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Animal behaviour

Paradox lost: variable colour-pattern geometry is associated with differences in movement in aposematic frogs

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Aposematic signal variation is a paradox: predators are better at learning and retaining the association between conspicuousness and unprofitability when signal variation is low. Movement patterns and variable colour patterns are linked in non-aposematic species: striped patterns generate illusions of altered speed and direction when moving linearly, affecting predators' tracking ability; blotched patterns benefit instead from unpredictable pauses and random movement. We tested whether the extensive colour-pattern variation in an aposematic frog is linked to movement, and found that individuals moving directionally and faster have more elongated patterns than individuals moving randomly and slowly. This may help explain the paradox of polymorphic aposematism: variable warning signals may reduce protection, but predator defence might still be effective if specific behaviours are tuned to specific signals. The interacting effects of behavioural and morphological traits may be a key to the evolution of warning signals.

1. Introduction

Some animals inform predators about their unprofitability with warning colour patterns (aposematism [1]). Because this relies on predators learning and remembering the relationship between colour patterns and unprofitability, selection should favour colour patterns with little or no variation [2]; patterns with low variation are easier to learn [2,3] and remember [4]. Paradoxically, within-population variation exists in some aposematic species [5,6]. Despite studies suggesting a natural–sexual selection interaction as a cause [5,7], the mechanisms by which within-population variation is maintained, and its ecological and behavioural correlates, are poorly understood. Attempts to explain the maintenance of variable aposematic signals have primarily addressed among-population variation [8,9] or involved laboratory studies with artificial prey or computer games. Here, we address it using a wild natural population.

In non-aposematic snakes with variable coloration, certain patterns seem to be more effective when accompanied by matched escape behaviours. For example, individuals with striped patterns benefit from fleeing from predators, because the pattern appears to remain stationary or move more slowly than the escaping animal [10–13], whereas individuals with spotted patterns tend to stay motionless and change direction during escape [10–13]. The use of computer games with human 'predators' has yielded similar results: moving targets with stripes running along the movement axis (elongated) are missed more often by observers than targets with other types of patterns [14], and targets moving over longer segments are more difficult to catch [15]. However, these studies do not consider aposematic species, which have different dynamics than cryptic species [2]. At the species level, birds can discriminate palatable

