PAT-GEOM: A Software Package for the Analysis of Animal Patterns

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Abstract

1. Colour patterns often influence how animals interact with one another, but the ability of researchers to quantify pattern *per se* is hampered by a lack of easily-accessible and user-friendly measurement software packages.

2. We address this issue by releasing PAT-GEOM, a free software package for use within ImageJ that allows users to measure seven properties of a pattern: (1) the shape of its markings, (2) the directionality in the shape of its markings, (3) the size of its markings, (4) the contrast of the pattern, (5) the distribution of its markings, (6) the directionality in the distribution of its markings, and (7) the randomness of the pattern.

3. We provide examples of how PAT-GEOM may be used, such as to visualise the 'average pattern' of a population of animals, or to compare the patterns on two animals. Using data from two case studies, we also demonstrate PAT-GEOM's ability to identify the specific aspects of an organism's pattern that match its background and to design artificial prey items that accurately resemble their model organism for use in predation experiments.

4. PAT-GEOM collates the tools to measure these seven diverse properties of animal colour patterns into one convenient, easy-to-use package. It can be employed in a wide range of studies on topics such as aposematism, camouflage and mimicry, and also has the potential to be applied to other research fields such as landscape ecology, botany and cellular biology.

Keywords

Animal colour patterns; aposematism; background matching; behavioural ecology; pattern geometry; sensory ecology; spatial pattern.

Introduction

Colour patterns influence many animal interactions (Cuthill et al., 2017), yet our ability to understand and quantify them remains limited. The visual information in colour patterns usually comprises several components, including colour, brightness, light polarisation properties, and pattern (the last being the spatial arrangement of the three preceding aspects), but most work has focused on colour or simple blocks of colour/brightness contrast. For example, the literature on animal colour vision (reviewed by Kelber, Vorobyev & Osorio, 2003) and colour spaces (reviewed by Renoult, Kelber & Schaefer, 2015) is comprehensive and measurement techniques are readily-available. Conversely, much less attention has been given to pattern.

There is growing awareness that pattern *per se* provides important information, e.g. in common European vipers *Vipera berus* Linnaeus, 1758, zig-zag patterns alone can produce aposematic effects (Wüster et al., 2004), and avian brood parasite hosts use colour and pattern to recognise parasitic eggs (Spottiswoode & Stevens, 2010). This is stimulating the development of measurement tools—especially digital imaging (Stevens et al., 2007)—and analysis techniques, e.g. pixel matrices (Todd et al., 2005), adjacency analysis (Endler, 2012), pattern identification algorithms (Stoddard, Kilner & Town, 2014), saliency maps (Pike, 2018) and boundary strength analysis (Endler, Cole & Kranz, 2018).

There remains, however, uncertainty regarding what pattern properties are quantifiable and which approaches are suited to different questions and pattern types (Pérez-Rodríguez, Jovani & Stevens, 2017). Furthermore, measurement tools are often not readily-available or located in separate software because their development stemmed from researchers working on disparate systems. It is generally inconvenient to measure multiple properties as images must be processed numerous times in different software, e.g. first with the MICA toolbox (Troscianko & Stevens, 2015) for measuring contrast, then in *NaturePatternMatch* (Stoddard, Kilner & Town, 2014) for size and orientation, and finally in R for shape using the *Momocs* package (Bonhomme et al., 2014). A coordinated effort is needed to (1) determine what pattern properties can or should be quantified, and (2) develop tools to help researchers accomplish this easily. Here, we address these issues by releasing a free software package: PAT-GEOM.

PAT-GEOM Overview

PAT-GEOM is a free-to-use suite of macros (programmes automating functions within a larger programme) based in ImageJ (Schneider, Rasband & Eliceiri, 2012) that analyse pattern in digital images. It measures seven pattern properties (illustrated in Fig. 1; example applications in Table 1): (1) the shape of its markings (i.e. the colour patches or mosaic elements within a pattern; *sensu* Endler, 1990), (2) the directionality in the shape of its markings, (3) the size of its markings, (4) the contrast of the pattern, (5) the distribution of its markings, (6) the directionality in the distribution of its markings, and (7) the randomness of the pattern.

