

## Distance transform: a tool for the study of animal colour patterns

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### Summary

1. The information in animal colour patterns plays a key role in many ecological interactions; quantification would help us to study them, but this is problematic. Comparing patterns using human judgement is subjective and inconsistent. Traditional shape analysis is unsuitable as patterns do not usually contain conserved landmarks. Alternative statistical approaches also have weaknesses, particularly as they are generally based on summary measures that discard most or all of the spatial information in a pattern.
2. We present a method for quantifying the similarity of a pair of patterns based on the distance transform of a binary image. The method compares the whole pattern, pixel by pixel, while being robust to small spatial variations among images.
3. We demonstrate the utility of the distance transform method using three ecological examples. We generate a measure of mimetic accuracy between hoverflies (Diptera: Syrphidae) and wasps (Hymenoptera) based on abdominal pattern and show that this correlates strongly with the perception of a model predator (humans). We calculate similarity values within a group of mimetic butterflies and compare this with proposed pairings of Müllerian comimics. Finally, we characterise variation in clypeal badges of a paper wasp (*Polistes dominula*) and compare this with previous measures of variation.
4. While our results generally support the findings of existing studies that have used simpler *ad hoc* methods for measuring differences between patterns, our method is able to detect more subtle variation and hence reveal previously overlooked trends.

**Key-words:** image analysis, individual recognition, MATLAB, mimicry, signalling, similarity

### Introduction

Colour patterns play a key role in many animal interactions (Cott 1940). Signal receivers must discriminate among patterns with significant information content, such as social dominance (Møller 1988) or toxicity (Brodie 1993). To understand such decisions fully, we need a way of extracting the information contained in the colour pattern observed. There exist effective standardised methods for comparing colours (Endler & Mielke 2005; Stoddard 2012), but ways of quantifying the spatial aspect of a pattern are less well developed.

Humans have extremely good visual capabilities and cognition (Pinker 1984) and hence, when studying colour patterns, often find it easy to make qualitative decisions about the information in a signal and classify the patterns appropriately. However, if asked to articulate rigorously and precisely the reasons behind such decisions, we find this far more difficult. This means that the outcome of such comparisons is subjective and inconsistent. Furthermore, humans, like all species, perceive the world in a way that is subtly constrained by their sensory and cognitive abilities. Differences in factors such as spatial resolution and spectral sensitivity, as well as higher

processing, mean that the human perception on which a judgement is based may not accurately reflect that of the most relevant signal receiver (Endler 1990).

An alternative is therefore to use automated, computer-based methods for comparing patterns. Traditional methods of shape analysis are inappropriate, as they rely on the selection, either manual or automatic (Boyer *et al.* 2011), of homologous landmarks. These are unlikely to exist in colour patterns, especially when comparing disparate taxa, such as will often be the case in examples of mimicry. To capture pattern information in a more flexible way, there are two main types of approach, both well developed in computer science. The first involves ‘feature extraction’: creating a statistical summary for each of the patterns under study, often based on properties of the image in the frequency domain using Fourier transforms (Zhang & Lu 2002), or on ‘moment invariants’ (Khotanzad & Yaw Hua 1990). A simple measure such as Euclidean distance between two summary vectors can then give the dissimilarity between two patterns (Zhang & Lu 2003). This type of method is commonly used in image retrieval algorithms, where an image is sought within a large database that shares similar properties to a target image (Rui, Huang & Chang 1999).

The second approach involves direct comparison of individual pixels or regions of pixels through, for example, cross-correlation (Briechele & Hanebeck 2001) or the sum of absolute

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differences (Goshtasby 2005). This method, known as template matching, is commonly used for image registration (Zitová & Flusser 2003). The key point here is that it compares whole images, pixel by pixel, before the information is summarised. By contrast, in feature extraction, the images are first summarised and then compared. Thus, template matching is sensitive to differences in specific features within the pattern (e.g. particular spots or stripes), while feature extraction is based on the overall attributes of the pattern.

In a biological context, several authors have used Fourier transforms to facilitate comparison of general pattern properties, such as in striped coats of mammals (Godfrey, Lythgoe & Rumball 1987), cuttlefish displays (Barbosa *et al.* 2008) and spots on bird eggs (Stoddard & Stevens 2010). Endler (2012) suggests a slightly different approach, counting the number of transitions from one colour to another in 'adjacency analysis'. However, all of these cases adopt an 'image retrieval'-type approach, in which the image is simplified to a few summary values before the comparison with another image takes place. In doing so, specific spatial information regarding the location of particular pattern features is discarded.

In some cases, this is not a problem and could even be an advantage. For example, in egg mimicry, the pattern on each host egg is unique. The mimetic egg therefore resembles the overall features and type of pattern of a host egg rather than the exact locations of pigment blotches (Stoddard & Stevens 2010), and so the use of summary variables is more appropriate than a consideration of individual pattern elements. On the other hand, for strongly stereotyped patterns, such as those seen in many insect mimicry complexes (see e.g. Cott 1940; Ruxton, Sherratt & Speed 2004), the precise shape and position of pattern features may be important. In summarising, we risk discarding pertinent information, and what is retained will depend upon the statistics chosen, increasing subjectivity.

By contrast, the 'template matching' approach of comparing specific features within an image has very rarely been applied to biological colour patterns. A simple version was used by Williams (2007), who subdivided bumblebee patterns into 27 regions, each of which, within individuals, is usually occupied by a uniform colour. The patterns can then be compared region by region. This approach can be effective, but is limited by the fact that colour boundaries in a given individual may not exactly coincide with the predefined regions; it relies upon creating discrete homologous categories in patterns which are often continuous in nature. Williams' (2007) division of a bumblebee pattern into 27 regions is able to detect the presence or absence of a spot or stripe, but not its exact outline.

