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*Chapter 6*

## **PATTERN FORMATION AND DIVERSITY IN BUTTERFLY WINGS: EXPERIMENTS AND MODELS**

*Toshio Sekimura*

Department of Biological Chemistry, Graduate School of Bioscience  
and Biotechnology, Chubu University, Kasugai, Aichi, Japan

### **ABSTRACT**

Butterfly wings are covered with thousands of monochromatic scale cells, which form regularly arranged patterns, that is, parallel rows of scale cells in the proximal-distal direction of the wing. Color pattern in butterfly wings is a kind of mosaic pattern of dozens to hundreds of finely-tiled monochromatic scale cells. The wing consists of two cell layers, a surface layer and a back layer. The two layers are completely separated by the middle tissues, and developmental phenomena in each layer occur independently of the other. Color pattern formation occurs in a single layer of the wing disc and it is essentially two-dimensional in space without cell movement. Therefore, the problem of color pattern formation could be solved by analyzing the underlying mechanism of how colored scales are settled at specific positions in a two-dimensional wing surface.

In this article, I review the progress of experiment-based mathematical modeling for color pattern formation and diversification in butterfly wings. First, I describe fundamental features of the wing color pattern, and then go into discussions on guiding principles or governing rules to derive from the so-called ground plan to diverse wing color patterns. In order to make the problem clear, I take up some specific butterfly species as model systems, of which color pattern and its diversity have been well investigated with both experiment and mathematical analysis. Finally, I would like to stress the importance of integration of different aspects such as gene and environment for understanding of the diversity in wing color patterns.

## 1. INTRODUCTION

Butterfly wing color patterns are one of the most colorful and spectacular examples of pattern in biology. The color patterns have evolved as mechanisms for visual communication such as camouflage, sexual signaling, warning coloration, and mimicry. Biologists have been interested in the developmental origin and evolutionary diversification of these patterns for more than a century. Comparative morphological studies revealed that a great unity underlies the diverse patterns of lepidopteran wings. Most butterflies have color patterns composed of discrete pattern elements whose homology can be traced across genera and family. The system of homologies among pattern elements is now called the nymphalid ground plan (Schwanwitsch, 1924; Süffert, 1927; Nijhout, 1991, see also section 8.1). Experimental work revealed that some of the pattern elements develop around discrete organizing centers, from which some signals affecting the spatial pattern of pigment synthesis are sent out in their vicinity. Recent experimental works on the developmental genetics of eyespot patterns have found out several genes related to the development of the eyespot organizing centers (e.g., Brunetti et al., 2001).

In parallel to the experimental work, several investigators have attempted to build experiment-based mathematical models for color pattern formation in butterfly wings (e.g., Murray, 1981; Bard & French, 1984; Sekimura et al., 2000 for global color patterns, and Nijhout, 1990 for eyespot pattern). In following sections, I review the progress of both experimental and mathematical researches on butterfly wing pattern and diversity.

## 2. DEVELOPMENT AND CHARACTERISTICS OF BUTTERFLY WING

Lepidopteran wings are composed of two monolayers of epithelial cells, which are separated by an extracellular space. Immediately after pupation, the epithelial cells of the wing are not differentiated and they are morphologically homogeneous. About one to three days after pupation (the timing depends on the insect and the temperature at which it develops), two cell types can be readily distinguished. The smaller cells are generalized epithelial cells (GECs) of the wing, and the larger cells are scale precursor cells (SPCs) that differentiate from GECs at the inception of adult development. SPCs are arranged in space such that they are separated from each other surrounded by GECs. Within a few hours of differentiation of the isotropically arranged SPCs, these cells become polarized along the proximodistal axis of the wing and begin to align into rows parallel to the anteroposterior axis of the wing. This row formation continues until a stable spatial periodicity of rows is established. These parallel rows of SPCs that are established at the beginning of adult development maintain their arrangement throughout adult development and represent the same rows of scales that appear on the surface of the adult wing (Nijhout, 1980; Nardi & Magee-Adams, 1986; Yoshida & Aoki, 1989). As a result, we could see thousands of scale cells in a highly ordered and intricate pattern on each surface of the adult wing.

### 3. TWO TYPES OF PATTERNS – COLOR PATTERN AND CELLULAR PATTERN

As noted in the previous section, thousands of monochromatic scale cells cover the wing in a highly ordered cellular pattern, that is, parallel rows of scale cells in the proximal-distal direction of the wing. The parallel rows can be easily seen through a simple magnifying glass. There exist two different kinds of patterns, color pattern and cellular pattern of scale cells (Figure 1). The colors on butterfly wings are due to the colors of regularly-arranged monochromatic scale cells. The overall color pattern is constructed as a fine-tiled mosaic of overlapping, monochromatic scales, and it is characteristic of each lepidopteran species. The arrangement pattern of scale cells, that is, cellular arrangement pattern occurs in the early stages of pupation, while pigment syntheses related to color patterns occur in the last stage of pupation after completion of cell rearrangement. It is known that the formation of the color pattern is independent of the arrangement pattern formation of scale cells. The timescales on which these two kinds of patterns are generated are different from each other (Nijhout, 1991). Color patterns have attracted the interest of taxonomists and developmental biologists and have also provided some of the most dramatic examples of morphological diversity. The color of scale cells is mainly due to chemical pigments, or it is sometimes due to the structural color that comes from diffraction of light in the physically fine-structured scale. On the other hand, cellular arrangement patterns of scale cells do not show species-specific patterns, but they are, in general, common to all lepidopteran wings. The arrangement of scale cells has a number of remarkable characteristics. Firstly, SPCs in the developing adult wings rearrange to form nearly parallel rows along the anteroposterior axis of the wing that are maintained throughout and after adult development. Secondly, these rows are arranged at regular spatial intervals along the proximodistal axis. Thirdly, these rows are continuous across the veins in the wing, and they are also continuous around the upper and lower monolayers of the wing.