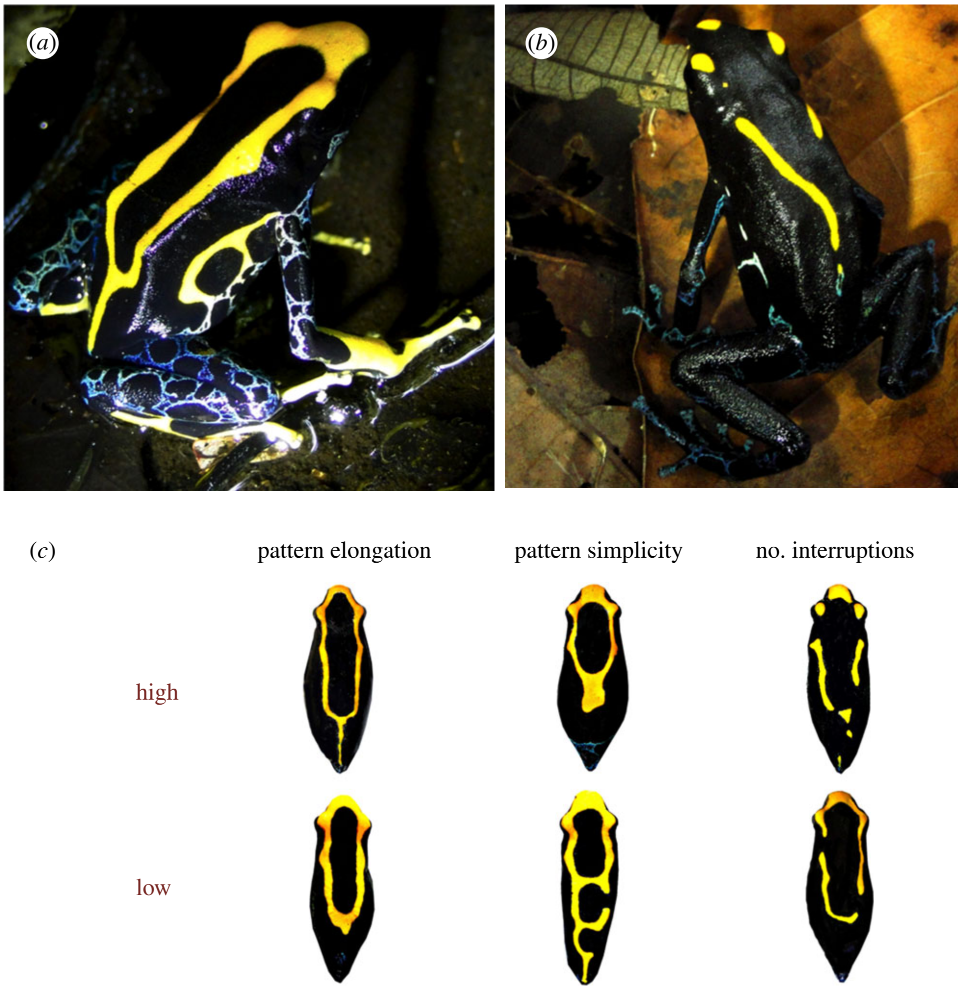

Figure 1. Colour patterns in *D. tinctorius*. A typical elongated pattern (*a*) and an interrupted one (*b*); and (*c*) examples of high and low values of the colour-pattern parameters measured.

from unpalatable butterflies on the basis of their flight [16]. Palatable butterflies fly fast or erratically and have inconspicuous coloration, whereas unpalatable species fly slowly or regularly and are conspicuously, warningly coloured [16]. That example, however, does not address variable aposematic coloration *within* a species. Here, we explore whether an association between behaviour and colour pattern would benefit aposematic species with variable aposematic signals. We tested this hypothesis in a natural population of the aposematic frog *Dendrobates tinctorius*, which has extensive colour-pattern variation [6].

2. Material and methods

(a) Study site and species

Dendrobates tinctorius (Dendrobatidae) is a diurnal frog occurring in primary forests in the Eastern Guiana Shield [17]. It exhibits striking variation in colour patterns (figure 1) [6,17], has skin alkaloid defences, and field experiments with Plasticine models suggest birds as major predators [18]. This study was carried out at Camp Pararé, Les Nouragues Reserve, French Guiana (3°59′ N, 52°35′ W, 120 m.a.s.l.), February–July 2011.

(b) Trajectories

Twenty-five females and 14 males were followed for two continuous hours each. This duration was chosen based on prior observations of individuals remaining motionless on the same spot for almost 1 h. Preliminary observations allowed us to determine the distance at which the frog would not show fleeing behaviour: about 2.5 m (less when the observer moved very slowly). This allowed us to observe the frogs without affecting their behaviour and record their pattern of movement under normal circumstances (i.e. not in response to a potential predator). We stuck a flag into the ground wherever the frog remained still for at least 5 s. After the 2-h observation period, we measured the distance and angle between pairs of flags (segments) and estimated the total linear distance travelled (between starting and endpoints), the average linear speed (total linear distance/2 h) and the path length of each individual by adding all segment lengths.

We calculated the mean angle and distance of displacement for each segment and ran Rayleigh tests [19] on the segment vectors for each frog in order to know whether their movement was random or directional. With this information, we created a new categorical variable, 'directionality'. A frog was classified as 'directional' if the Rayleigh test was significant at the 5% level, indicating a significant directional vector, and 'random' if not.