To make the subdivision method more sensitive, we can use more regions. The logical extreme is to divide the pattern into as many separate regions as possible; in practical terms, for a pattern recorded as a 2D digital image, regions would be the individual pixels of the image. We can then score the dissimilarity at each pixel location and take the sum of all such values; this method is known as the sum of absolute differences (Goshtasby 2005). The dissimilarity score could be a simple match or mismatch for binary images or a difference in brightness for greyscale. Unfortunately, increasing the number of

regions introduces a different problem, which is that common features between the two images must line up exactly to be recognised as similar. A stripe offset by just a few pixels would be counted as just as strong a mismatch as its complete absence – in fact more so, since both locations will count as mismatches. Although the method has been used to produce a rough measure of mimetic accuracy in hoverflies (Dittrich *et al.* 1993; Azmeh *et al.* 1998), it gives several anomalies. For example, Azmeh (1999) observed that all-black hoverflies are given unrealistically high measures of similarity to wasps.

Here, we describe a new, holistic method for the measurement of similarity between two or more biological colour patterns. The method is similar to the sum of absolute differences in that it uses information from the whole pattern at the level of the individual pixel, but it is more robust to small spatial variations among images because it is based on the distance transform (Borgefors 1986). In the distance transform of a binary image, each pixel is weighted by the minimum distance to the nearest white pixel. Distance transforms have been used on one previous occasion to analyse biological colour patterns (Anderson *et al.* 2010) but to make qualitative decisions (identification) rather than the quantitative comparisons of similarity we seek. The method we describe is applicable to any pattern that consists of clearly separated colours (as opposed to colours blending from one to another), for example, those seen in the abdominal patterns of many insects, cetacean markings, wing patterns of many butterflies and moths, amphibian aposematic signals and body patterns of reef fish. We demonstrate the utility and versatility of the method by applying it to three ecological examples.

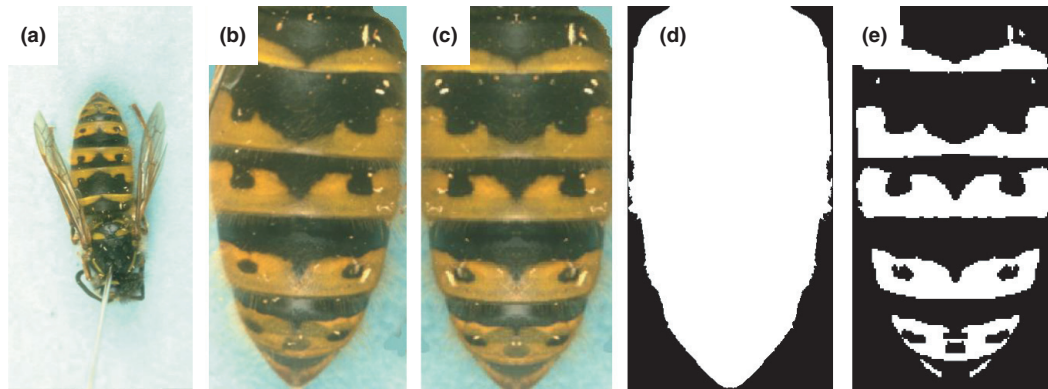
## General methods

We carried out automated image analysis in MATLAB (2010); for the full code along with detailed annotations and instructions, see Appendices S3 and S4. Some early steps in image pre-processing (such as alignment of images and selection of the region of interest) required user input, but this was kept to a minimum and rarely exceeded a couple of minutes for a single image.

### IMAGE PREPROCESSING

The distance transform method assumes that good-quality 2D images of the patterns of interest are available. These images should reflect the pattern as seen by the relevant signal receiver (see e.g. Endler & Mielke 2005). For simplicity, we have used RGB digital photographs, but our method is equally applicable to images produced using other methods, such as those including ultraviolet (UV) components.

The input images (Fig. 1a) must first be 'registered', that is, transformed in order to match in size and alignment, and cropped down to the region of interest (Fig. 1b,c). Ideally, this should be achieved by the use of fixed landmarks within the image; however, homologous landmarks common to all images within a set may be difficult or impossible to define. If true landmarks exist, the images can be transformed



**Fig. 1.** Image preprocessing. (a) An original image. (b) Rotated and cropped. (c) Symmetry detected and image made symmetrical (this step is optional). (d) Mask. White pixels correspond to the region of interest. (e) Binary image of one segment, with white areas corresponding to yellow parts of the pattern.

automatically to line up these ‘control points’ as closely as possible – see *Heliconius* example below. In other cases, more approximate visual landmarks such as the line of symmetry can be used. This may add a small level of subjectivity to the analysis, but we show our method to be robust to small errors of rotation and scaling, as well as to noise (see Appendix S2, Figs S1–4).

Next, each image must be simplified by converting into a set of binary images, hereafter ‘segments’, one for each distinct colour, with values 1 for presence and 0 for absence (Fig. 1e; see also Fig. 2b). A key assumption here is that each segment represents a distinct colour category in the perception of the signal receiver. To define segments appropriately, it is necessary to understand both the properties of the device used to acquire the image (Stevens *et al.* 2007) and the sensory capabilities of the receiver (Endler & Mielke 2005). We have chosen examples in which the lack of UV sensitivity of standard digital cameras, and differences in colour perception between humans and the most important signal receivers, are unlikely to have an important effect on the definition of the segments, but these issues must be considered carefully. For example, if the receiver and its visual properties are known, then it is possible to calculate what constitutes a ‘just noticeable difference’ in colour and to separate the pattern into regions differing by at least this amount (two ways of doing this are suggested by Endler 2012). If a typical viewing distance can be estimated, the image could also be converted to a resolution equivalent to that experienced by the receiver (again, see Endler 2012).

The methods used to separate a pattern into its segments will vary according to the organisms under study and the source and quality of the images. Consequently, specific details are to be found accompanying the three examples; see also Appendix S1.