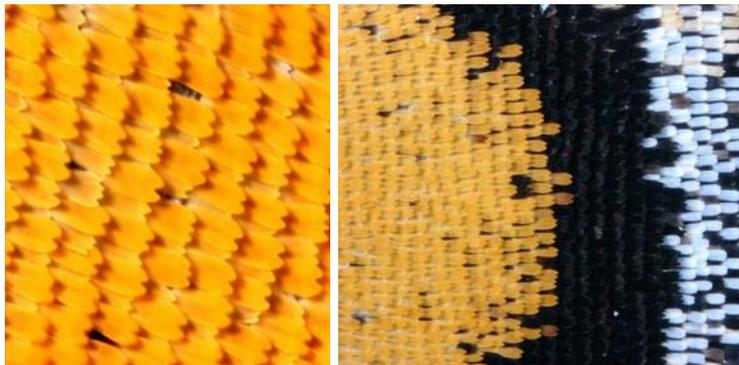


Figure 1. Cellular pattern and color pattern. (Left) Cellular pattern: parallel rows of monochromatic scale cells in the proximal-distal direction of the wing. (Right) Color pattern: mosaic pattern of dozens to hundreds of finely-tiled monochromatic scale cells, which is characteristic of each lepidopteran species.

I first describe cellular pattern formation of scale cells as the basis of all patterns in butterfly wings and then go to color pattern formation with mathematical models.

## 4. CELLULAR PATTERN FORMATION – EXPERIMENTS AND MODEL

I first summarize experimental evidences (i) ~ (v) to construct a mathematical model for cellular pattern formation in the lepidopteran wing, and then present a mathematical model and computer simulation results (Sekimura et al., 1998,1999).

### 4.1. Experimental Evidences

- i. Cell rearrangement to form parallel rows occurs in a monolayer  
 There are two monolayers (the upper and lower ones) of epithelial cells in the lepidopteran wing. During rearrangement of epithelial cells in the wing, these two monolayers are separated by an extracellular space. Cell rearrangement in the developing adult wing has been assumed to occur within each monolayer without influence from the other monolayer (Nardi, 1992, 1994). These findings suggest that the process of scale row formation in the lepidopteran wing does not involve complex three-dimensional interactions among epithelial cells, but instead involves simpler two-dimensional interactions.
- ii. Lateral inhibition probably forms the uniform pattern of SPCs  
 Before epithelial cells rearrange their spatial positions in the developing adult wing, SPCs differentiate from GECs. SPCs are distributed in an uniform hexagonal pattern, in which they do not come in contact with each other. The initial isotropic spacing of SPCs is strongly suggestive of patterns generated by a process referred to as lateral inhibition. The inhibitory signal emanates from the scale precursor cell.
- iii. Short-range interaction mediated by differential cell adhesion  
 A few kinds of surface proteins have been found in the developing adult wing monolayer during rearrangement of epithelial cells. The expression of these proteins changes dramatically in space and time during morphogenesis of the wing pattern. SPCs show a different pattern of protein expression from GECs during cell rearrangement. These proteins are assumed to be cell adhesion molecules (CAMs), which mediate differential cell adhesion between different cell types (short-range interaction) to drive cell movement.
- iv. Origin-dependent cell adhesion  
 Grafting experiments within the pupal wing monolayer have been done to test differences in affinity properties of epithelial cells along the proximodistal axis of the wing. Those experiments have revealed that the greater the distance separating host and graft cell populations along the proximodistal axis, the more circular and constricted the interface between graft and host cells. This result shows that affinity properties of cells depend on their positions, or more precisely, the distance between their original positions along the proximodistal axis of the wing.
- v. Long-range interaction mediated by basal processes  
 During rearrangement of epithelial cells, both SPCs and GECs extend basal processes. As the alignment of SPCs into rows proceeds, extension of processes from the basal surfaces of the epithelial cells simultaneously occurs. These processes can extend for distances of several cell diameters and can establish contacts not only with adjacent cells but with cells that are four or five cell diameters away. This long-range

interaction is mediated by basal filopodia which extend over several cell diameters in many directions (Nardi & Magee-Adams, 1986).

## 4.2. Mathematical Model for Cellular Arrangement Pattern of SPCs

Based on observation on cellular pattern formation, we begin by presenting a general model for aggregation in which cells move up gradients of adhesivity. Since cells can respond directly to non-adjacent neighbors, we use integrals to represent the local average adhesivity to which a cell responds. In this model we focus on only one cell type (SPCs) and assume that cells interact with each other according to the distance between their original locations (as well as the distance between their current locations).

Let  $n(\vec{x}, a, t)$  denote the cell density at position  $\vec{x}=(x, y)$  at time  $t$  for the cells of a given adhesivity  $a$  that originate from a position that is a distance  $a$  away from the body axis (the basal part of the wing). We assume that cell movement is due to two processes; diffusion, and advection (directed movement) in response to gradients of adhesivity. Due to the evidence for long-range interactions, we consider a cell to respond to gradients in a spatially averaged adhesivity. As an evolution equation for cell density in space, we write

$$n_t = D \nabla^2 n - \nabla \cdot (n \vec{c}) \quad (1)$$

where  $D$  is the diffusion coefficient. The advection velocity,  $\vec{c}$ , is given by

$$\vec{c} = C \nabla \left[ \iiint n(\vec{x} - \vec{y}, a - s) w(\vec{y}, s) ds dy_1 dy_2 \right]$$

where  $\vec{x}=(x, y)$  and  $\vec{y}=(y_1, y_2)$  are position variables, and  $C$  is a positive constant. The integral represents the spatially averaged adhesivity. The degree of adhesivity as a function of distance,  $\vec{y}$ , and adhesivity distance (distance in adhesivity space),  $s$ , are incorporated in the kernel  $w(\vec{y}, s)$ . For simplicity, we assume that this is separable (i.e., that the effects of distance in physical and adhesivity space are independent of each other). We therefore write

$$w(\vec{y}, s) = g(\vec{y}) h(s)$$

We assume that  $g$  displays rotational symmetry in the two spatial dimensions, and  $h$  is symmetric in the adhesivity difference. Notice the threshold in adhesivity marking a transition from attraction to repulsion. Also, the spatial kernel is such that very short range attraction is weaker than middle distance attraction. This reflects the fact that scale cells appear not to come into contact with one another during reorganization.

