellow rim was probably learnt preferentially because it provided more internal pattern edges.

The amount of edge within a pattern and pattern repetition are tightly correlated. In our study, fish learnt to avoid a single circle (Group F) more quickly than a single spot (Group B), which differed

only in the amount of pattern edge and not in shape repetition (Figure 1c), suggesting that the amount of pattern edge is more important than regularity or repetition alone. Indeed, in a previous study, birds learnt to avoid prey featuring the same amount of edge at a similar rate, regardless of whether stimuli were regularly or irregularly striped (Aronsson and Gamberale-Stille 2013). However, in our study, the combined effect of increased edge contrasts, repetition and enhanced symmetry may have improved learning.

Although large signal size increases conspicuousness and effectiveness of warning signals (Remmel and Tammarub 2011), many defended prey such as bees, wasps, lady beetles, and nudibranch molluscs are constrained by a small size and limited body surface. In terms of geometry, the ratio of edge to colored area is higher for stripes (rectangles) compared with spots (circles); indeed, in our study, we required 9 spots to equal the edge of 4 stripes (Table 1). Stripes therefore offer the advantage of more conspicuous edge transitions when the size of a warning signal is constrained, or when color is provided by a rare or costly pigment. However, the evolution of pattern geometry is complex, and influenced by additional factors such as body shape. For example, nudibranch mollusks have elongated, elliptical bodies, and so longitudinal stripes emphasize their primary body axis. In comparison, small, circular animals such as ladybirds may evolve spots because they provide patterns that replicate their body shape and provide curved edges, which run parallel to their circular body outline to enhance conspicuousness (Troscianko et al. 2017).

We used wild caught fish and therefore unfortunately cannot account for their prior experience of colored stimuli, which may have influenced our results. However, we are unaware of aposematic species at the collection site of our fish with color patterns similar to those used in our experiment. Ideally, it would have been preferable to use wild caught reef fish raised from light-trapped larvae; however, large fish such as triggerfish are rarely caught using such methods (Thorroid 1992). Our sample sizes were relatively small ( $n = 7-10$  per group, total = 58 fish), which was the maximum number that could be housed and trained in separate aquaria simultaneously. However, for pairs where a nonsignificant result was expected, the results were very similar between groups (between “Circles”: Group E and F,  $P = 0.554$ ) and for Groups A, C, E, and F,  $P = 0.36$ ), and so we were unlikely to get a significant result with a larger sample size.

In conclusion, we have provided evidence that warning signals that display increased internal pattern edges, provided by spots, stripes, or circles, improve predator avoidance learning. Our research provides new insights into the value and function of patterns in warning signals and has important implications for signal evolution and pattern geometry. Understanding how signal receivers respond to different components of visual signals will help us to reveal how complex color signals evolve and are maintained.

## AUTHORS' CONTRIBUTIONS

N.F.G. and H.H.U. carried out the behavioral work, N.F.G. carried out the statistical analyses; N.F.G., C.v.d.B., N.J.M., and K.L.C. conceived and designed the study, coordinated the study and drafted the manuscript. All authors gave final approval for publication.

## FUNDING

This work was supported by the Australian Research Council (funder ID 501100000923, grant number DP150102710 awarded to K.L.C. and N.J.M.).

We would like to thank Natalie Wood, Emily Guevara, Laurie Mitchell, Fabio Cortesi, Sara Stieb, and Fanny de Busserolles for assistance in the field, and the staff at Lizard Island Research Station for logistic support. The authors have no competing interests.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Green et al. (2018).

