Modelling butterfly wing eyespot patterns

Rui Dilão^{1,2*} and Joaquim Sainhas^{1,3}

¹*Grupo de Dinâmica Não-Linear, Instituto Superior Técnico, Departamento de Física, Avenida Rovisco Pais, 1049-001 Lisbon, Portugal*

²*Institut des Hautes Études Scientifiques, Le Bois-Marie, 35, route de Chartres, F-91440 Bures-sur-Yvette, France*

³*Faculdade de Motricidade Humana, Estrada da Costa, 1495-688 Cruz Quebrada, Lisbon, Portugal*

Eyespots are concentric motifs with contrasting colours on butterfly wings. Eyespots have intra- and inter-specific visual signalling functions with adaptive and selective roles. We propose a reaction–diffusion model that accounts for eyespot development. The model considers two diffusive morphogens and three non-diffusive pigment precursors. The first morphogen is produced in the focus and determines the differentiation of the first eyespot ring. A second morphogen is then produced, modifying the chromatic properties of the wing background pigment precursor, inducing the differentiation of a second ring. The model simulates the general structural organization of eyespots, their phenotypic plasticity and seasonal variability, and predicts effects from microsurgical manipulations on pupal wings as reported in the literature.

Keywords: butterfly; eyespot; pattern formation; morphogenesis; reaction–diffusion systems

The most remarkable phenotypic features of butterflies are their wing patterns, which have adaptive functions ranging from passive camouflage and mimicry to competitive self-advertising (Nijhout 1991). They display great plasticity and adaptability to seasonal and environmental changes (Smith 1978; Koch 1992). Butterfly wing eyespots, or *ocelli*, are concentric rings with intense and contrasting coloration. They mimic the global appearance of vertebrate eyes and have active signalling functions against predators, as well as sexual signalling roles for mate competition (Nijhout 1981, 1996; Vane-Wright & Boppré 1993). Selection studies have shown that eyespot patterns reveal high heritability (Brakefield & van Noordwijk 1985; Monteiro *et al.* 1997; Brakefield & French 1999). The biology of butterfly wing patterns involves different levels of description, ranging from molecular mechanisms of development to selection and pattern evolution. In fact, the link between developmental patterns and evolution processes is an important biological problem (Nijhout 1991; Brakefield & French 1999).

Insect wings have two independent global morphogenic fields, corresponding to the epithelial monolayers of the wing surfaces. As lepidopteran patterns are different in both wing surfaces, morphogenic signals responsible for pattern formation propagate within each monolayer through gap junctions, facilitating diffusive intercellular communication (Unwin & Zampighi 1980; Nijhout 1990). Global wing patterns result from the combination of a few elementary motifs. On a local scale, the areas delimited by wing veins correspond to independent morphogenic fields, where elementary patterns such as ocelli emerge. Wing patterns have a discrete structure formed by overlapping scales distributed along parallel rows. The scales are flattened protrusions of specialized epithelial cells that differentiate among homogeneous pigmentary colours or light-diffracting microstructures (Nijhout 1991; Vukusic *et al.* 1999).

Some aspects of the genetic mechanisms of eyespot ontogeny have been clarified (Brakefield & French 1999; Beldade & Brakefield 2002). Eyespot development begins at the larval stage, and is initiated by the expression of wing-patterning genes at predetermined points (foci) of the developing wings (Carroll *et al.* 1994; Brakefield *et al.* 1996; Beldade & Brakefield 2002). These foci create positional information that regulates the developmental pathway of eyespots, defining a cellular territory that corresponds to the emergent eyespot (French & Brakefield 1992, 1995; Nijhout 1994, 1996; Brakefield & French 1995).

In experiments with *Bicyclus anynana*, the inducing action of focus cells in eyespot formation is corroborated by surgical experiments that involve the grafting and destruction of focus cells in early pupal stages. In the first case, eyespot development occurs in the new non-programmed areas. In the second case, an inhibition of normal eyespot development is observed (Nijhout 1994; French & Brakefield 1995). Both situations show the role of the eyespot focus as a source of a diffusing morphogenic signal that triggers the developmental pathway of eyespots (French & Brakefield 1992, 1995; Brakefield & French 1995; Brunetti *et al.* 2001).