#### PAIRWISE DISSIMILARITY CALCULATION

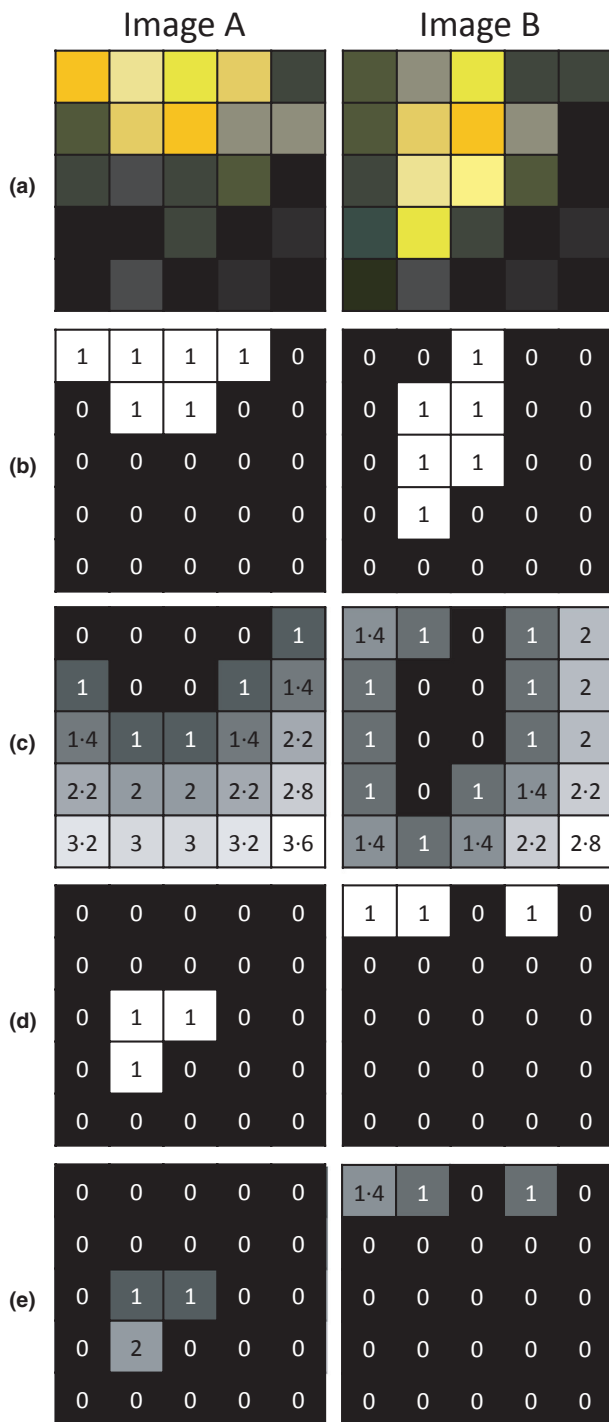
For the sake of simplicity, we describe here how to compare two images, A and B, each with two different segments (A1 and A2, B1 and B2; see example in Fig. 2); the method is easily

extended to cases with more than two segments. A distance transform of A1 is carried out, such that all pixel locations are assigned a value calculated as the smallest distance to a pixel of value 1 (Borgefors 1986). This distance is zero for pixels which have a value of 1 in A1, and a real number, at least one, for all other pixels, calculated using Euclidean distance (Fig. 2c). The distance transform values for all pixels are then multiplied by the raw values for equivalent pixels in B1. The result is that any pixel locations that are the same in both images (a match) will carry a value of 0, while any nonmatching pixel locations will be weighted in proportion to the minimum distance to a matching pixel (Fig. 2e). Total mismatch for the whole image is simply the sum of these pixel values.

Next, the process is repeated with image roles reversed (A1 multiplied by the distance transform of B1), and repeated in both directions for A2 and B2. To standardise the dissimilarity value between zero and one, we take the mean of these four values and divide it by the maximum possible total for that size of image. The greatest mismatch should in theory be between an entirely white and an entirely black image of the same size as A and B, but a distance transform cannot be obtained from an image which is composed entirely of zeros. A single white pixel is therefore placed in the corner of the otherwise black image, and vice versa, before calculation of the dissimilarity as described above, giving the maximum value obtainable.

#### OPTIMISATION

Two very similar patterns might not be classified as such if they are misaligned. An optimisation procedure is therefore used whereby one image is shifted relative to the other by varying small amounts horizontally and vertically, to find the lowest possible dissimilarity value given these various alignments. For example, in Fig. 2, if image B is translated one pixel upwards, the total mismatch of the yellow segments would be reduced from 7.4 to 4, giving a more optimal alignment. In preliminary testing, a maximum translation of 15% of the image size was sufficient to include the optimum value in the vast majority of cases. If the images are symmetrical and have already been



**Fig. 2.** A demonstration of the distance transform dissimilarity method on two five-by-five pixel patterns. (a) Two pattern images to be compared. (b) Binary images representing the yellow 'segments' A1 and B1, in which pixels that are above a defined 'yellowness' threshold carry a value of one. The black segments A2 and B2 would be the inverse (ones and zeros interchanged – not shown). (c) Distance transforms (to one decimal place) of the binary segments in (b). (d) Locations of mismatches between the segments in (b). (e) Mismatches in (d) weighted by distance transform values from (c). Total weighted mismatch for this pair of segments is 7.4. The process would then be repeated on the black segments.

aligned along their lines of symmetry, then translation perpendicular to that line is not necessary.

A further possibility is to vary the aspect ratio of the image, as well as horizontal and vertical alignment. This involves stretching or compressing one image relative to the other. In repeating patterns, such as stripes, this accounts for slight variation in scale. Again, a maximum of 15% change in height was sufficient in preliminary testing. Other forms of transformation could potentially be used in order to optimise image alignment further; for example, rotation of the images could be useful in cases without clear landmarks by which the images can be aligned.

