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Automatic recognition and measurement of butterfly eyespot patterns

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ABSTRACT

A favorite wing pattern element in butterflies that has been the focus of intense study in evolutionary and developmental biology, as well as in behavioral ecology, is the eyespot. Because the pace of research on these bull's eye patterns is accelerating we sought to develop a tool to automatically detect and measure butterfly eyespot patterns in digital images of the wings. We used a machine learning algorithm with features based on circularity and symmetry to detect eyespots on the images. The algorithm is first trained with examples from a database of images with two different labels (eyespot and non-eyespot), and subsequently is able to provide classification for a new image. After an eyespot is detected the radius measurements of its color rings are performed by a 1D Hough Transform which corresponds to histogramming. We trained software to recognize eyespot patterns of the nymphalid butterfly *Bicyclus anynana* but eyespots of other butterfly species were also successfully detected by the software.

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1. Introduction

Work in behavioral ecology, in evolutionary and developmental biology and in quantitative and functional genetics of butterfly wing patterns has been accelerating in recent years but researchers in these fields still lack an efficient measuring tool to quantify wing pattern variation. Until now most quantifications of wing pattern variability performed in butterflies are done by mouse-clicking landmarks around each of the targeted wing pattern elements, either using an image analysis software and a photograph of each animal, or using the live animal positioned under a microscope with a camera lucida attachment and clicking on a digitizing pad. Due to the slowness of manually scoring wing patterns, in experiments where hundreds of individuals must be scored, only a few of these wing patterns are usually measured in each individual, and analysis of the complete set of patterns are seldomly done.

Most of the research on quantitative genetics of butterfly wing patterns has focused on the circular eyespot patterns that are present along the border on the wing in a variety of nymphalid butterflies including two main model species, the buckeye, *Junonia (Precis) coenia*, and the squinting bush brown, *Bicyclus anynana*. Other nymphalids, however, such as *Heliconius* species (Joron et al., 2006), and the specked wood, *Pararge aegeria* (Breuker et al., 2007), are also targets of similar large-scale quantitative approaches. Research questions regarding the eyespot patterns have ranged from (1) quantifying the effect of environmental temperature and other variables on changes in the size of the eyespots (Kooi et al., 1996; Roskam and Brakefield, 1996); (2) determining patterns of eyespot covariation (Allen, 2007; Paulsen and Nijhout, 1993; Monteiro et al., 1994, 1997; Beldade and Brakefield, 2003); (3) discovering the extent to which each of these eyespots is free to vary independently of the others by using artificial selection or mutagenesis experiments (Beldade et al., 2002; Monteiro et al., 2003); (4) discovering which genes underlie eyespot pattern variation via linkage association studies (Monteiro et al., 2007; Beldade et al., 2002); (5) estimating the effect of ectopic expression or knockdown of candidate developmental genes on eyespot morphology (Monteiro and Chen, in prep.); (6) and understanding the evolution of eyespot number (Monteiro, in press). In order to accelerate the pace of investigation on butterfly quantitative and functional genetics we developed software that automatically recognizes and measures several of the color rings in each of the eyespot patterns using digital photographs of wings. We first trained and tested the software on a set of images from dissected wings of *B. anynana* but later tested the software on images from other *Bicyclus* species to estimate its flexibility in recognizing eyespot patterns in general. This software was specifically developed to recognize circular patterns on images and can potentially be of broader applicability within or outside the biological sciences. The main goals for the software were to (1) recognize all eyespot pattern elements on an image and count the number of eyespots on each wing surface, and (2) measure the radius of the different color rings in each eyespot.

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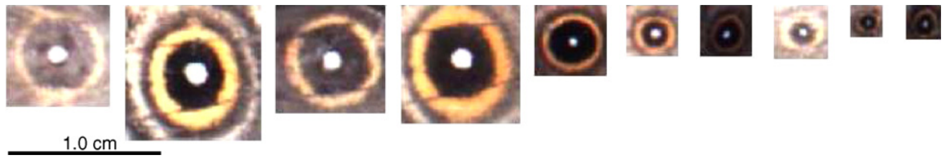

Fig. 1. Diversity of eyespots patterns in *Bicyclus anynana*. Different eyespots have different sizes, number of rings, color and contrast.

2. Materials and methods

The automatic eyespot recognition software was developed in Matlab 7.1, using the Image Processing Toolbox and the Statistical Pattern Recognition Toolbox and is made available at http://www.isr.ist.utl.pt/ msilveira/eyespot_recognition.htm.

2.1. The Butterflies

The wings of 250 *B. anynana* individuals, reared at 28 °C and 80% humidity, were separated from the body and photographed using a Nikon SMZ1500 dissecting microscope at 3.8× magnification and a digital camera (Qimaging Micropublisher RTV). The wings were lit from left and right sides with a fiber optic double gooseneck connected to a cold light source. The photos were taken at 300 dpi and are 5.74 cm × 4.33 cm in size; they were saved as Tiff files.