We propose the action of a minimum of two diffusive signalling substances, or morphogens (Turing 1952), as a model for eyespot development. The first morphogen M_1 is produced in a specific region of the wing by an initial precursor A . The spatial distribution of the initial precursor $A = A(x, y, t = 0)$ at time zero defines the localization of foci. The differentiation of the first ring (dark region) and the production of the second morphogen M_2 are induced by M_1 reacting with a wing background pigment precursor P_0 and modifying its chromatic properties. The second morphogen M_2 reacts with the wing background pigment precursor P_0 , generating a second ring or light aureole. The final pigmentation is always defined by the highest local concentration of the pigment precursors.

* Author for correspondence (rui@sd.ist.utl.pt).

Figure 1. Schematic diagram showing the several kinetic mechanisms considered in our model for the formation of butterfly wing eyespot patterns. Morphogens or diffusive substances are represented by M_1 and M_2 ; P_0 , P_1 and P_2 are pigment precursors; k_1 to k_5 are the rate constants for each elementary process. Applying the mass-action law to the elementary processes and adding diffusion to signalling substances or morphogens, we obtain equation (1.1), which describes the space and time evolution of eyespot development. In this mechanism, pigment precursors may be considered as belonging to the same chemical category.

Figure 2. Solution of the reaction–diffusion system of equation (1.1) in a square of 101×101 cells for (a) time $t = 15$ and (b) the steady state ($t \rightarrow \infty$). The graphs represent the concentration profiles of the model variables taken along a cross-section passing through the focus. All variables are represented in dimensionless form. The squares at the top left indicate the concentration levels of pigment precursors. The highest P_0 concentration areas are represented in brown. Black and yellow represent the highest concentrations of P_1 and P_2 , respectively. The white spots represent focal points. Patterns emerge as the steady-state solutions of equation (1.1) for the pigment precursors. At the steady state, the morphogen concentrations are zero. Simulations were carried out with zero-flux boundary conditions and the following initial conditions: $P_1(x, y, 0) = P_2(x, y, 0) = 0$ and $M_1(x, y, 0) = M_2(x, y, 0) = 0$. The focus region corresponds to a square of 5×5 cells and is characterized by the initial conditions $A(x, y, 0) = 20$ and $P_0(x, y, 0) = 0$. Outside the focal region, $A(x, y, 0) = 0$ and $P_0(x, y, 0) = 0.2$. We selected the following parameters: $k_1 = 1.0$, $k_2 = 0.05$, $k_3 = 4.0$, $k_4 = 0.01$, $k_5 = 4.0$, $D_1 = D_2 = 1.0$, $\Delta t = 0.1$ and $\Delta x^2 = 6D_1\Delta t$.

Figure 3. (a–c) The evolution of eyespot patterns over time as a function of the focus area for the same parameter values as in figure 2. In (a) the focus region corresponds to one cell; in (b) to a square of 3×3 cells, and in (c) to a square of 5×5 cells. According to experimental observations, the larger the focus area the larger the eyespot. (d,e) Demonstrate that the variability and typical interaction patterns of eyespots are those predicted by the model equation (1.1) for point (d) and arc (e) foci. The patterns in (d,e) are steady-state solutions of equation (1.1).

This model can be implemented with the following kinetic pathways. (i) Focal cells release a primary diffusive morphogen M_1 . The simplest conceivable kinetic mechanism is the following:

where k_1 and k_2 are reaction rates, A is the morphogen precursor, and where the second reaction represents morphogen degradation. (ii) The morphogen M_1 reacts with the background pigment precursor P_0 in the surrounding wing area, producing a new pigment precursor P_1 and a secondary morphogen M_2 :

The pigment precursor P_0 is responsible for wing background pigmentation and P_1 for pigmentation of the first ring. (iii) The diffusive morphogen M_2 produces a chemical modification in the pigmentation of the wing background:

where P_2 is the pigment precursor of the second ring. In figure 1, we schematically show this developmental mechanism.