(c) Colour patterns

Each frog was photographed against graph paper for scale. Its colour pattern was analysed with a method that uses grid transects across colour patterns and allows the estimation of geometric parameters based on the distribution of the number of transitions between adjacent colours (here yellow and black) [20]. The parameters used were *pattern simplicity* (mean distance between colour transitions, longitudinally and transversally), and *pattern elongation* (ratio of transition densities along and perpendicular to the body axis [20]). Pattern analyses were done

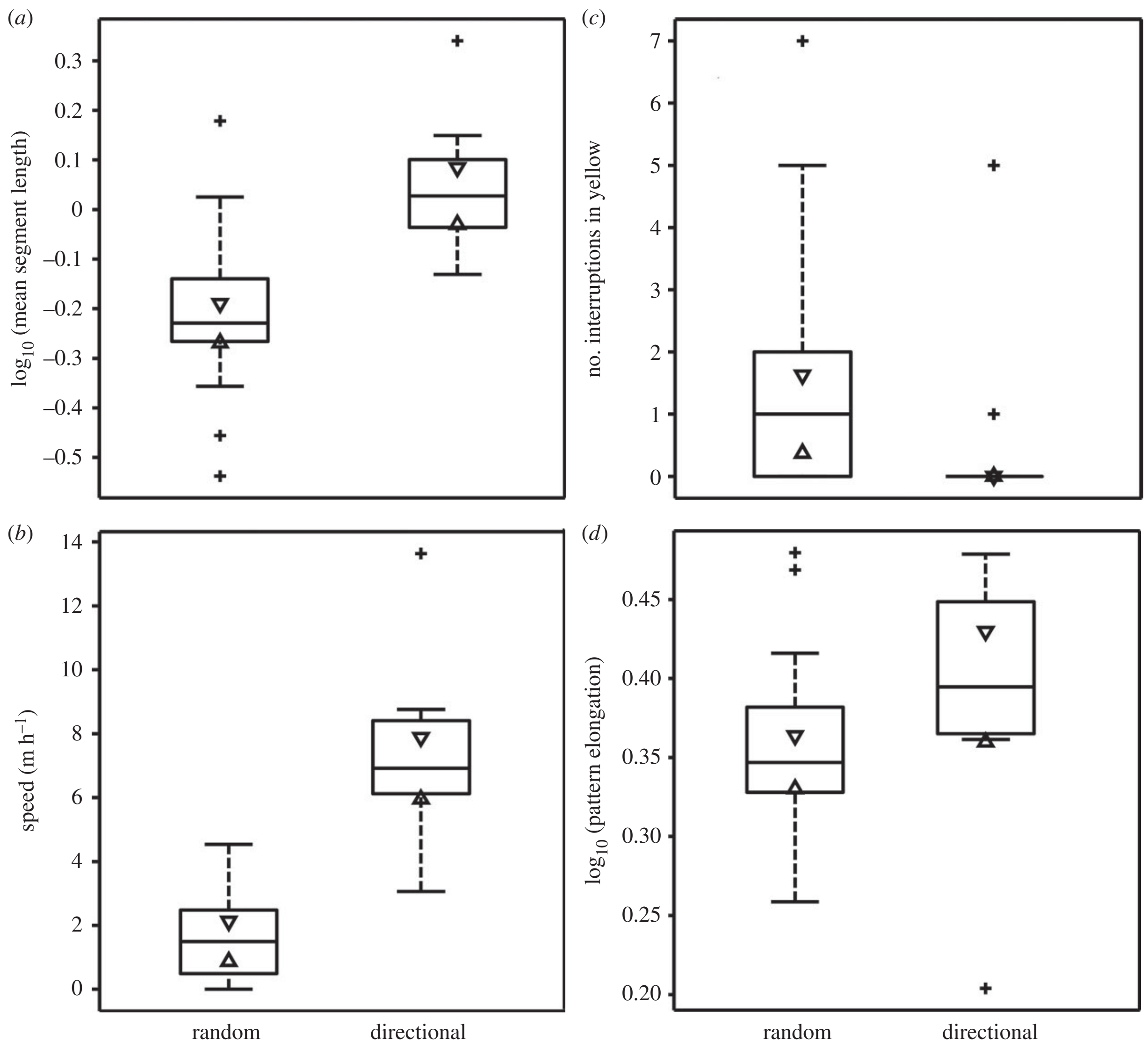

Figure 2. Differences in movement and colour-pattern geometry between frogs moving directionally and randomly. (*a*) Mean segment length; (*b*) linear speed; (*c*) number of interruptions and (*d*) pattern elongation. Triangles enclose segments ('notches') which, if they do not overlap between directional and random plots, indicate that the medians are different at the 5% level or better [21].

with MATLAB. We also recorded the number of interruptions of yellow on each frog's back (figure 1*c*).