#### STATISTICAL METHODS

The output of the process this far will be a dissimilarity value between a pair of images. If a similarity value is required, we can use  $\text{similarity} = 1 - \text{dissimilarity}$ . In the likely case that more than two images are to be compared, the process is repeated for all possible pairs, leading to a matrix of pairwise dissimilarity values (such as Table 1). For more than a few patterns, this matrix is very difficult to interpret directly, and it is useful to summarise the information using a form of ordination. Using nonmetric multidimensional scaling (NMDS; Legendre & Legendre 1998), each pattern is represented by a point in Euclidean space, with the distance between any two points being approximately equal to their dissimilarity value. Usually, the first two dimensions represent an appropriate visualisation (see Fig. 4). Note that, while useful for visualising trends, ordinations of relationships between multiple patterns are approximations of the true situation, and reference should also be made to the raw dissimilarity values.

#### Applications

##### HOVERFLY AND WASP ABDOMINAL PATTERNS

Many species of hoverfly (Diptera: Syrphidae) are thought to gain protection through resemblance to stinging Hymenoptera such as wasps (Rotheray & Gilbert 2011). This is an example of Batesian mimicry – predators mistake the palatable mimic (hoverfly) for an unpalatable model (wasp), and thus, the attack rate on the mimic is reduced (Bates 1862). Although we would expect natural selection to favour those with the highest level of mimetic accuracy, some hoverflies seem to resemble wasps better than others. Why are some hoverflies inaccurate mimics of wasps (Edmunds 2000; Gilbert 2005)?

Of course, we cannot answer this question without being able to define what we mean by 'inaccurate mimic': some authors have used human judgement (Penney *et al.* 2012), pigeon *Columba livia* judgement (Dittrich *et al.* 1993) or the sum of absolute differences (see above; Dittrich *et al.* 1993; Azmeh *et al.* 1998). The most successful approach to date has been the multivariate method used by Penney *et al.* (2012). They calculated a dissimilarity value from a combination of six



**Table 1.** Similarity matrices for images of 10 butterfly wings taken from Joron *et al.*'s (2006) study. (a) Images scaled to a height of 350 pixels with shape unchanged. (b) Shape standardised using control point registration (see Appendix S2)

	Hnarc	Mpha	Hnaur	Mril	Hnbic	Mmot	Hnsil	MLud	Hntar	Mmen
<i>(a)</i>										
Hnarc		0.7504	0.6154	0.5844	0.2062	0.3577	0.4240	0.2774	0.3036	0.3024
Mpha	0.7504		0.4062	0.6959	0.0140	0.3858	0.3800	0.2277	0.2879	0.3698
Hnaur	0.6154	0.4062		0.6102	0.6157	0.6056	0.3801	0.4037	0.3337	0.3560
Mril	0.5844	0.6959	0.6102		0.4357	0.6657	0.5309	0.3968	0.2265	0.4818
Hnbic	0.2062	0.0140	0.6157	0.4357		0.8411	0.0622	0.0220	0.0000	0.1058
Mmot	0.3577	0.3858	0.6056	0.6657	0.8411		0.2614	0.1404	0.2543	0.4005
Hnsil	0.4240	0.3800	0.3801	0.5309	0.0622	0.2614		0.8031	0.2889	0.3692
MLud	0.2774	0.2277	0.4037	0.3968	0.0220	0.1404	0.8031		0.3671	0.4305
Hntar	0.3036	0.2879	0.3337	0.2265	0.0000	0.2543	0.2889	0.3671		0.7679
Mmen	0.3024	0.3698	0.3560	0.4818	0.1058	0.4005	0.3692	0.4305	0.7679	
<i>(b)</i>										
Hnarc		0.8127	0.7490	0.5971	0.3983	0.4144	0.4505	0.3128	0.3379	0.3898
Mpha	0.8127		0.7062	0.7577	0.3108	0.4916	0.3737	0.3454	0.3926	0.5458
Hnaur	0.7490	0.7062		0.8332	0.6474	0.6642	0.5385	0.4279	0.3822	0.5025
Mril	0.5971	0.7577	0.8332		0.5912	0.7298	0.4711	0.4463	0.3090	0.5614
Hnbic	0.3983	0.3108	0.6474	0.5912		0.8675	0.1854	0.0335	0.2009	0.3480
Mmot	0.4144	0.4916	0.6642	0.7298	0.8675		0.2489	0.1691	0.2790	0.4871
Hnsil	0.4505	0.3737	0.5385	0.4711	0.1854	0.2489		0.8550	0.3366	0.4775
MLud	0.3128	0.3454	0.4279	0.4463	0.0335	0.1691	0.8550		0.3599	0.5349
Hntar	0.3379	0.3926	0.3822	0.3090	0.2009	0.2790	0.3366	0.3599		0.8120
Mmen	0.3898	0.5458	0.5025	0.5614	0.3480	0.4871	0.4775	0.5349	0.8120	

Traditionally proposed mimetic pairings are adjacent (i.e. 1 and 2, 3 and 4, etc.). Highlighted cells show the highest similarity for a given column. Identification codes: Hnarc, *Heliconius numata* f. *arcuella*; Hnaur, *H. n. f. aureus*; Hnbic, *H. n. f. bicoloratus*; Hnsil, *H. n. f. silvana*; Hntar, *H. n. f. tarapotensis*; MLud, *Melinaea ludovica ludovica*; Mmen, *M. menophilus* ssp. nov.; Mmot, *M. marsaeus mothone*; Mpha, *M. m. phasiana*; Mril, *M. m. rileyi*.

different morphometric features plus summary pattern features such as mean red, green and blue values from the RGB image. This difference measure was found to correlate with human estimates of similarity and enabled the authors to test a number of the proposed explanations for the existence of inaccurate mimicry. However, abdominal pattern was only approximately represented in the form of six summary values, possibly a major shortcoming given that pattern is one of the most immediately recognisable features of a wasp. The distance transform method enables us to examine in more detail the similarities and differences between wasp and hoverfly colour patterns.