PROPERTY 1: MARKING SHAPE

Shape measurements of appendages or whole organisms are important in behavioural studies and biology (e.g. Fitzpatrick, 1998; McLellan & Endler, 1998) but their application to colour pattern markings is relatively new. PAT-GEOM quantifies the shape of any Region of Interest (ROI; an area of the image to be measured) demarcated by users (manually using ImageJ’s drawing tools or automatically using its built-in

"Analyze Particles" function) using elliptical Fourier analysis (EFA), a landmark-independent technique that approximates the ROI's outline with a series of harmonically-related trigonometric functions (Kuhl & Giardina, 1982). For each harmonic, the x- and y-coordinates of the outline with increasing displacement, t, from a starting point, $x(t)$ and $y(t)$, are described by:

$$x(t) = \sum_{n=1}^{N} \left[A_n \cos\left(\frac{2\pi nt}{T}\right) + B_n \sin\left(\frac{2\pi nt}{T}\right) \right] \qquad \text{(eqn 1)}$$

and

$$y(t) = \sum_{n=1}^{N} \left[C_n \cos\left(\frac{2\pi nt}{T}\right) + D_n \sin\left(\frac{2\pi nt}{T}\right) \right] \qquad \text{(eqn 2)}$$

Where: N = total number of harmonics

n = harmonic number

T = total displacement

t = displacement along outline

Elliptical Fourier descriptors (EFDs) for each harmonic are calculated from the coefficients, A_n, B_n, C_n and D_n, utilising the Fourier Shape Analysis plugin (Boudier & Tupper, 2016) which needs only be downloaded and placed in the ImageJ plugins folder. These EFDs are scale-, rotation- and translation-invariant and insensitive to variation in trace start point (Nixon & Aguado, 2008). Taken together, the EFDs of a shape's harmonics uniquely describe it, i.e. they correspond to only that shape. Shapes with similar descriptors are also similar graphically (Nixon & Aguado, 2008), and EFDs may be used to compare shapes, e.g. using Principal Components Analysis (see Fig. 2D).

PROPERTY 2: MARKING SHAPE DIRECTIONALITY

Directionality in pattern elements is known to affect neuronal activity in animal visual processing (Van Kerkoerle et al., 2014). PAT-GEOM quantifies the directionality in marking shape by fitting ellipses onto ROIs and computing their aspect ratio (major axis divided by minor axis) and orientation (angle of the major axis, rotating clockwise from the image's x-axis; Fig. 1). It is important to standardise image orientation if comparing orientation across images, but not when comparing aspect ratio or variation in orientation. To standardise images, users should rotate ROIs (e.g. using ImageJ's Rotate function) so that their reference axis (i.e. the axis the user wishes to represent an orientation of 0°) is parallel to the image's x-axis. This will likely differ in every study, but could be the animal's long axis or a line connecting two points on the organism.

PROPERTY 3: MARKING SIZE

The influence of marking size in animal signals is well-established (e.g. Spottiswoode & Stevens, 2010) but studies rarely use centroid size (the root-sum-squared distance between a shape's centroid and the landmarks along its outline): the only independent measure of size (Bookstein, 1991). To compare shapes using centroid size, however, they must have the same number of landmarks. This is problematic because animal markings typically have no homologous features and may be drawn using different numbers of points. PAT-GEOM solves this by using averaged centroid size ($S_{c,ave}$), i.e. centroid size divided by the square root of the number of points on an ROI's outline:

$$S_{c,\,ave} = \sqrt{\frac{1}{N}\sum_{n=1}^{N} {d_n}^2} \qquad \text{(eqn 3)}$$

Where: N = total number of points on the outline

d_n = distance of point n from the ROI's centroid

A worked example is included in the Supporting Information. Alternatively, PAT-GEOM also outputs size in square pixels. An example where furrowed crabs *Xantho hydrophilus* (Herbst, 1790) are compared to their background substrate is shown in Fig. 2C.