**Handling editor:** John Skelhorn

## REFERENCES

- Allen WL, Cuthill IC, Scott-Samuel NE, Baddeley R. 2011. Why the leopard got its spots: relating pattern development to ecology in felids. *Proc Biol Sci.* 278:1373–1380.
- Aronsson M, Gamberale-Stille G. 2008. Domestic chicks primarily attend to colour, not pattern, when learning an aposematic coloration. *Anim Behav.* 75:417–423.
- Aronsson M, Gamberale-Stille G. 2009. Importance of internal pattern contrast and contrast against the background in aposematic signals. *Behav Ecol.* 20:1356–1362.
- Aronsson M, Gamberale-Stille G. 2013. Evidence of signaling benefits to contrasting internal color boundaries in warning coloration. *Behav Ecol.* 24:349–354.
- Barnett JB, Scott-Samuel NE, Cuthill IC. 2016. Aposematism: balancing salience and camouflage. *Biol Lett.* 12. doi:10.1098/rsbl.2016.0335
- Blest A. 1957. The function of eyespot patterns in the Lepidoptera. *Behaviour.* 11:209–256.
- Bruce V, Green PR, Georgeson MA. 1996. Visual perception: physiology, psychology and ecology. East Sussex (UK): Psychology Press.
- Champ C, Wallis G, Vorobyev M, Siebeck U, Marshall J. 2014. Visual acuity in a species of coral reef fish: *Rhinecanthus aculeatus*. *Brain Behav Evol.* 83:31–42.
- Cheney KL, Newport C, McClure EC, Marshall NJ. 2013. Colour vision and response bias in a coral reef fish. *J Exp Biol.* 216:2967–2973.
- Cott HB. 1940. Adaptive coloration in animals. United Kingdom (Methuen): Oxford University Press.
- Cuthill IC, Stevens M, Sheppard J, Maddocks T, Párraga CA, Troscianko TS. 2005. Disruptive coloration and background pattern matching. *Nature.* 434:72–74.
- Cuthill IC, Székely A. 2009. Coincident disruptive coloration. *Philos Trans R Soc Lond B Biol Sci.* 364:489–496.
- Cox DR. 1972. Regression models and life tables (with discussion). *J R Statist Soc B.* 34:187–220.
- De Bona S, Valkonen JK, López-Sepulcre A, Mappes J. 2015. Predator mimicry, not conspicuousness, explains the efficacy of butterfly eyespots. *Proc Biol Sci.* 282:20150202.
- Dolenská M, Nedvěd O, Veselý P, Tesařová M, Fuchs R. 2009. What constitutes optical warning signals of ladybirds (Coleoptera: Coccinellidae) towards bird predators: colour, pattern or general look? *Biol J Linn Soc.* 98:234–242.
- Driver J, Baylis GC. 1996. Edge-assignment and figure-ground segmentation in short-term visual matching. *Cogn Psychol.* 31:248–306.
- Endler JA. 2006. Disruptive and cryptic coloration. *Proc Biol Sci.* 273:2425–2426.
- Endler JA. 2012. A framework for analysing colour pattern geometry: adjacent colours. *Biol J Linn Soc.* 107:233–253.
- Enroth-Cugell C, Pinto LH. 1972. Properties of the surround response mechanism of cat retinal ganglion cells and centre-surround interaction. *J Physiol.* 220:403–439.
- Fox J, Weisberg S. 2011. An R companion to applied regression, 2nd Ed. Thousand Oaks (CA): SAGE publications.
- Godfrey D, Lythgoe JN, Rumball DA. 1987. Zebra stripes and tiger stripes: the spatial frequency distribution of the pattern compared to that of the background is significant in display and crypsis. *Biol J Linn Soc.* 32:427–433.
- Green NF, Urquhart HH, van den Berg CP, Marshall NJ, Cheney KL. 2018. Data from: pattern edges improve predator learning of aposematic signals. Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.jd1t3jk>.
- Guilford T. 1986. How do warning colours work? Conspicuousness may reduce recognition errors in experienced predators. *Anim Behav.* 34:286–288.
- Guilford T, Dawkins MS. 1991. Receiver psychology and the evolution of animal signals. *Anim Behav.* 42:1–14.
- Hailman JP. 1977. Optical signals: animal communication and light. London (UK): Indiana University Press.
- Harris RA, Graham P, Collett TS. 2007. Visual cues for the retrieval of landmark memories by navigating wood ants. *Curr Biol.* 17:93–102.
- Hegna RH, Saporito RA, Gerow KG, Donnelly MA. 2012. Contrasting colors of an aposematic poison frog do not affect predation. *Ann Zool Fennici.* 48:29–38.