### Materials and methods

We obtained 39 high-resolution photographs (in TIFF format) of 10 species of hoverfly and eight photographs of the wasps *Vespula vulgaris* L. 1758 and *V. rufa* L. 1758 (images used previously by Dittrich *et al.* 1993). In order to facilitate comparison with the study by Penney *et al.* (2012), we also took high-resolution scans of illustrations of 20 hoverfly species from a field guide (Stubbs & Falk 2002), chosen to correspond to as many as possible of the 'wasp mimics' in Penney *et al.*'s Fig. S1 (2012).

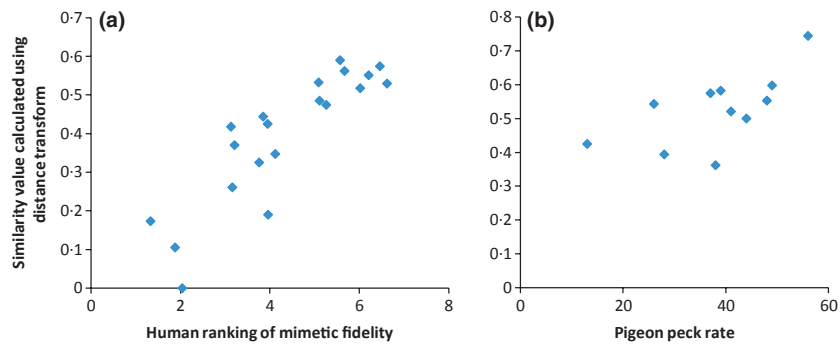
The abdomen was selected as the region of interest, and images aligned along their lines of symmetry. Hoverfly and wasp patterns typically appear to have two discrete colours to the human eye, well represented in RGB digital photographs. Spectrometry suggests that the patterns do not include hidden

UV elements (Gilbert 2005) and hence that likely predators (birds) also perceive them as consisting of two colour classes. We therefore segmented the images into yellow and black components (for full details, see Appendix S1; see also Fig. 1).

Similarity was calculated as described in 'General methods' above, with optimisation using vertical translation and vertical scaling. The lowest similarity to *Vespula* spp. in the data set came from the hoverfly *Syrirta pipiens* (L. 1758), and for ease of interpretation, this was assigned a similarity value of 0 in relation to *Vespula* spp., with all other similarity values expressed relative to this. This scaling has no effect on the outcome of the statistical tests. Correlation with similarity estimates from other sources was calculated using one-tailed Pearson's correlation.

### Results

Mean similarity values between images of each species of hoverfly and the eight wasp images, calculated with the distance transform method, correlate strongly with human estimates of similarity, which were based on separate photographs of hoverflies and *V. vulgaris* (Fig. 3a;  $r = 0.87$ ,  $P < 0.0001$ ,  $n = 20$ ; human data from Penney *et al.* 2012). There is also a significant correlation between distance transform similarity and similarity as assessed by pigeons in the form of peck rates (Fig. 3b;  $r = 0.65$ ,  $P = 0.014$ ,  $n = 11$ ; pigeon data from Dittrich *et al.* 1993).



**Fig. 3.** Correlation between distance transform similarity and other measures. (a) Compared against human assessment of similarity of hoverflies to *Vespa vulgaris*, given as a score out of 10 (Penney *et al.* 2012). (b) Compared against peck rate from pigeons trained to peck at images of wasps, but not at those of flies (Dittrich *et al.* 1993).

#### MÜLLERIAN MIMICRY IN HELICONIINAE

A number of tropical butterfly species (Lepidoptera: Nymphalidae) are highly distasteful to predators and employ aposematic coloration on their wings to advertise their unprofitability (Brown 1981). In a given geographical location, we often see convergent evolution of patterns from well-separated taxa, sometimes to an extraordinary degree. This convergence first led Müller (1879) to formulate the theory of mimicry that bears his name.

The butterfly *Heliconius numata* (Cramer 1780; Nymphalidae: Heliconiinae) exists in a number of distinct forms, each with its own characteristic colour pattern (Joron *et al.* 1999; Heliconius Genome Consortium 2012). Each form is thought to mimic a particular species or subspecies of another group of butterflies, the genus *Melinaea*. However, to our knowledge, mimetic pairings have always been chosen simply on the basis of the author's perception and geographical coincidence. Similarities between proposed Müllerian comimics certainly appear striking to the human eye, but it would be informative to take a more objective view of their similarities and differences.

#### Materials and methods

Images of five forms of *H. numata* and five species/subspecies of *Melinaea* were taken from Fig. 1 in the study of Joron *et al.* (2006). Nomenclature follows Lamas *et al.*'s (2004) study. Each image was cropped to a rectangle just large enough to contain both the fore- and hind-wing of the right-hand side. Other features such as antennae or parts of the head encroaching on the defined area were removed. Two alternative methods were used to register the images, giving slightly different results. In one case, images were scaled to a standard height of 350 pixels, preserving aspect ratio, with no other transformation. In the other, four control points were selected, using wing veins as landmarks, and images were transformed to match the control points to each other as closely as possible (see Appendix S1).