PROPERTY 4: PATTERN CONTRAST

Contrast is recognised as an important element of animal signals (e.g. Sandre, Stevens & Mappes, 2010; Cole & Endler, 2015). PAT-GEOM measures contrast using the Coefficient of Variation (CoV) of the pixel values in an ROI, i.e. their standard deviation divided by their mean. Because many biological patterns tend to exhibit higher variance with increasing mean values, this correction makes patterns of different luminance levels more comparable:

$$\text{CoV Contrast} = \frac{1}{\bar{I}}\sqrt{\frac{1}{cr}\sum_{i=0}^{c-1}\sum_{j=0}^{r-1}\left(I_{ij}-\bar{I}\right)^2} \quad \text{(eqn 4)}$$

Where: c = width of the ROI in pixels

r = height of the ROI in pixels

i = pixel's x-coordinate, where $0 \leq i \leq c-1$

j = pixel's y-coordinate, where $0 \leq j \leq r-1$

I_{ij} = luminance of pixel (i, j)

$\bar{I}$ = average luminance of all pixels in the ROI

PROPERTY 5: DISTRIBUTION OF MARKINGS

Marking distribution, i.e. the spatial location of the markings within a colour pattern, has been used to identify pattern variation amongst different populations of a species (Todd et al., 2005). PAT-GEOM measures marking distribution by the position of their component pixels: an approach developed by Todd et al. (2005) and automated here. Images should be standardised for area, orientation and resolution, e.g. by matching the lowest resolution manually using ImageJ's Scale function or using the MICA toolbox's automated function. Low resolution images where the pattern of interest is unclear should be excluded. PAT-GEOM converts thresholded images into matrices of '1's (pixels representing markings) and '0's (pixels representing the background) and outputs individual or cumulative matrices and heat maps (Fig. 3).

PROPERTY 6: DIRECTIONALITY OF MARKING DISTRIBUTION

In addition to marking shape directionality (Property 2), directionality in marking distribution can also affect visual processing (Van Kerkoele et al., 2014). To measure this property, PAT-GEOM draws a linear best fit line through all the marking centroids and measures: (1) the line's angle (rotating clockwise from the image's x-axis) for orientation; and (2) its R^2 value for alignment (Fig. 1). As elongated bodies tend to have more directional patterns, users should compare animals of similar shape or standardise images for aspect ratio and orientation, e.g. using ImageJ's Size and Rotate functions.

PROPERTY 7: PATTERN RANDOMNESS

The randomness of patterns in visual scenes is known to influence animal behaviour, especially in camouflage, e.g. in blue tits (Dimitrova & Merilaita, 2009), but it is rarely quantified. For a measure of randomness (i.e. algorithmic complexity; Kolmogorov, 1965), PAT-GEOM outputs the size of the gif file that would be required to encode the ROI, corrected for header size. A fully random pattern contains the highest algorithmic complexity and therefore requires the largest file size, whereas one with repeating parts is less random and requires a smaller file (Lempel & Ziv, 1976; Kaspar & Schuster, 1987). The nature of compression in gif files (Bolliger, Sprott & Mladenoff, 2003) and the suitability of this measure (Leeuwenberg, 1968; Donderi, 2006a; 2006b) are well studied. It was first applied in landscape ecology (e.g. Bolliger, Sprott & Mladenoff, 2003) to measure the complexity of landscapes with patches of different land uses, which are analogous to markings in an animal colour pattern, and PAT-GEOM automates the process of deriving the file size. To compare ROIs, they should have identical sizes and sensitivity (ISO) settings (higher settings can introduce noise which artificially increases measurements).

OTHER TOOLS

In addition, PAT-GEOM contains tools to facilitate repetitive image processing steps, e.g. detecting ROIs, randomly sampling pixel values (Fig.4, Step 1), creating randomly-positioned copies of an ROI and calculating the percentage coverage of markings on an animal (Fig. 4, Step 3).