At the beginning of the developmental process, the primary pigment determinant P_0 is spatially distributed along the wing surfaces, except at focal cells. Distribution of the foci is described by the spatial distribution of the morphogen precursor A : focal areas are characterized by positive values of A , whereas $A=0$ outside focal areas. All other pigment determinants P_1 and P_2 are absent at the beginning of the developmental process.

Applying mass-action-law formalism to the above elementary reactions, we obtain the following system of partial differential equations:

$$\left. \begin{aligned} \partial A / \partial t &= -k_1 A, \\ \partial M_1 / \partial t &= k_1 A - k_2 M_1 - k_3 M_1 P_0 + D_1 \nabla^2 M_1, \\ \partial M_2 / \partial t &= k_3 M_1 P_0 - k_4 M_2 - k_5 M_2 P_0 + D_2 \nabla^2 M_2, \\ \partial P_0 / \partial t &= -k_3 M_1 P_0 - k_5 M_2 P_0, \\ \partial P_1 / \partial t &= k_3 M_1 P_0, \\ \partial P_2 / \partial t &= k_5 M_2 P_0, \end{aligned} \right\} \quad (1.1)$$

where D_1 and D_2 are the diffusion coefficients of morphogens M_1 and M_2 on the two-dimensional wing region, k_1 to k_5 are the rate constants of the elementary kinetic processes and $\nabla^2 = (\partial^2 / \partial x^2 + \partial^2 / \partial y^2)$ is the two-dimensional Laplace operator. We consider that the pigment precursors P_i do not diffuse and that local wing colour is determined by the pigment precursor with the highest concentration.

It follows from system (1.1) that pigment precursors obey a mass conservation law of the form $P_0(x, y, t) + P_1(x, y, t) + P_2(x, y, t) = \text{constant}$, and that the spatial dependence of the pigment precursors is induced by diffusion of the morphogens. In the steady state ($t \rightarrow \infty$), the concentrations of morphogens M_1 and M_2 vanish and stable patterns emerge, obeying the conservation law $P_0(x, y, t=0) = P_0(x, y, t=\infty) + P_1(x, y, t=\infty) + P_2(x, y, t=\infty)$. The spatial distribution of the pigment precursors may be considered a pre-pattern that determines subsequent patterning events. The model presumes a competitive selection of exclusive pigment synthesis pathways within scale cells, which agrees with the experimental results (Koch 1991; Koch & Kaufmann 1995).

To test this model, we numerically integrate equation (1.1) with an explicit finite-difference method, minimizing the global numerical error and avoiding spurious symmetries induced by the two-dimensional integration lattice (Dilão & Sainhas 1998). The simulations were performed with zero-flux boundary conditions in a two-dimensional spatial region of 101×101 cells. The simulations start with $A > 0$ and $P_0 = 0$ at focal points. We set the uniform concentration of the primary pigment precursor away from focal points to be $P_0 > 0$, and $A = 0$. At time zero, all other variables in the model are null over the entire spatial range. If we let $P_0 > 0$ at focal points, the patterns emerging from the steady-state solutions of equation (1.1) remain unchanged, but focal points are not differentiated. In fact, in different butterfly species, focal cells of ocelli can be white or unspecified. In figure 2, we depict the evolution of morphogens and pigment precursors according to the model equation (1.1).

During the growth of the first ring (dark region), the morphogen concentration M_2 increases, promoting the formation of the second ring (figure 2). The grafting and destruction of focal cells are modelled by changing the initial concentration of the morphogen precursor A . In the

event of the destruction of focal cells, $A = 0$, wing colour is determined by the wing background pigment precursor, $P_0(x, y, t=0) = P_0(x, y, t=\infty)$, and no eyespots appear. Grafting of focal cells is modelled by the local introduction of A . The light ring increases with the area of the focal region, as observed in the wing patterns of different butterfly species (figure 3a–c). In this model, it is the initial distribution of the morphogen precursor A that determines the phenotypic differentiation of butterfly wings, one of the main features associated with natural selection.