The pattern was segmented into black and red, and a third category of yellow/white that was present in only six of 10

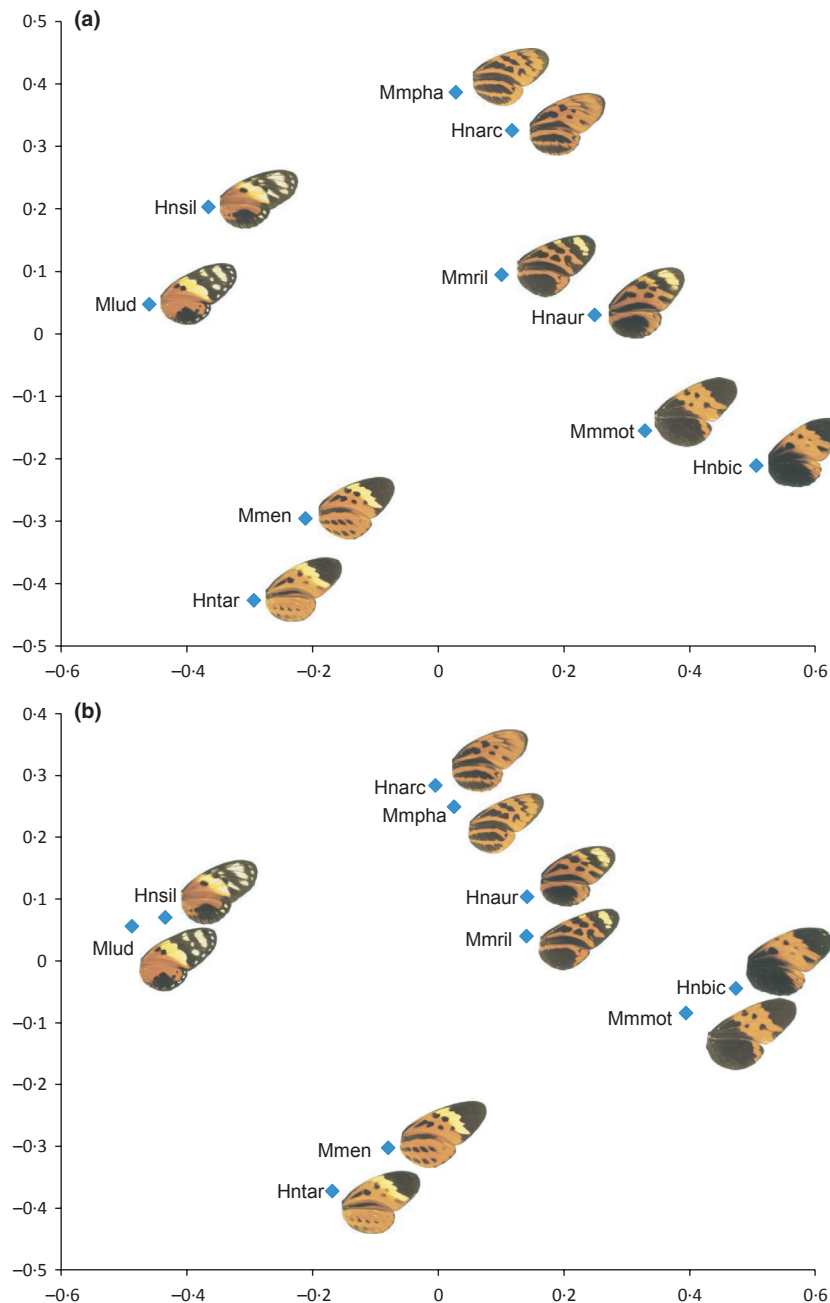
images. Yellow and white were not separated, as they appeared much less distinct to the human eye than the other major colour divisions, and often shaded from one into the other. The most important signal receivers in this system (most likely avian predators) may perceive the boundaries between the colours on the wing differently. In the absence of more complete information, however, we focus here on illustrating how the results of our technique for comparing the spatial arrangement of colour patches on the wing compare with the existing classification of mimetic pairs, which was based on human perceptions of the patterns. For full details of image preprocessing, see Appendix S1.

Since not all patterns contained a yellow component, pairwise dissimilarity measures were calculated using the black and red segments only. However, this did still allow for variation in the yellow parts to be considered, since these were represented as areas scoring zero on both the red and the black segments. Optimisation was carried out using horizontal and vertical translation. For ease of interpretation, similarity values were scaled to span the range 0–1.

#### Results

Figure 4a shows the ordination of the 10 species/morphs based on images with a preserved shape. The similarity values (Table 1) show that in eight out of 10 cases, the highest similarity value for a given pattern corresponds to the proposed comimic. However, *M. marsaeus rileyi* is more similar to its sister subspecies *phasiana* and *mothone* than to the proposed comimic *H. numata f. aurora*, and likewise, the latter is slightly more similar to both conspecific forms *bicoloratus* and *arcuella* than to *M. m. rileyi*.

By inspection, the main feature driving this result appears to be the shape of the wing rather than the colour pattern itself, and this can be confirmed through control point registration. If, during the preprocessing stage, the images are transformed to line up wing landmarks, then the results are slightly different (Fig. 4b). Importantly, comimics are now much more closely associated, with the highest similarity value in each case corresponding to the comimic.