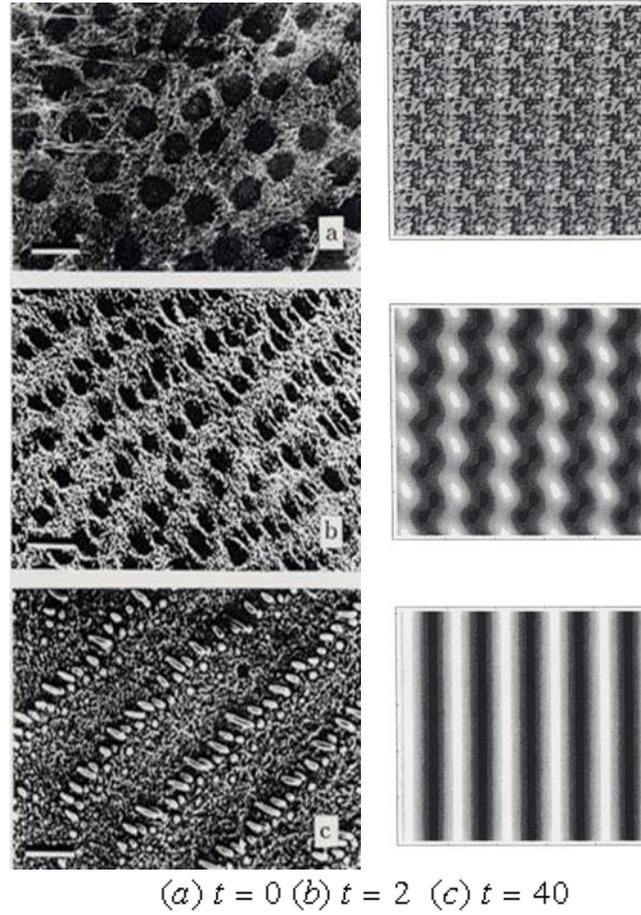


Figure 2. Experiments and numerical simulations. (Left) Parallel row formation of SPCs on the moth *Manduca* wing disc (Nardi & Magee-Adams, 1986). Bar in the figure =  $50 \mu\text{m}$ . (Right) Numerical simulation results on the row formation by the mathematical model Eq. (2) (Sekimura et al., 1998). The lighter color represents high cell density of SPCs, while the darker color represents low cell density. In numerical simulations, a random cell distribution is assumed as the initial condition, and periodic boundary conditions have been imposed on the boundary.

Below, we assume that  $|\bar{y}|$  and  $|s|$  are both much smaller than one. Biologically, this corresponds to the assumption that the adhesive interaction takes place over short distances in comparison to the size of the domain and that changes in the magnitude of adhesivity are very small. Using the assumption that  $|\bar{y}| \ll 1, |s| \ll 1$ , we can simplify the integral representation of the advection velocity,  $\bar{c}$ , by Taylor expanding, and finally we could have a following partial differential equation:

$$n_t = D\nabla^2 n - C\nabla \cdot [n\nabla(n + \gamma\nabla^2 n + \beta n_{aa})] + O(s^4 + |\bar{y}|^4)$$

where,

$$\gamma = \frac{1}{2} \int |\bar{y}|^2 g(\bar{y}) dy_1 dy_2, \beta = \frac{1}{2} \int s^2 h(s) ds$$

The above equation includes parameters  $\gamma$ ,  $\beta$ , which represent two different kinds of adhesivity effects, physical distance effect and adhesive distance effect, respectively. We further assume that  $n_{aa} = E n_{xx}$  ( $E$ : constant) and we neglect the last term. The equation for cellular pattern (parallel row) formation of SPCs with origin-dependent adhesivity is,

$$n_t = D \nabla^2 n - C \nabla \cdot [n \nabla (n + \gamma \nabla^2 n + \beta^* n_{xx})] \quad (2)$$

where,  $\beta^* = E \beta (\leq 0)$ . Here we assume an inequality  $\gamma + \beta^* \geq 0$ , showing that due to the effect of origin-dependent adhesivity, SPCs receive a kind of reduction in diffusive movement in the  $x$ -direction (the proximal-distal direction of the wing). According to our non-linear analysis of the equation, we have found following three main results. The first result is that due to the effect of origin-dependent adhesivity, the region in the parameter space of  $D, C, \gamma$  to generate stable parallel rows of SPCs is largely extended. The second result is more important, that is, due to the effect of origin-dependent adhesivity, the direction of parallel rows is correctly predicted (Figure 2). This means that due to the effect, scale rows parallel to the body axis seen in real butterflies could be correctly reproduced. The third result is that if the effect is sufficiently strong, only parallel rows of SPCs could be generated without spotted patterns.

## 5. COLOR PATTERN FORMATION

### 5.1. General Features of Color Pattern Formation – Color Pattern and Pre-Pattern

Butterfly color patterns are two-dimensional patterns of pigment synthesis. A fraction of the epidermal cells on each wing surface differentiate into scale cells. These cells send out a large flat appendage, the scale, into which pigments are secreted. With few exceptions, each scale cell synthesizes a single type of pigment. The overall color pattern is thus constructed as a fine-tiled mosaic of colored scales. One of the problems of pattern formation is how a particular scale cell is induced to synthesize the right pigment for its particular location on the wing.

Recently, antibody fluorescent techniques revealed that the pattern formation has started at least in the late larval stage of development. The butterfly wing originates from the wing disc in the larva, which appears first in the larval stage and continues to develop during the pupal stage. The wing disc is transparent and colorless throughout the larval and early pupal stages of development. However, antibody fluorescent techniques for the corresponding genes have revealed existence of the so-called “pre-pattern” on the wing disc, which overlaps

completely with the color pattern of the adult butterfly wing (e.g., Brunetti et al., 2001). It is generally known that the pre-pattern on the wing disc is the original feature of the color pattern. Pattern determination in butterflies has been considered to occur in two stages. The first stage consists of the specification of organizing or signaling centers on the wing surface. In species where this has been studied, this process occurs during the early to mid portions of the larval stadium in the still growing wing imaginal disc (Nijhout, 1991; Carroll et al., 1994). The second stage consist of the definition of the boundaries of the future patterned synthesis of each of the pigments that will make up the color pattern, which are established by signals generated by the organizing centers. This second stage results in an invisible spatial pre-pattern of cell commitment to a particular pigment synthetic pathway. Actual pigment synthesis does not begin until the end of the pupal stage, a few days before emergence of the adult butterfly. We therefore assume that the problem of color pattern formation is essentially that of pre-pattern formation in the wing disc, but not simple color setting by pigmentation in the latest pupal stage. Since the wing disc grows in size and shape, we in deed have to understand the time-course of development of pre-pattern formation during the time period of larval and pupal stages.