Considerations when using PAT-GEOM

The ability to quantify the properties listed above should be useful for studying pattern in various organisms and topics. However, two important issues require consideration: how to collect image data rigorously and how to select properties to analyse.

RIGOROUS DATA COLLECTION

All digital image-based analysis using any software (including, but not limited to, PAT-GEOM) requires properly standardised images of sufficient resolution to capture the pattern being quantified (Stevens et al., 2007). A useful guide is that the shortest length measured should comprise at least two pixels. Calibration to correct for differing light conditions and non-linear sensor responses to radiance is also needed and the MICA toolbox (Troscianko & Stevens, 2015) in ImageJ produces mspec images corrected for these biases. It can also produce composite images with both ultraviolet and human visible wavelengths and convert pixel values based on animal vision models to reflect what animals might see. Usage of the MICA toolbox is recommended and PAT-GEOM was designed for compatibility with its mspec images. Nevertheless, PAT-GEOM is able to analyse any image format readable by ImageJ.

WHAT PROPERTIES TO ANALYSE

The choice of properties to analyse depends on the specific research question and study system. Table 1 provides usage guidelines and examples where it may be advisable to measure each property in PAT-GEOM.

Summary and Future Directions

Colour patterns are an important part of animal interactions, yet researchers' ability to quantify pattern *per se* is poorly developed (Pérez-Rodríguez, Jovani & Stevens, 2017) and techniques to measure specific properties are lacking or difficult to implement. To address this, we developed PAT-GEOM, a suite of free-to-use macros (available at *www.ianzwchan.com/my-research/pat-geom* or *https://doi.org/10.5281/zenodo.1834035*) that quantitatively describe seven pattern properties: Marking Shape, Marking Shape

Directionality, Marking Size, Pattern Contrast, Marking Distribution, Marking Distribution Directionality and Pattern Randomness.

Whilst five of the properties can be measured using other programmes (although usually using different metrics), a key benefit of PAT-GEOM is that the tools are in one package, making it convenient to measure multiple properties. For example, *NaturePatternMatch* measures only marking size and orientation; HANGLE, HMATCH and HCURVE (Crampton & Haines, 1996) measure only shape; and although some R packages take similar measurements (e.g. EFA with *Momocs*), these must be separately installed. Moreover, because these examples are distinct programmes, images must be processed multiple times to perform all measurements, whereas with PAT-GEOM processing needs to be done only once. PAT-GEOM also complements a recently-released R package *patternize* (Van Belleghem et al., 2017); while *patternize* investigates overall pattern variation by analysing raster objects representing entire colour patterns, PAT-GEOM quantifies specific properties that contribute to this variation.

Being based in ImageJ, PAT-GEOM is highly versatile: it will analyse any image that ImageJ can open, including jpg, bmp, tif, gif, mspec and nef). It is also convenient to conduct analyses using other ImageJ-based programmes, e.g. granularity analysis with the MICA Toolbox and measuring fractal dimension with FracLac (Karperien, 1999). Finally, PAT-GEOM is not limited to patterns on animals and can potentially be applied to patterns across diverse fields, including landscape ecology (e.g. quantifying land plot randomness), botany (e.g. measuring leaf shape), and cellular biology (e.g. measuring occlusion body size in diseased cells).

It remains important, however, to improve our fundamental understanding of pattern and identify which measurable properties are biologically meaningful (Endler & Mappes, 2017; Pérez-Rodríguez et al., 2017). This would direct future work, including developing guidelines on what properties to measure in different situations and standardising the techniques used so that results are comparable across studies. It is an exciting time for researchers in this field: interest in the effects of pattern *per se* on animal behaviour, ecology, and evolution is growing, and our ability to quantify pattern using programmes such as PAT-GEOM is developing rapidly (Endler & Mappes, 2017).

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Author's Contributions

I.Z.W.C. wrote the software and conducted the case studies. All authors conceived the ideas for the software and contributed to manuscript drafts.