Eyespot variability may be understood within this framework. For example, by changing the position and width of a focus, it is possible to simulate different structures for eyespot patterns, as in figure 3d,e. Seasonal polyphenism and phenotypic plasticity are simulated by changing the rate constants of the model equations. In nature, this adaptability depends on environmental factors, such as temperature, relative humidity and photoperiod (Smith 1978; Nijhout 1991; Koch 1992), changing the reaction rates of kinetic mechanisms. Generalizing the cascading model presented here, additional concentric rings may be modelled by the inclusion of new morphogens and pigment precursors.

One of the novelties of this model is based on the assumption that the reaction–diffusion mechanism associated with the morphogens is the process that triggers the synthesis of the non-diffusive pigment precursor responsible for patterning. In the steady state, the concentration of morphogens is zero. This contrasts with the classical Turing reaction–diffusion approach, where patterning is due to spatial distribution of the morphogens at the steady state (Turing 1952; Murray 1989; Nijhout 1991).

The classical model of pattern formation in butterflies is based on the assumption of simple diffusion and threshold mechanisms of pigments (Murray 1989; Nijhout 1990; Monteiro *et al.* 2001). According to Nijhout (2001, p. 221), ‘[p]igment bands are formed at several threshold levels of a symmetrical gradient of pigment determination’ and ‘each threshold becomes the origin of a new symmetrical gradient of pigment determination’. In the model presented here, pigments are non-diffusive substances, morphogens are diffusive and reacting substances (Turing 1952), and we have no threshold assumptions. By contrast, one of the undetermined issues of the classical model (Monteiro *et al.* 2001) is whether the focal signal is a constant-level or constant-rate source of morphogen. In the model presented here, the focal signal is determined by a localized morphogen precursor (A) that decays during the developmental process.

In conclusion, we have shown that the development of butterfly wing patterns may be explained by a reaction–diffusion mechanism with two diffusive morphogens, triggering different pathways from a common pigment precursor P_0 , in a cascading process. At the beginning of the developmental process, the pigment precursor P_0 is equally distributed along pupal wings, and we have considered that its dynamics is non-diffusive. This is in agreement with the chemistry of the major pigments in butterflies (Nijhout 1991). Changing the kinetic parameters of the model and the initial distribution of the morphogen precursor A results in an abundant diversity of patterns, as observed in lepidopteran wings. This model

consistently predicts the major features of the development of butterfly eyespot patterns, their structural organization, phenotypic plasticity, seasonal variability and the effects of microsurgical manipulations.

The authors thank Noah Hardy for carefully reading and editing the text. This work has been partly supported by Fundação para a Ciência e a Tecnologia (Portugal), under the framework of the POCTI Project /FIS/13161/1998 and by the Institut des Hautes Études Scientifiques (Bures-sur-Yvette, France).