## 5.2. Two Types of Color Patterns – Local Color Pattern and Global Color Pattern

Local color pattern means localized pattern in a restricted area of the entire wing surface such as the eyespot pattern. Global color pattern, on the other hand, means the whole pattern covering whole dorsal and ventral wing monolayers and it is sometimes used for identification of butterfly species (Figure 3).



Figure 3. Local color pattern and global color pattern. (Left) Local eyespot patterns with colored concentric rings on the forewing of *Micalesis gotama*. (Right) Global color patterns on fore- and hindwings of *Papilio xuthus*.

Global color pattern includes local color patterns as parts of all pattern elements composing the global wing pattern. We should have in mind that research on local color patterns is not independent of research on the global color pattern. Recent research on the genetics of color patterns has been mostly concentrated on the local color pattern. On the other hand, genetic research on the global pattern has not been so far advanced. Recently,

however, great progresses have been made using mimetic butterflies such as *Papilio dardanus*, *Heliconius* butterflies, and *Papilio polytes*.

However, the problem of color pattern formation in wings is still not sufficiently solved through both experiment and theory. A few mathematical models based on experimental data have been proposed to account for specific features of the pattern and I review these models briefly with experimental data in following sections.

## 6. LOCAL COLOR PATTERN FORMATION – EXPERIMENTS AND MODELS

### 6.1. Genetic Research on Eyespot Pattern Formation

Among local color patterns, the eyespot pattern has been studied most at both molecular and genetic levels. Carroll et al. (1994) found eye-spot foci in the wing disc of a species *Precis coenia* by using antibody fluorescent techniques for the gene *Distal-less*. The positions of the foci on the wing disc were found to correspond completely to eyespot centers on the adult butterfly (Brakefield et al., 1996). Brunetti et al. (2001) found that two different transcription factors, *spalt* and *engrailed* are expressed in the wing disc of the African species *Bicyclus anynana*. The expression patterns of concentric rings, that is, eyespot pre-patterns correspond completely to the eyespot patterns on the adult butterfly. Some genes associated with pigmentation have also been investigated and identified so far. Current researching trends for eyespot pattern formation are in the direction of detailed genetic analyses, which have taken into account newly founded gene regulatory networks. Based on these findings, the mechanism of eyespot formation has been assumed to consist of following three developmental stages,

- (i) Determination of the eyespot focus, or signaling center in the wing disc, from which some signaling chemicals, or morphogens originate. The focus point on the disc is determined by complex genetic regulatory networks.
- (ii) Spreading out of morphogens into the surroundings of the focus point through diffusion, and activation of corresponding genes (e.g., *Distal-less*, *engrailed*) to establish concentric eyespot pre-patterns on the disc.
- (iii) Activation of pigmentation genes (e.g., *DDC*, *GTP-CH1*, *cinebar*) depending on pre-patterns, and then, color differentiation of scale cells in the adult wing.

### 6.2. Genetic Research for Local Color Patterns with Respect to Evolution

Reed & Serfas (2004) analyzed changes in the *Notch/Distal-less* intervenous expression patterns in wing discs of 8 different butterfly species such as *Vanessa cardui*, *Precis coenia* and *Bicyclus anynana*, with respect to evolution of pattern elements. They concluded that the combination of the *Notch/Distal-less* expression patterns is a good index or tool for understanding the evolutionary pathway of butterfly species. Monteiro et al. (2006) did a comparative homology research on saturniid moth and nymphalid butterfly eyespots with

concentric rings of color, in which two transcription factors, *Distal-less* and *engrailed*, are found to express in the center of the future pattern. They also found that the ligand *Wingless*, and an activated signal transducer, a phosphorylated Smad protein, are expressed, but neither these proteins nor the previous two proteins are found in pierid spot centers that consist of a single patch of color. Both butterfly wing patterns, however, express a third transcription factor, *Spalt*, a portion of whose expression domain maps to the black-colored scales on the adult wing.

On the other hand, there are few mathematical models for local color pattern formation except for eyespot formation. Next, I would like to review mathematical models for eyespot formation.

### 6.3. Mathematical Models for Eyespot Pattern Formation

#### 6.3.1. Activator–Inhibitor Model – A Model for Determination of the Eyespot Focus

Nijhout (1990) found that computer simulations by using a mathematical model could reproduce a focal point on the wing cell as a stable point-like solution of the model equation. The wing cell, in this case, means a localized area composed of scale cells between two adjacent veins near the wing margin, but not a biological single cell.

Eyespots develop around small groups of cells that act as signaling or inducing sources, which are sometimes called “organizing or signaling centers”. Nijhout (1990) showed that point-like patterns can be produced in the exact locations of the organizing centers by an activator–inhibitor reaction-diffusion mechanism that assumes the existence of two chemicals: an activator,  $a(\vec{x}, t)$  at space  $\vec{x}$  and time  $t$ , whose synthesis is subject to positive feedback (autocatalysis), and a inhibitor,  $h(\vec{x}, t)$ , which suppresses autocatalysis around an area of activator production. The system is described by the following equations:

$$\frac{\partial a}{\partial t} = \frac{ca^2}{h} - k_1 a + D_a \nabla^2 a$$

$$\frac{\partial h}{\partial t} = ca^2 - k_2 h + D_h \nabla^2 h$$

where parameters  $c$ ,  $k_1$ , and  $k_2$  are reaction constants;  $D_a$  and  $D_h$  are diffusion constants; and the equations were solved in a two-dimensional space. In numerical simulations, it is assumed that the wing veins act as fixed boundary conditions for the activator and as reflecting boundaries for the inhibitor. In biological terms this implies that the veins, or cells associated with the wing veins, are sources of one of the diffusing reactants. Variation in parameter values can displace the positions of these point-like patterns, and mimic the effect of certain mutations on the positions of the centers of eyespots (e.g., Brakefield, 1998).