Conflict of Interest Declaration

The authors declare that we have no conflict of interest.

Data Accessibility

The PAT-GEOM software package and its User Guide are available from the first author's personal website (*www.ianzwchan.com/my-research/pat-geom*) or the Zenodo repository, *https://doi.org/10.5281/zenodo.1834035* (for the software package; Chan, Stevens & Todd, 2018a) and *https://doi.org/10.5281/zenodo.1835291* (for the User Guide; Chan, Stevens & Todd, 2018b). Datasets and R code are also available from Zenodo, *https://doi.org/10.5281/zenodo.1831671* (Chan, Stevens & Todd, 2018c).

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Table 1. Guidelines and application examples for the seven properties measured by PAT-GEOM.

Property	Technique	Guidelines	Usage Examples
Marking Shape	Elliptical Fourier Analysis	- Can be used in most, if not all situations where there are discrete pattern components.	- Comparing the shape of the spots on a cuckoo egg to those on its host's eggs. - Comparing average marking shape in two populations of a species (e.g. giraffes *Giraffa camelopardalis*). - Identifying individuals in species with unique colour patterns (e.g. whale sharks *Rhincodon typus*). - Comparing carapace patterns of a furrowed crab *Xantho hydrophilus* to the patterns in its background in putative background matching (see Fig. 2).
Marking Shape Directionality	Aspect Ratio and Orientation	- More useful for patterns with elongated markings. - May need to first standardise for orientation, size and shape.	- Comparing the markings found on hoverflies versus wasps. - Comparing an animal's stripes to stripe-like patterns in its background, e.g. in zebras *Equus quagga*. - Measuring changes in butterfly wing or eyespot shape due to genetic manipulation or selection pressures, e.g. in the squinting bush-brown butterfly *Bicyclus anynana*. - Measuring variation in stripe shape in tigers *Panthera tigris*, e.g. photographed using camera traps.
Marking Size	Averaged Centroid Size	- Better for discrete markings, vis-à-vis mottled patterns where granularity analysis (Troscianko & Stevens, 2015) is preferable.	- Comparing the markings of artificial prey items and their model organism, e.g. for predation experiments with the monarch caterpillar *Danaus plexippus*. - Comparing average spot size in two populations of the same species, e.g. the seven-spot ladybird *Coccinella septempunctata*. - Comparing the size of the markings on an animal to those on its background.
Pattern Contrast	Coefficient of Variation	- For use on non-thresholded images. - Can measure the whole or part of an animal.	- Determining if a flounder's (suborder Pleuronectidae) colour pattern matches a random sample of its background substrate. - Comparing two different parts of an animal which can change its appearance rapidly such as the common cuttlefish *Sepia officinalis*.
Marking Distribution	Pixel Matrix	- Areas to be compared must be of the same dimensions (in pixels).	- Visualising the "average pattern" of a population of animals, e.g. shore crabs *Carcinus maenas.* - Designing realistic prey items, e.g. to test putative aposematic coloration in the pink warty sea cucumber *Cercodemas anceps* (Figs. 3 & 4).
Marking Distribution Directionality	Angle and Alignment	- May need to first standardise for orientation, size and shape of the animal's body.	- Determining if a particular population of organisms is developing more linearly positioned markings in response to a selection pressure, e.g. the spots of the queen fish *Scomberoides commersonianus*, or the eyespots of the squinting bush-brown butterfly *Bicyclus anynana*. - Comparing the patterns of two species with similar overall body shapes.
Pattern Randomness	Gif File Size	- For non-thresholded images. - Areas to be compared must have the same dimensions (in pixels) and ISO settings.	- Comparing patterns on different morphotypes of a species, such as button snails *Umbonium vestiarium*. - Determining mimic quality, e.g. the eggs of the common cuckoo *Cuculua canorus* and those of its host. - Comparing an animal (e.g. shore crabs *Carcinus maenas)* to its background.

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