2.2. Automatic Recognition Software

Our approach for the automatic eyespot recognition was to train a machine learning algorithm which assigned one of two possible labels (eyespot or non-eyespot) to each image pixel based on measurements obtained in the neighborhood of that pixel. Those measurements were collected into a feature vector x of length n and the algorithm output was based on a discriminant function $g(x)$ that partitions the feature space $\mathbb{R}^n$ into two decision regions:

$$g(x) = w^T x + b \tag{1}$$

where $b \in \mathbb{R}$ and $w \in \mathbb{R}^n$ are the coefficients of the linear discriminant function which had to be learned from examples of images with both labels (eyespot and non-eyespot). These example images corresponded to smaller square areas of the original digital images, including individual eyespots (Fig. 1) or background wing patterns. The background images were randomly generated throughout the wings in order to capture the diversity of the wing texture. The function yields positive values for eyespot examples and negative values for non-eyespot. Thus, training examples from the two different classes are separated by the hyperplane $g(x) = w^T x + b = 0$.

2.2.1. Features

The eyespot patterns are approximately circular and formed by concentric rings, but they have different sizes, number of rings, brightness values and contrast (Fig. 1). In the particular case of *B. anynana*, one of the rings in the eyespots has a distinctive gold color that could be used as a feature but we refrained from using color features because we intend to use the software with other species.

The features we used exploit the fact that the eyespots are circular and symmetric relative to their center. We obtained good results with a very reduced number of features, which were carefully selected. One set of features measures circularity and is inspired by the convergence index filter (Kobatake and Hashimoto, 1999) which was designed to detect rounded regions. This filter measures the degree of convergence of gradient vectors in the neighborhood of the pixel of interest. Let p denote the center pixel of a region R and q denote an arbitrary pixel in R with relative coordinates from the center pixel $q = (k, l)$. The gradient of image $I(q)$ is denoted $g(q) = (g_x(q), g_y(q))$. From $g_x(q)$ and $g_y(q)$ the gradient magnitude and orientation can be calculated:

$$\|g(q)\| = \sqrt{g_x(q)^2 + g_y(q)^2} \tag{2}$$

$$\phi(q) = \arctan \frac{g_y(q)}{g_x(q)} \tag{3}$$

The angle $\theta(q)$ measures the orientation of the gradient vector $g(q)$ with respect to the line $\overline{pq}$ and the degree of convergence of $g(q)$ is given by $\cos\theta$ (Fig. 2a). The convergence index output is the average of the convergence indices at all pixels in R:

$$c(p) = \frac{1}{M} \sum_{q \in R} \cos\theta(q) \tag{4}$$

where M is the number of pixels in region R. We adapted the convergence index filter because in our case, the eyespots have both dark and light rings, so some gradient vectors will point towards the pixel of interest and others will point away from it. In the first case the values of $c(q)$ will be positive and in the second case they will be negative. Therefore, we divided the pixels in region R into two sets based on their angle $\theta(q)$:

$$R^+ = \{q \in R | \cos\theta(q) \geq 0\} \tag{5}$$

$$R^- = \{q \in R | \cos\theta(q) < 0\} \tag{6}$$

We calculated $\cos\theta(q)$ efficiently by using the following normalized dot product:

$$\cos\theta(q) = \frac{g(q).v(q)}{\|g(q)\|\|v(q)\|} \tag{7}$$

where v is the vector $q - p$.

The filter output is multiplied by the gradient magnitude to give more weight to the more contrasted points. This is done because gradient elements with small magnitude have less reliable orientation. In addition, the output is scaled by the total gradient magnitude in order to obtain a measure adapted to local contrast:

$$c^+(p) = \frac{\sum_{q \in R^+} \|g(q)\| \cos\theta(q)}{\sum_{q \in R^+} \|g(q)\|} \tag{8}$$

$$c^-(p) = \frac{\sum_{q \in R^-} \|g(q)\| \cos\theta(q)}{\sum_{q \in R^-} \|g(q)\|} \tag{9}$$

Another feature measures radial gradient and was used in Daugman (2004) to localize and recognize a human iris. It is an integrodifferential operator that calculates at center coordinates, p, in the image domain, the blurred partial derivative with respect to increasing radius, r, of the normalized contour integral of I along a circular arc ds of radius r:

$$rg(r, p) = G_\sigma * \frac{\partial}{\partial r} \int_{r,p} \frac{I(q)}{2\pi r} \mathrm{d}s \tag{10}$$

The symbol $*$ denotes convolution and G_σ is a smoothing function such as a Gaussian of scale σ. As our feature we use the average of the radial gradient $rg(r, p)$ computed for all values of the radii r.

Two additional features exploit the pattern's gradient symmetry relative to the center point. Using symmetry as a feature is important to avoid false detections from the curved chevron patterns present along the border of the wing. Moreover, this symmetry feature is useful to detect eyespots with elliptic shapes. We used one of the symmetry features proposed in Loy and Zelinsky (2003) but calculated dark and bright symmetry separately.

We calculate at each radius r two projection images O_r+ and O_r-, that will collect evidence of dark and bright symmetry, respectively. To create these images, for each point p we calculate the pixel p_+ that the gradient vector $g(p)$ is pointing

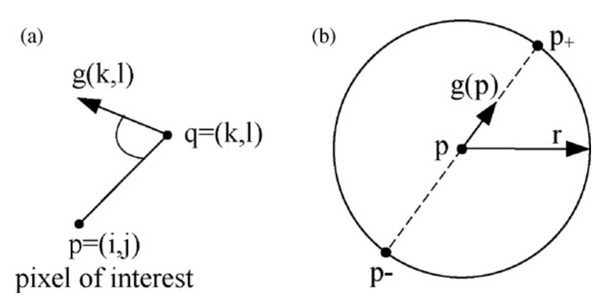

Fig. 2. Gradients and angles used in the circularity and symmetry features. (a) circularity; (b) symmetry.

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