The mathematical model also predicted a rather complex time-evolution of the spatial pattern of activator concentration, before it settles to point-like pattern at the stable steady state (Nijhout, 1990). Several years later, Carroll et al. (1994) demonstrated that the gene

*Distal-less* is expressed uniquely in the cells of the organizing centers of eyespots. Moreover, the spatial pattern of expression of *Distal-less* undergoes exactly the same complex changes as those predicted by the mathematical model (Carroll et al., 1994; Nijhout, 1994).

### **6.3.2. Other Mathematical Models for Eyespot Pattern Formation**

Current researching trends for eyespot pattern formation are in the direction of detailed genetic analyses, which have taken into account newly founded gene regulatory networks. Mathematical modeling trends are also in the same direction. Evans & Marcus (2006) presented a mathematical model for determination of the eyespot focus with gene regulatory networks.

As noted in section 6.1, the mechanism of eyespot pattern formation consists of three ((i), (ii), and (iii) stages in section 6.1) developmental stages. After determination of focus points, that is, after the first stage, morphogens spread out from the focus point into the surroundings through diffusion, and then, corresponding genes are activated to form pre-patterns, and finally, color differentiation occurs in scale cells just one or two days before emergence of adult butterflies. Dilão & Sainhas (2004) presented a reaction-diffusion model for eyespot pattern formation, which includes two diffusive morphogens and three non-diffusive pigment precursors. The model has focused on the process of the second and third stages of development for eyespot formation. Assuming that the focus point is given a priori in a specific region of the wing, the model has simulated successfully two concentric dark- and light rings of the pattern, the structural organization of eyespots, their phenotypic plasticity and seasonal variability.

## **7. GLOBAL COLOR PATTERN FORMATION: EXPERIMENTS AND MODELS**

### **7.1. Genetic Research on Global Color Patterns**

Recent research on the genetics of color patterns has been mostly concentrated on the local color pattern and genes such as *Distal-less* and *engrailed* controlling the eyespot pre-pattern have already been identified (e.g., Brunetti et al., 2001). On the other hand, genetic research on the global pattern has not been advanced so far. Recently, however, great progresses have been made by using mimetic butterflies such as *Papilio dardanus*, *Heliconius* butterflies, and *Papilio polytes*. Among them, I select here two mimetic butterfly species, *Papilio dardanus* and *Heliconius erato*, which have been of special interest to biologists for over a century because of the adaptive variation in their wing patterns.

#### **7.1.1. *Papilio Dardanus***

*Papilio dardanus* is a species of swallowtail butterfly widely distributed across sub-Saharan Africa. *P. dardanus* is well known for the spectacular phenotypic polymorphism in females. The females of different geographic races have evolved more than a dozen different wing color patterns and have come to mimic different species of unpalatable butterflies and moths in their specific regions (Batesian mimicry). The males, on the other hand, are

monomorphic and strikingly different from the females, exhibiting a characteristic yellow and black color pattern and tailed hind wing (see Figure 4).

The breeding work of Clarke & Sheppard (1959, 1960) showed that the female-limited polymorphism is controlled by a single genetic locus, termed *H*, with at least 10 distinct alleles. Recently, Clark et al. (2008) demonstrated that the transcription factor *invected*, and its closely linked paralogue *engrailed*, are candidates for the mimicry locus *H*. In addition to genetic studies, other work using phylogenetics (Vane-Wright et al., 1999), morphometrics (Nijhout, 2003) and mathematical modeling (Sekimura et al., 2000) has been done to clarify the function and genetic architecture of *H*.



Figure 4. Polymorphism in females and male of *P. dardanus*. Top left photo shows male pattern, and other eight photos show different female forms of a species *P. dardanus*. (Courtesy of Mr. Steve Collins of African Butterfly Institute, Nairobi, Kenya)

### 7.1.2. *Heliconius* Butterfly

It is known that the species *Heliconius erato* is a mimetic butterfly with more than 20 different wing color pattern variants adapted to different regions across Central and South America (Müllerian mimicry). Recently, genetic analyses of *Heliconius* have started to unearth the characteristics of global wing color patterns. Reed & Gilbert (2004) studied the relationship between wing venation and *Distal-less* expression in *Heliconius* displaying a mutant phenotype with a severe vein deficiency, and found that major color pattern elements develop independently of wing venation. This finding lends support to previous theoretical models suggesting that proximal-distal pre-patterns can be sufficient for establishing complex whole-wing color patterns in butterflies (Sekimura et al., 2000). Reed et al. (2008) investigated expression of candidate genes potentially involved with a red/yellow forewing

band polymorphism in *H. erato*. They found that a transcript *cinnabar* expression was associated with the forewing band regardless of pigment color, and another *vermilion* expression changed spatially over time in red-banded butterflies, but was not expressed at detectable levels in yellow-banded butterflies, suggesting that regulation of *vermilion* may be involved with the red/yellow polymorphism.

I next review some mathematical models for global color pattern formation in peific moth and butterfly wings.

## 7.2. Mathematical Models for Global Color Pattern Formation

### 7.2.1. Diffusing-Morphogen-Gene-Activation Model (Murray, 1981)

A simple model for the development of the commonly observed crossbands of pigmentation shortly after pupation was proposed by Murray (1981). This model is based on a diffusing-morphogen-gene-activation system and extends the idea of a determination stream proposed by Kühn & von Engelhardt (1933), namely, that the anterior and posterior margins of the wing are sources from which emanates a wave of morphogen concentration. As the morphogen diffuses, the morphogen is assumed to be degraded via first-order kinetics. The governing equation for the morphogen concentration  $S(\vec{x}, t)$  at space  $\vec{x}$  and time  $t$  is then

$$\frac{\partial S}{\partial t} = D \nabla^2 S - \kappa S$$

where  $D(\text{cm}^2\text{s}^{-1})$  is the diffusion coefficient and  $\kappa(\text{s}^{-1})$  the degradation rate constant. Boundary conditions for the morphogen at the wing edges are taken to be zero flux.