- Lau KK, Roberts S, Biro D, Freeman R, Meade J, Guilford T. 2006. An edge-detection approach to investigating pigeon navigation. *J Theor Biol.* 239:71–78.
- Lythgoe JN. 1979. The ecology of vision. Oxford (NY): Clarendon Press.
- Newport C, Green NF, McClure EC, Osorio DC, Vorobyev M, Marshall NJ, Cheney KL. 2017. Fish use colour to learn compound visual signals. *Anim Behav.* 125:93–100.
- Osorio D, Jones CD, Vorobyev M. 1999. Accurate memory for colour but not pattern contrast in chicks. *Curr Biol.* 9:199–202.
- Osorio D, Srinivasan MV. 1991. Camouflage by edge enhancement in animal coloration patterns and its implications for visual mechanisms. *Proc Biol Sci.* 244:81–85.
- Palmer SE, Ghose T. 2008. Extremal edges: a powerful cue to depth perception and figure-ground organization. *Psychol Sci.* 19:77–84.
- Phillips GAC, How MJ, Lange JE, Marshall NJ, Cheney KL. 2017. Disruptive colouration in reef fish: does matching the background reduce predation risk? *J Exp Biol.* 220:1962–1974.
- Pignatelli V, Champ C, Marshall J, Vorobyev M. 2010. Double cones are used for colour discrimination in the reef fish, *Rhinecanthus aculeatus*. *Biol Lett.* 6:537–539.
- Poulton EB. 1890. The colors of animals: their meaning and use, especially considered in the case of insects. New York: Appleton.
- Randall JE. 1981. Underwater guide to hawaiian reef fishes. Newtown Square (Pennsylvania): Harwood Books.
- R Core Team. 2015. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. <http://www.R-project.org>.
- Rommel T, Tammarub T. 2011. Evidence for the higher importance of signal size over body size in aposematic signaling in insects. *J Insect Sci.* 11:4.
- Schaefer HM, Stobbe N. 2006. Disruptive coloration provides camouflage independent of background matching. *Proc Biol Sci.* 273:2427–2432.
- Stevens M. 2005. The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biol Rev Camb Philos Soc.* 80:573–588.
- Stevens M, Cantor A, Graham J. 2009. The function of animal ‘eyespot’: Conspicuousness but not eye mimicry is key. *Curr Zoo.* 55:319–326.
- Stevens M, Cuthill IC, Windsor AM, Walker HJ. 2006. Disruptive contrast in animal camouflage. *Proc Biol Sci.* 273:2433–2438.
- Stevens M, Winney IS, Cantor A, Graham J. 2009. Outline and surface disruption in animal camouflage. *Proc Biol Sci.* 276:781–786.
- Thayer GH. 1909. Concealing coloration in the animal kingdom: an exposition of the laws of disguise through colour and pattern; being a summary of Abbott H. Thayer’s discoveries. New York: Macmillan.
- Therneau TM. 2015. A package for survival analysis in S. 2.38 ed. <http://CRAN.R-project.org/package=survival>.
- Thorrold SR. 1992. Evaluating the performance of light traps for sampling small fish and squid in open waters of the central Great Barrier Reef Lagoon. *Mar Ecol Prog Ser.* 89:277–285.
- Troscianko T, Benton CP, Lovell PG, Tolhurst DJ, Pizlo Z. 2009. Camouflage and visual perception. *Philos Trans R Soc Lond B Biol Sci.* 364:449–461.
- Troscianko J, Skelhorn J, Stevens M. 2017. Quantifying camouflage: how to predict detectability from appearance. *BMC Evol Biol.* 17:7.
- Turing AM. 1952. The chemical basis of morphogenesis. *Philos Trans Royal Soc B.* 237:37–72.
- Webster RJ, Callahan A, Godin JG, Sherratt TN. 2009. Behaviourally mediated crypsis in two nocturnal moths with contrasting appearance. *Philos Trans R Soc Lond B Biol Sci.* 364:503–510.
- Webster RJ, Hassall C, Herdman CM, Godin JG, Sherratt TN. 2013. Disruptive camouflage impairs object recognition. *Biol Lett.* 9:20130501.
- Winters AE, Green NF, Wilson NG, How MJ, Garson MJ, Marshall NJ, Cheney KL. 2017. Stabilizing selection on individual pattern elements of aposematic signals. *Proc Royal Soc. B.* 284. doi:10.1098/rspb.2017.0926
- Wüster W, Allum CS, Bjargardóttir IB, Bailey KL, Dawson KJ, Guenioui J, Lewis J, McGurk J, Moore AG, Niskanen M, et al. 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. *Proc Biol Sci.* 271:2495–2499.