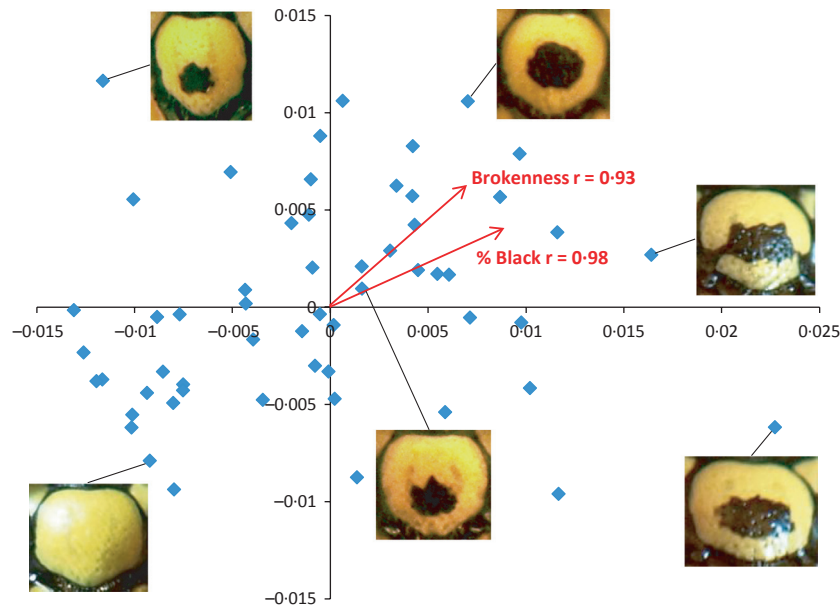


**Fig. 4.** Ordination of dissimilarity data for five forms of *Heliconius numata* along with their comimics. Positions were computed using nonmetric multidimensional scaling (NMDS) in two dimensions. Images from Joron *et al.*'s (2006) study. (a) Each image scaled to a height of 350 pixels but aspect ratio preserved. Stress = 0.125. (b) Shape standardised using control point registration. Stress = 0.076. For an explanation of identification codes, see Table 1.

#### FACE BADGES OF *POLISTES DOMINULA*

The front of the head of many wasp species of the genus *Polistes* (Hymenoptera: Vespidae) bears a black-and-yellow pattern that varies greatly among individuals. For example, *P. dominula* (Christ 1791) generally has a black 'badge' on its otherwise yellow clypeus (except in some cases where the clypeus is entirely yellow; see examples in Fig. 5). This badge is highly variable both within and among populations (Tibbetts *et al.* 2011), and it has been proposed that the badge is a signal of competitive ability (Tibbetts & Dale 2004; Tibbetts & Curtis 2007).

Variation in badge size and shape has so far been quantified almost exclusively by the use of a single summary variable: the 'brokenness' of the black region. Brokenness is defined (Tibbetts & Dale 2004) as the variation in the vertical extent of the central section of the badge. However, this measure has had mixed success in capturing an association between pattern and social dominance, depending on the population studied (Cervo *et al.* 2008; Green & Field 2011; Tibbetts *et al.* 2011). It may be that the clypeal badge is a signal of quality in some populations and not in others, but it is also possible that the brokenness summary measure is



**Fig. 5.** Nonmetric multidimensional scaling (NMDS) ordination of dissimilarity data from shapes of clypeal badges of 56 individuals of *Polistes dominula* (one extreme outlier is omitted for the sake of clarity). Red arrows show the direction and relative strengths of correlation of the axis scores with two variables: brokenness and proportion of the clypeus that is black (% black). Inserts show the clypeal badge for six data points as examples.

not sufficient to capture the relevant signal variation in all cases.

We need a more robust way of quantifying badge variation if we are to understand fully the geographical differences between populations of *P. dominula*. Even within a single population, it is conceivable that a more holistic pattern measure might be able to account for more of the variation in characters such as body size (which is a useful predictor of dominance; Tibbetts & Dale 2004). We have therefore compared the use of the distance transform method with the brokenness measure on images from a Portuguese population of *P. dominula*.

### Materials and methods

Fifty-seven foundresses of *P. dominula* were taken from their nests at Quinta de Sao Pedro, Portugal. Wet body mass was measured using an electronic balance immediately after capture. Their faces were photographed with an Olympus E420 DSLR. The image was cropped to a rectangle containing the clypeus and segmented into black and yellow components; for full details, see Appendix S1. As with the hoverfly/wasp example above, it seems reasonable to assume that the two-colour (black and yellow) pattern, which is clear to the human eye when viewing the *P. dominula* clypeus in both living specimens and RGB photographs, is also perceived by the intended receivers of the signal (other wasps) in this system. Wasp patterns appear not to have any UV components (Gilbert 2005).

Dissimilarity values were calculated as described in General methods, but with one modification. Some individual wasps had no black badge on the clypeus whatsoever, making a distance transform of the black segment of the image impossible. The dissimilarity for all image pairs was therefore calculated from the yellow segment alone rather than the mean of the

values from both the yellow and the black segments. Optimisation was carried out using horizontal and vertical translation. Two-dimensional NMDS was then used to provide  $x$  and  $y$  scores, giving a bivariate summary of the facial pattern.

Brokenness values were calculated as described in the study of Tibbetts *et al.* (2011) – see Appendix S1. A second summary variable, proportion of clypeus that is black, was also calculated. Correlations between variables were calculated using two-tailed Pearson's correlation tests.

### Results

Brokenness and proportion black both correlate significantly with both  $x$  and  $y$  axes from NMDS ( $P < 0.01$  for each pairing; Table 2 and Fig. 5), showing that the distance transform measure is sensitive to the type of variation captured by the other summary measures. Indeed, since the  $x$  and  $y$  axes are orthogonal, we can combine the  $r$  values in two dimensions to give  $r = 0.93$  between brokenness and the 2D ordination, and  $r = 0.98$  for proportion black and the ordination (both

**Table 2.** Pearson correlation coefficients between variables relating to clypeal badges of *Polistes dominula* and the body mass of the wasp.  $x$  and  $y$  are the axis scores from NMDS of dissimilarity data; percentage black is the proportion of the clypeus that is black

	$x$	$y$	% Black	Brokenness
Mass	-0.097	0.122	-0.030	0.065
Brokenness	0.699***	0.620***	0.901***	
% Black	0.898***	0.400**		
$y$	0.000			

NMDS, nonmetric multidimensional scaling.

$n = 57$ . \*\*Significant at  $P < 0.01$ , \*\*\*significant at  $P < 0.001$ .