(d) Statistics

Both coloration and movement variables were tested for normality (Shapiro–Wilks tests with $p > 0.05$) and log-transformed when this assumption was not met. Colour-pattern parameters and movement variables (path length, mean segment length and linear speed of individual trajectories) were compared between the two directionality groups with MANOVA, or Mann–Whitney tests when the variables violated normality after transformation. Statistical analyses were performed with SPSS v. 19.0 for Mac, and all tests were two-tailed.

3. Results

Thirty-six per cent of the frogs were classed as 'directional' and 64% 'random'. We found no differences between the number of females and males in each directionality group ($\chi^2 = 0.986$, d.f. $= 1$, $p > 0.05$).

'Directional' frogs have trajectories with longer segments and higher average linear speeds than 'random' frogs (figure 2*a*,*b*; MANOVA on directionality: Pillai's trace $= 0.727$, $F_{4,33} = 17.56$, $p < 0.001$; log path length $F_{1,38} = 4.80$, $p = 0.035$; log mean segment length $F_{1,38} = 25.33$, $p < 0.001$; linear speed $F_{1,38} = 78.37$, $p < 0.001$). Note that average speeds underestimate the speed over each segment; if we just use the time moving, the speeds are 1.14 ± 0.24 cm s^{-1} (for directional frogs) and 0.31 ± 0.07 cm s^{-1}. This still underestimates the true speed as it does not account for the very brief pauses between the fast jerks.

Colour-pattern geometry was significantly different between the two movement groups. 'Random' frogs have more interruptions in their yellow patches (figures 1*b* and 2*c*; Mann–Whitney $U = 88.0$, $p = 0.006$, $n = 39$), whereas 'directional' frogs have more elongated patterns [20] (figures 1*a* and 2*d*; $F_{1,39} = 4.88$, $p = 0.034$). Pattern elongation is significantly negatively correlated with the number of interruptions of yellow (Spearman's $\rho = -0.489$, $p = 0.002$, $n = 39$), but not correlated with pattern simplicity (Spearman's $\rho = 0.053$, $p = 0.750$, $n = 39$). Simplicity did not differ between the two directionality groups ($F_{1,39} = 1.27$, $p = 0.27$).

4. Discussion

Polymorphic aposematic species could use polymorphic behavioural strategies to mitigate the disadvantages of variable colour patterns, which could be particularly strong when exposed to naive predators. Within a single population, frogs with more elongated patterns move continuously in a given direction rather than randomly. This pattern–movement combination might create the illusion of a static pattern or a pattern with a greatly reduced speed that affects predators' abilities to track the trajectory of moving individuals and predict their attack angle [11,14]. This may be more pronounced when movements occur at a higher speed [22,23] and over longer segments [15], as in these frogs. Frogs moving randomly, with unpredictable changes of direction, have more interrupted patterns and move at a lower average speed, over shorter segments than directional frogs. Interrupted patterns may be visually disruptive [11] or cryptic at a distance [24], and the combination of disruptive patterns and slower movements, or alternating movement and freezing, might be advantageous for the avoidance of motion-oriented predators [12,13,25].