As the morphogen diffuses across the wing surface, the cells react in response to the local morphogen concentration, and a gene activated by  $S$  produces a product  $g$ . The kinetics of the gene product is assumed to exhibit a biochemical switch behavior produced by a standard cubic-like form.

$$\frac{dg}{dt} = k_1 S + \frac{k_2 g^2}{k_4 + g^2} - k_3 g$$

where  $k_1 \sim k_4$  are positive parameters. Here, the product  $g$  is produced linearly by the morphogen  $S$ , by the product in a nonlinear positive feedback way and linearly degraded proportional to itself. It is noteworthy to notice that  $g$  is a function of position through the morphogen  $S(\vec{x}, t)$ .

Murray's model hypothesizes that the morphogen activates a gene product which, in turn, determines color pattern. The above model equations are solved either on a sector of a circle, representing a wing, or a rectangle, representing a wing cell with appropriate boundary conditions (see Murray, 1981, 2003). Murray showed that this simple model could exhibit a wide variety of observed patterns. For example, it exhibits patterns consistent with those observed after microcautery surgery. The theoretical results are consistent with the

observation of Schwantwitsch (1924). Varying the scale and geometry of the model wing leads to more complicated patterns that are consistent with those observed on certain butterfly and moth wings, while considering the model on a wing cell and varying the source strengths also leads to commonly observed patterns.

### 7.2.2. Simple Diffusion Model (Bard & French, 1984)

A simple diffusion mechanism of a single morphogen was proposed for the formation of the wing pigmentation patterns of three species of butterflies by Bard & French (1984). Their model is based on three main assumptions. First, there may be morphogen sources in the foci of eyespots and morphogen sinks at some parts of the wing margin. Second, the morphogen has a finite half-life and diffuses simply and freely away from the sources throughout a wing of hexagonally packed cells. Third, that the overt pattern derives from cells interpreting the local morphogen concentration with respect to thresholds which determine scale colors. The final pattern follows lines of constant morphogen concentration and may, depending on the distribution of sources, comprise rings, curves, or bands.

In the model, the morphogen was assumed to move through the wing epithelium by simple, membrane-limited diffusion. The morphogen was also assumed to be lost exponentially with time either by simple decay or by a first-order biochemical degradation. Thus, the concentration of the morphogen  $C(\vec{x}, t)$  at space  $\vec{x}$  and time  $t$  is given by the equation

$$\frac{\partial C}{\partial t} = \mu \nabla^2 C - k C$$

where  $\mu$  is the morphogen diffusion constant and  $\kappa$  is the decay constant. Since relatively few parameters are required to specify the system, the diffusion constant  $\mu$ , the decay constant  $\kappa$  and the threshold values are the most important. They chose the diffusion constant to be  $5 \times 10^{-7}$  as a biologically reasonable value. Values of the morphogen half life and thresholds were chosen by trial and error. With a biologically realistic diffusion constant and a morphogen half-life less than 6 hours, the model could generate many features of butterfly wing patterns as stable patterns of morphogen concentration, the compound spots of *Tenaris domitilla*, the large rings of *Diaethria marchalii* and the pattern of eyespots, rings and asymmetric bands of *Ragadia minoa*.

### 7.2.3. Reaction Diffusion Model (Sekimura et al., 2000)

A reaction diffusion model on a geometrically accurate wing domain was first presented for the formation of global pigmentation patterns in the butterfly wing of *Papilio dardanus* by Sekimura et al. (2000). As noted in section 7.1.1, the female wing patterns look very complicated in their appearance and at first glance it seems difficult to find an underlying logical relationship between them even within the single species. However, Nijhout (1991) proposed an idea that the black color pattern elements in the wing constitute the principle pattern elements, even though the background color attracts our attention most. The elements differ in size depending on the mimetic form and this can have dramatic effects on the overall

appearance of the pattern. The problem is, then, largely simplified and the goal is to present a mechanism that can account for only the black pattern elements.

The mathematical model is based on the idea that a system of reacting and diffusing chemicals could evolve from an initially uniform spatial distribution to concentration profiles that vary spatially – the diffusion driven instability (Turing, 1952). The model equations is the non-dimensionalised reaction-diffusion system with Gierer-Meinhardt (1972) reaction kinetics,

$$\frac{\partial u}{\partial t} = \gamma \left( a - bu + \frac{u^2}{v(1 + ku^2)} \right) + \nabla^2 u \quad (3)$$

$$\frac{\partial v}{\partial t} = \gamma(u^2 - v) + d \nabla^2 v \quad (4)$$

where  $u(\bar{x}, t)$  and  $v(\bar{x}, t)$  are chemical concentrations at position  $\bar{x}$  and time  $t$ ;  $a, b, d, K$  and  $\gamma$  are positive parameters.

#### [Result 1] Mimetic and Non-Mimetic Patterns in Females

Mathematical and computational analyses were made first with nine forms of different wing color patterns, that is, four mimetic forms (f. *trophonius*, f. *cenea*, f. *planemoides*, and f. *hippocooides*) (see Figure 5), four non-mimetic forms (f. *natalica*, f. *niobe*, f. *salaami*, and f. *leigh*) of the female, and the male of *P. dardanus* (Sekimura et al., 2000). The idea for the analysis is based on the so-called ground plan of *P. dardanus* (Nijhout, 1991), which consist of three regions on the forewing and one region on the hindwing, where (i) the black pattern elements are major parts of the wing patterns, even though the background color attracts our attention most, and (ii) the black pattern elements can increase or decrease in size, depending on the phenotypic form of each female.

By mathematical analysis and computer simulations of the model equations: Eqs. (3) & (4), Sekimura et al. suggested that the global wing coloration is essentially due to underlying stripe-like patterns of some pigment inducing morphogen. The result is consistent with experimental results on the *Heliconius* butterfly (Reed & Gilbert, 2004). Furthermore, their results suggest that different patterns in females are similar to each other and they could be generated essentially by a fixed set of kinetic and diffusion parameter values in a reaction-diffusion system. This result could be also important from the genetic point of view, because it agrees with the finding that different forms of the females are controlled by a single genetic locus (Clarke & Sheppard, 1959, 1960).