$P < 0.0001$ ). However, none of the measures correlate significantly with body mass (brokenness:  $r = 0.07$ ,  $P = 0.63$ ; proportion black:  $r = -0.03$ ,  $P = 0.82$ ;  $x$  axis:  $r = -0.10$ ,  $P = 0.47$ ;  $y$  axis:  $r = 0.12$ ,  $P = 0.37$ , all  $n = 57$ ). Mass is a useful proxy for social dominance (Tibbetts & Dale 2004). Therefore, in this population, no association between pattern and dominance is detected by any of the methods used, despite the extra information contained in the two-dimensional ordination compared with the one-dimensional summary measures.

## Discussion

We have demonstrated the use of distance transforms to generate a measure of similarity between two colour patterns. This is not the first use of distance transforms for image comparison in biology. However, in the only other instance of which we are aware, it was used in the context of individual recognition, to detect whether two patterns of spots were the same or different (Anderson *et al.* 2010). In this qualitative approach, the pattern variation was used as a means for identifying individuals rather than being of interest in itself. We have sought to show that there is much more potential in the method for studying variation in patterns. To our knowledge, this is the first such study to use distance transforms where the magnitude of the difference is biologically relevant.

Our method captures far more pattern information than in previous analyses of the three systems studied here, since it does not characterise a pattern using summary variables. Instead, it uses the full pattern, pixel by pixel, to calculate a similarity value. The advantage gained is clear when we look at the hoverfly example: despite using information only from the abdominal colour pattern, the distance transform method gave an assessment of similarity that correlated extremely well with human perception of whole insect similarity ( $r = 0.87$ ). The multivariate measure from Penney *et al.* (2012), which included summary variables for the pattern, as well as morphometric data from other body parts, still gave a significant correlation but explained a lower proportion of the variance ( $r = 0.56$ , from Penney *et al.* 2012). It is worth noting, though, that even if the calculated dissimilarity did not correlate with a predator's perception of the pattern, it would still form a useful comparison; we could then ask the question: why does the predator *not* make use of the available information to distinguish models from mimics?

Similarly, in the case of *Polistes* clypeal badges, the brokenness measure (Tibbetts & Dale 2004) captures only a limited amount of information about the shape of the badge. In the case of the Portuguese population used in this study, the distance transform method retains almost all of the brokenness information, while adding more detail along a second dimension (Fig. 5). Despite the extra information, we still detect no association between mass and badge shape. As the measure we have used is more comprehensive, it strengthens the conclusion that the lack of a detected association is due to the genuine absence of a link rather than failure to capture the relevant variation. This supports the findings of Cervo *et al.* (2008) and Green *et al.* (2013) in other European populations. A further

advantage of the distance transform method is that, because it is based on an image of the whole clypeus rather than just the badge, it captures variation in clypeus outline as well as the badge itself. This may be especially useful in populations where a large proportion of individuals have no black badge at all (Cervo *et al.* 2008).

The relatively objective nature of our technique helps minimise a researcher's reliance on assumptions based on their own perception. The data we present on Müllerian mimicry in heliconiines largely confirm previous assumptions (Joron *et al.* 1999), but do reveal that in absolute terms, a few mimetic pairings may not be as clear cut as they initially appear. In particular, a consistent difference is picked up between subspecies of *Melinaea marseus* and their *Heliconius numata* comimics (Fig. 4a). This difference is connected largely with shape rather than pattern, since it is reduced considerably when shape is standardised (Fig. 4b). The difference may or may not be relevant to a consideration of mimicry since predators may not attend to shape information, difficult to interpret reliably with different wing angles at rest. Predators, such as birds, may well perceive the same striking pairings as suggested by humans, but this should be explicitly tested, given that alternative groupings, or a more continuous mimicry ring, also form plausible descriptions. This example is also a good demonstration of the potential scope of the technique; it can compare patterns with more than two colours and detect differences of outline as well as pattern.

We recognise that in our example analyses, the use of RGB photographs and illustrations may introduce a bias towards the human perception of colour patterns and that a more thorough analysis would take into account the visual abilities of the likely signal receivers. However, our focus is on the method of pattern comparison, whatever technique was used to collect and prepare the images. Furthermore, there is no evidence in any of the examples to suggest that human perception of the colour boundaries is any different from that of other animals. In the case of hoverflies, for example, responses of pigeons were no different when presented with naturally lit specimens (Green *et al.* 1999) as opposed to RGB photographs (Dittrich *et al.* 1993).

One limitation of the distance transform method is that it captures spatial variation in patterns only, not variation in colour hue. This is because the image is converted into binary format, with a colour classed as either present or absent; any more subtle information on hue or brightness is lost. While restricting the scope of the method slightly, this also brings benefits, since brightness of colours can in some cases vary over time, both during an animal's lifetime and after death. If images are taken from museum specimens, then fading of colours can be a major problem. However, provided major colour boundaries are still detectable, fading will not affect the binary images produced. If data on hue or brightness are thought to be relevant to the system under study, for example, the brightness of an aposematic signal, then this information would need to be included through a separate analysis such as recording of spectral reflectance values (Endler & Mielke 2005).

While we have aimed to develop an objective and consistent similarity measure, it will never be possible to remove subjectivity from the process entirely. The very process of representing a 3D colour pattern in a 2D digital image inevitably changes the nature of the pattern in some ways. The choice of colours used to segment the pattern (as in the *Heliconius* example) will also inevitably alter the outcome.

## Conclusions

The use of distance transforms to calculate dissimilarity is a broadly applicable, consistent, powerful tool in the comparative study of animal colour patterns. It is able to cast new light on well-studied systems, to challenge assumptions and has the potential to open up new avenues of investigation.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Supplementary methods.

**Appendix S2.** Testing robustness of the distance transform method. Includes Figs S1–S4.

**Appendix S3.** MATLAB code.

**Appendix S4.** Readme for MATLAB code.