REFERENCES

- Beldade, P. & Brakefield, P. M. 2002 The genetics and evolution of butterfly wing patterns. *Nature Rev. Genet.* **3**, 442–452. (DOI 10.1038/nrg818.)
- Brakefield, P. M. & French, V. 1995 Eyespot development on butterfly wings: the epidermal response to damage. *Dev. Biol.* **168**, 98–111.
- Brakefield, P. M. & French, V. 1999 Butterfly wings: the evolution of development of colour patterns. *BioEssays* **21**, 391–401.
- Brakefield, P. M. & van Noordwijk, J. 1985 The genetic of spot pattern characters in the meadow brown butterfly *Maniola jurtina* (Lepidoptera: Satyridae). *Heredity* **54**, 275–284.
- Brakefield, P. M., Gates, J., Keys, D., Kesbeke, F., Wijngaarden, P. J., Monteiro, A., French, V. & Carroll, S. 1996 Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**, 236–242.
- Brunetti, C. R., Selegue, J. E., Monteiro, A., French, V., Brakefield, P. M. & Carroll, S. B. 2001 The generation and diversification of butterfly eyespot color patterns. *Curr. Biol.* **11**, 1578–1585.
- Carroll, S. B., Gates, J., Keys, D. N., Paddock, S. W., Panganiban, G. E. F., Selegue, J. E. & Williams, J. A. 1994 Pattern formation and eyespot determination in butterfly wings. *Science* **265**, 109–114.
- Dilão, R. & Sainhas, J. 1998 Validation and calibration of models for reaction–diffusion systems. *Int. J. Bifurc. Chaos* **8**, 1163–1182.
- French, V. & Brakefield, P. M. 1992 The development of eyespot patterns on butterfly wings: morphogen sources or sinks? *Development* **116**, 103–109.
- French, V. & Brakefield, P. M. 1995 Eyespot development on butterfly wings: the focal signal. *Dev. Biol.* **168**, 112–123.
- Koch, P. B. 1991 Precursors of pattern specific ommatin in red wing scales of the polyphonic butterfly *Araschnia levana* L.: haemolymph and 3-hydroxykynurenine. *Insect Biochem.* **21**, 785–794.
- Koch, P. B. 1992 Seasonal polyphenism in butterflies: a hormonally controlled phenomenon of pattern formation. *Zool. J. Physiol.* **96**, 227–240.
- Koch, P. B. & Kaufmann, N. 1995 Pattern specific melanin synthesis and dopa decarboxylase activity in a butterfly wing of *Precis coenia* Hubner. *Insect Biochem. Mol. Biol.* **25**, 73–82.
- Monteiro, A., Brakefield, P. M. & French, V. 1997 The relationship between eyespot shape and wing shape in the butterfly *Bicyclus anynana*: a genetic and morphometrical approach. *J. Evol. Biol.* **10**, 787–802.
- Monteiro, A., French, V., Smit, G., Brakefield, P. M. & Metz, J. A. J. 2001 Butterfly eyespot patterns: evidence for specification by a morphogen diffusion gradient. *Acta Biotheor.* **49**, 77–88.
- Murray, J. D. 1989 *Mathematical biology*. New York: Springer.
- Nijhout, H. F. 1981 The color patterns of butterflies and moths. *Sci. Am.* **245**, 145–151.
- Nijhout, H. F. 1990 A comprehensive model for color pattern formation in butterflies. *Proc. R. Soc. Lond. B* **239**, 81–113.
- Nijhout, H. F. 1991 *The development and evolution of butterfly wing patterns*. Washington, DC: Smithsonian Institution Press.
- Nijhout, H. F. 1994 Genes on the wing. *Science* **265**, 44–45.
- Nijhout, H. F. 1996 Focus on butterfly eyespot development. *Nature* **384**, 209–210.
- Nijhout, H. F. 2001 Elements of butterfly wing patterns. *J. Exp. Zool.* **291**, 213–225.
- Smith, A. G. 1978 Environmental factors influencing pupal colour determination in Lepidoptera. I. Experiments with *Papilio polytes*, *Papilio demoleus* and *Papilio polyxenes*. *Proc. R. Soc. Lond. B* **200**, 295–329.
- Turing, A. M. 1952 The chemical basis of morphogenesis. *Phil. Trans. R. Soc. Lond. B* **237**, 5–72.
- Unwin, P. N. T. & Zampighi, G. 1980 Structure of the junction between communicating cells. *Nature* **283**, 545–549.
- Vane-Wright, R. I. & Boppré, M. 1993 Visual and chemical signalling in butterflies. *Phil. Trans. R. Soc. Lond. B* **340**, 197–205.
- Vukusic, P., Sambles, J. R., Lawrence, C. R. & Wootton, R. J. 1999 Quantified interferences and diffraction in single morpho butterfly scales. *Proc. R. Soc. Lond. B* **266**, 1403–1411. (DOI 10.1098/rspb.1999.0794.)