The model does not need to assume supplementary morphogen sources and sinks in some parts of the wing to get patterns. Sekimura et al. also stressed the importance of some key factors to have realistic patterns in computer simulations such as parameter values for mode selection, threshold values which determine color, wing shape and boundary conditions.



Figure 5. Mimetic forms in females of *P. dardanus* and numerical simulation results. (Left) Photographs of mimetic female forms of *P. dardanus* (f. *trophonius* (top left), f. *cenea* (top right), f. *planemoides* (bottom left), and f. *hippocoonideas* (bottom right)), (Courtesy of Dr. Alfried Vogler of Natural History Museum, and Imperial College, UK). (Right) Numerical simulation results by the mathematical model (Eqs. (3) & (4)) of corresponding forms: f. *trophonius*, f. *cenea*, f. *planemoides*, and f. *hippocoonideas*, respectively.

### [Result 2] Male and Male-Like Female Patterns

To our surprise, females exhibit male-like forms with wing tails in some populations. Difference between male and male-like female patterns is, however, very clear when we look at both front wing patterns closely. Yellow-colored patterns of male front wing are very simple and circularly outlined on the distal margin. Male-like female patterns, on the other hand, are somewhat structured and characteristic on the anterior margin of yellow pattern, showing a black colored hollow from outside into the center of the wing, which usually specify the phenotypic indication of male-like female forms. The problem of difference between male and male-like female patterns has been solved mathematically as follows.

From mathematical point of view, the male patterns are essentially different from the male-like female patterns. It was shown that the male patterns are related to parameter values  $(d, \gamma)$  of the equations to isolate the (1,0) mode on a square domain. On the other hand, the structured male-like female patterns are assumed to relate to parameters with the (3,0) mode which could generate other female patterns such as that of the form f. *hippocoonides* (Nijhout et al., 2003).

## 8. FACTORS OF DIVERSIFICATION OF WING COLOR PATTERNS

### 8.1. Ground Plan, a Hypothetical Origin of Global Color Patterns

Owing to the pioneering work of Schwanwitsch (1924) and Süffert (1927) on the nymphalid ground plan, the global color patterns on the wings can be understood as a composite of a relatively small number of pattern elements. For example, (1) the symmetry system consists of color bands that run anterior to posterior across the wing; (2) the border ocelli system consists of a series of eyespots in the distal half of the wing; (3) the marginal bands are a pair of narrow bands near the wing margin; (4) the dependent patterns are venous stripes, that is, a color pattern of the outline of the wing veins; (5) the ripple patterns run

proximal to distal of the wing in a manner similar to the ripples in windblown sand (Figure 6).

It is generally assumed that diverse global color patterns originate from the specific ground plan of each species through morphogenetic changes caused by some factors. In another word, species-specific color patterns develop because of the selective expression or suppression of individual pattern elements, and also the developmental regulation of the exact shape and pigmentation of each element.

I next describe major key factors that produce diverse global color patterns.

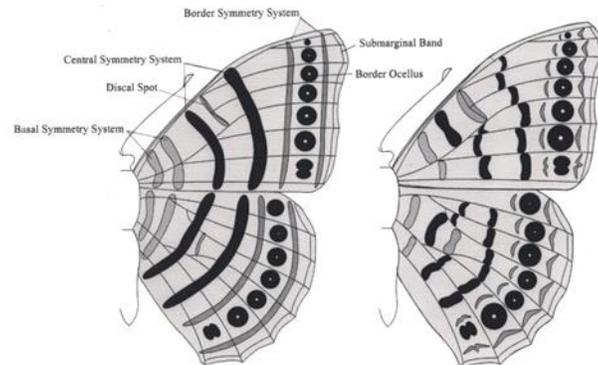


Figure 6. The Nymphalid ground plan according to Nijhout (1991). The ground plan consists of a system of pattern elements such as bands and spots that run from the anterior to the posterior margin of the wing. The pattern elements are classified and named as symmetry systems, submarginal bands, border ocelli, and discal spot.

## 8.2. Major Factors of Diversification of Global Color Patterns

In order to understand the diversity in global color patterns, we must know the vein system and vein positions in the wing, which play significant roles in generating the diversity. Precursors of wing veins, that is, pre-veins, appear first in the larval wing disc and develop throughout the larval and pupal stages until the appearance of adult butterfly. We focus our attentions on the relationship between the pre-veins and the pre-pattern formation in relation to the diversification of global wing patterns. Following factors (i)~(v) are known to have major effects to diversification of global wing patterns (see also Figure 7).

### (i) *Disruption*

Disruption means that pre-veins divide pattern elements such as marginal bands running parallel to the wing margin into fragmental elements such as the border ocelli mentioned above. As an example, we can see in the species *Bicyclus anynana* a mutant without a wing vein showing that two adjacent eyes-pots merge into a single continuous pattern element (Brakefield et al., 1996).

### (ii) *Dislocation*

Dislocation means that pre-patterns running parallel to the wing margin lose their continuity over pre-veins by a kind of sliding movement along the pre-veins.

For example, Koch & Nijhout (2002) found a naturally occurring veinless mutant of the swallowtail butterfly *Papilio xuthus* showing extremely aberrant patterns, where the loss of veins changes normal dislocated marginal bands into continuous bands running parallel to the wing margin.

(iii) *Venous and intervenous patterns*

The venous pattern appears precisely in the line of the wing veins, and intervenous pattern, by contrast, consists of a pigmented stripe that lies on the middle of two adjacent veins.

(iv) *Vein-independent patterns*

This pattern is completely different from the above vein-dependent ones, and is usually based on pattern elements such as the central symmetry system running in the central part of the wing far from the wing margin. For example, in *Heliconius* butterflies, the major pattern elements, that is, stripe-like patterns running through the middle of the wing, appear to be vein-independent.