A possible explanation for the match between movement behaviour and colour pattern could be correlational selection, which favours specific combinations of traits expressed simultaneously in a given individual without necessarily altering the distribution of each trait on its own [26,27]. The presence of the 'wrong' combinations of colour pattern and behaviour of every generation favours the evolution of genetic correlations between their gene loci, because genetic correlations reduce the frequency of the lower fitness combinations [26,28]. For the association to strengthen over evolutionary time, one or more factors causing genetic correlations would have to occur so that the phenotypic correlations would not have to be reformed from scratch every generation. Possible mechanisms include gametic phase or linkage disequilibrium, pleiotropy among loci, physical linkage or epistasis from a gene affecting both traits. Note that some of these genetic factors could occur in the absence of correlational selection. Plasticity can also favour phenotypic correlations. If predator misses were frequent enough, as is possible for aposematic species, inefficient predators would favour developmental plasticity or active learning to move in particular patterns. In addition, positive assortative mating for colour patterns would incidentally favour or reinforce the association.

Both directional movements paired with striped patterns and random movements paired with broken patterns may be equally good alternative strategies, allowing variation in both. Moreover, correlational selection often results from frequency-dependent interactions such as those between predators and prey, or parasites and hosts [28]; frequency-dependence can easily result in stable polymorphisms [2] and stable quantitative variation [29]. Provided that phenotypic correlations are consistent and based upon some genetic correlation, this can favour variation in both traits. How distinct and how variable each alternative phenotypic cluster can be depends on the local combination of correlational selection and genetics. The system may evolve to two distinct phenotypic combinations if the selection gradients are steep and genetic correlations very high, but if they are low then there may be more variation and less distinct alternatives, as we find in *D. tinctorius*.

One characteristic of a population at a polymorphic equilibrium is equality of fitness among the forms [2]. However, the mechanisms of fitness equality differ. The elongated pattern with directional movement might make it difficult for a predator to track the frog, hence increasing the probability of a missed attack by attacking in the wrong place. The broken pattern might be relatively more cryptic from a distance and forms a disruptive pattern up close, reducing the number of attacks rather than redirecting them. The elongated pattern shows off the aposematic pattern even when it is moving, but the broken pattern may show off the aposematic pattern better when it is not moving and the predator is close enough to resolve the separate yellow spots. Further experimental research is needed to support either possibility.

The evolutionary factors contributing to the maintenance of polymorphisms in aposematic species are more intricate than originally thought; a match between behaviour and colour patterns may be as important as predation and sexual selection in preserving different aposematic signals within populations. This study highlights the importance of considering behaviour–pattern interactions in future attempts to understand the paradox of intra-populational warning signal diversity, and signal evolution in general.

The study complied with local environmental legislation.

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References

1. Poulton EB. 1890 *The colours of animals: their meaning and use*. London, UK: Kegan Paul.
2. Endler JA, Mappes J. 2004 Predator mixes and the conspicuousness of aposematic signals. *Am. Nat.* **163**, 532–547. (doi:10.1086/382662)
3. Ruxton GD, Sherratt TN, Speed MP. 2004 *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. Oxford, UK: Oxford University Press.
4. Speed MP. 2000 Warning signals, receiver psychology and predator memory. *Anim. Behav.* **60**, 269–278. (doi:10.1006/anbe.2000.1430)
5. Nokelainen O, Hegna RH, Reudler JH, Lindstedt C, Mappes J. 2012 Trade-off between warning signal efficacy and mating success in the wood tiger moth. *Proc. R. Soc. B* **279**, 257–265. (doi:10.1098/rspb.2011.0880)
6. Rojas B, Endler JA. 2013 Sexual dimorphism and intra-populational colour pattern variation in the aposematic frog *Dendrobates tinctorius*. *Evol. Ecol.* **27**, 739–753. (doi:10.1007/s10682-013-9640-4)
7. Maan ME, Cummings ME. 2009 Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. *Proc. Natl Acad. Sci.* **106**, 19 072–19 077. (doi:10.1073/pnas.0903327106)
8. Rudh A, Breed MF, Qvarnstrom A. 2013 Does aggression and explorative behaviour decrease with lost warning coloration? *Biol. J. Linn. Soc.* **108**, 116–126. (doi:10.1111/j.1095-8312.2012.02006.x)
9. Willink B, Brenes-Mora E, Bolaños F, Pröhl H. 2013 Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. *Evolution* **67**, 2783–2794. (doi:10.1111/evo.12153)

10. Brodie ED. 1989 Genetic correlations between morphology and antipredator behaviour in natural populations of the garter snake *Thamnophis ordinoides*. *Nature* **342**, 542–543. (doi:10.1038/342542a0)
11. Jackson JF, Ingram W, Campbell HW. 1976 Dorsal pigmentation pattern of snakes as an anti-predator strategy: multivariate approach. *Am. Nat.* **110**, 1029–1053. (doi:10.1086/283125)
12. Creer DA. 2005 Correlations between ontogenetic change in color pattern and antipredator behavior in the racer, *Coluber constrictor*. *Ethology* **111**, 287–300. (doi:10.1111/j.1439-0310.2004.01062.x)
13. Allen WL, Baddeley R, Scott-Samuel NE, Cuthill IC. 2013 The evolution and function of pattern diversity in snakes. *Behav. Ecol.* **24**, 1237–1250. (doi:10.1093/beheco/art058)
14. Stevens M, Searle WTL, Seymour JE, Marshall KLA, Ruxton GD. 2011 Motion dazzle and camouflage as distinct anti-predator defenses. *BMC Biol.* **9**, 81. (doi:10.1186/1741-7007-9-81)
15. Sherratt TN, Rashed A, Beatty CD. 2004 The evolution of locomotory behavior in profitable and unprofitable simulated prey. *Oecologia* **138**, 143–150. (doi:10.1007/s00442-003-1411-4)
16. Chai P. 1986 Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biol. J. Linn. Soc.* **29**, 161–189. (doi:10.1111/j.1095-8312.1986.tb01772.x)
17. Noonan BP, Gaucher P. 2006 Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. *Mol. Ecol.* **15**, 4425–4435. (doi:10.1111/j.1365-294X.2006.03074.x)
18. Noonan BP, Comeault AA. 2009 The role of predator selection on polymorphic aposematic poison frogs. *Biol. Lett.* **5**, 51–54. (doi:10.1098/rsbl.2008.0586)
19. Zar JH. 1996 *Biostatistical analysis*, 3rd edn. Upper Saddle River, NJ: Prentice-Hall.
20. Endler JA. 2012 A framework for analysing colour pattern geometry: adjacent colours. *Biol. J. Linn. Soc.* **107**, 233–253. (doi:10.1111/j.1095-8312.2012.01937.x)
21. McGill R, Tukey JW, Larsen A. 1978 Variations of boxplots. *Am. Stat.* **32**, 12–16.
22. Stevens M, Yule DH, Ruxton GD. 2008 Dazzle coloration and prey movement. *Proc. R. Soc. B* **275**, 2639–2643. (doi:10.1098/rspb.2008.0877)
23. von Helversen B, Schooler LJ, Czienskowski U. 2013 Are stripes beneficial? Dazzle camouflage influences perceived speed and hit rates. *PLoS ONE* **8**, e61173. (doi:10.1371/journal.pone.0061173)
24. Endler JA. 1978 A predator's view of animal colour patterns. *Evol. Biol.* **11**, 319–364.
25. Hatle JD, Salazar BA, Whitman DW. 2002 Survival advantage of sluggish individuals in aggregations of aposematic prey, during encounters with ambush predators. *Evol. Ecol.* **16**, 415–431. (doi:10.1023/a:1020814110102)
26. Endler JA. 1986 *Natural selection in the wild*. Princeton, NJ: Princeton University Press.
27. Brodie ED. 1992 Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution* **46**, 1284–1298. (doi:10.2307/2409937)
28. Sinervo B, Svensson E. 2002 Correlational selection and the evolution of genomic architecture. *Heredity* **89**, 329–338. (doi:10.1038/sj.hdy.6800148)
29. Mani GS, Clarke BC, Shelton PR. 1990 A model of quantitative traits under frequency-dependent balancing selection. *Proc. R. Soc. Lond. B* **240**, 15–28. (doi:10.1098/rspb.1990.0024)