(v) *Expansion, shrinking, and elimination of pattern elements*

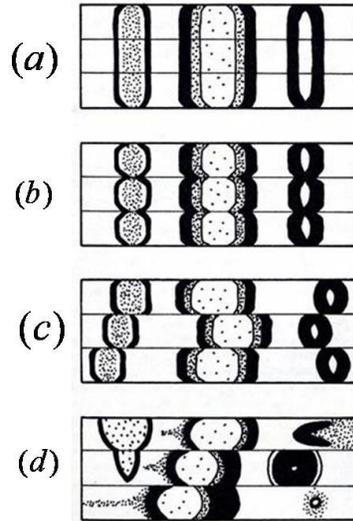


Figure 7. Major factors of diversification of color patterns. (a) Symmetry system of color bands that run anterior to posterior across the wing; (b) Disruption; (c) Dislocation; (d) Expansion, shrinking, elimination, and other deformation of pattern elements. (Courtesy of Prof. Fred Nijhout, Duke University)

### 8.3. Ecological Aspect: Factors Affecting Diversification of Global Color Patterns

Global color patterns are sometimes used for identification of butterfly species, because it is considered that diverse wing color patterns have been caused or derived from various types of adaptation to surrounding environments such as mimicry and behavior of females to choose food plants on which to lay eggs, and so on. Among ecological factors associated with

evolutionary strategies of butterflies, I next take up the relationship between fore- and hindwing patterns, population dynamics of the mimetic butterfly *Papilio polytes*.

### 8.3.1. Fore- and Hindwing Patterns

One of the most striking phenomena about wing color patterns is the close match between patterns of fore- and hindwing when wings are held at their resting position (Figure 8). This phenomenon is known as the Oudemans' principle (Oudemans, 1903). The overall unified pattern of both wings is usually explained by their functional significance, that is, the adaptation to the environment.



Figure 8. The close match between color patterns of fore- and hindwings (of *L. japonica*). White and black color bands running in the anterior-posterior direction of fore- and hindwings look like to be closely related to each other.

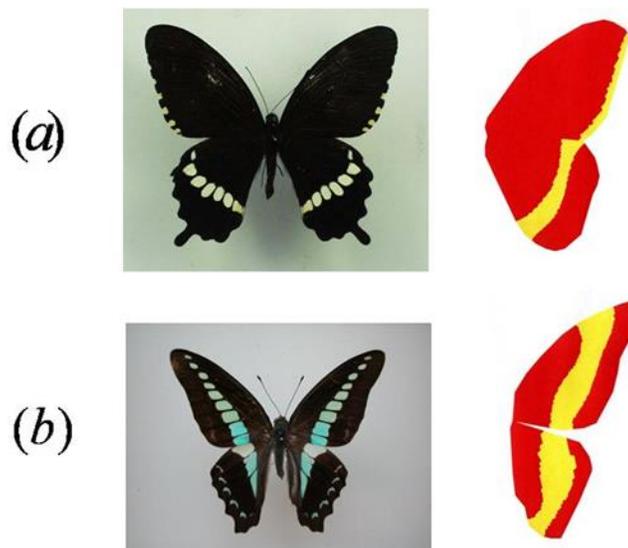


Figure 9. Numerical simulations of patterns of fore- and hindwings of (a) *Papilio polytes* & (b) *Graphium sarpedon* by the mathematical model (Eqs. (3) & (4)). The patterns formed on the forewing need not correlate to those of the hindwing in the sense that the formation mechanism is the same for both patterns. The independence of both patterns means that the coordination of unified patterns of fore- and hindwings is accidental, but not adaptive.

Sekimura et al. (2007) investigated the relationship between fore- and hindwings through mathematical modeling and computational analysis of *P. dardanus* and *P. polytes*. One of the main results is that the patterns formed on the forewing need not correlate to those of the

hindwing in the sense that the formation mechanism is the same for both patterns (see Figure 9). The independence of both patterns means that the coordination of unified patterns of fore- and hindwings is accidental, but not adaptive. This is remarkable, because from Oudemans' principle, patterns appearing on the exposed surface of fore- and hindwing at the natural resting position are often integrated to form a composite and unified adaptive pattern with their surrounding environment. However, the result does not always mean that coordinated unified patterns of fore- and hindwings are not affected by their surrounding environment.

### 8.3.2. Population Dynamics of *P. Polytes*

*P. polytes* is a species of swallowtail butterfly widely distributed across the Oriental region including the Sakishima Islands (southern islands group of Japan). It is known that the female is polymorphic with four forms (f. *cyrus*, f. *polytes*, f. *romulus*, and f. *theseus*), whereas the male is always monomorphic. In the Sakishima Islands, however, there exist only two female forms, f. *cyrus* (non-mimic) and f. *polytes* (mimic). The form f. *cyrus* resembles the male, and the other form f. *polytes* resembles an unpalatable butterfly *Pachliopta aristolochiae* (model). Interestingly, in their distribution range, particular islands do not have *P. aristolochiae* and f. *polytes*, or *P. aristolochiae*.

Based on published records, Uesugi (1991) reviewed temporal changes in the occurrence of f. *polytes* and its model *P. aristolochiae* in the Sakishima Islands. He found that there had not been any records of *P. aristolochiae* in the Sakishima Islands from 1907 to 1967, but it was first recorded in 1968 in the Yaeyama Islands, the southernmost islands group of the Sakishima Islands and the records of f. *polytes* (mimic) increased rapidly after 1972 there. He also found that on Miyako-jima Island, a northern island of the Sakishima Islands, the number of the mimic butterflies has increased after establishment of the model butterfly in 1975. The increase in the population of f. *polytes* was discussed and explained in the context of adaptive significance of the female-limited Batesian mimicry (Uesugi, 1996).

Recently, Sekimura et al. (2013) have investigated the population dynamics of f. *polytes* by use of mathematical modeling, that includes four major variables, i.e., population densities of three kinds of butterflies (two female forms f. *cyrus*, f. *polytes* and the unpalatable butterfly *P. aristrochiaie*) and their predator (submitted).

## 9. INTEGRATION OF KEY ASPECTS OF DIVERSITY IN WING PATTERNS

In order to understand how the diversity in wing patterns has been derived, we first have to analyze specific aspects (e.g., genetic aspect, ecological aspect, evolutionary aspect, and so on) underlying the pattern formation mechanisms and evolution. We then have to integrate them by new comprehensive methods including mathematical and computational analyses. A hint or way how to do this is for us to understand that the butterfly wing is an organ of a living butterfly surviving under various kinds of environmental restrictions such as various natural conditions, interspecific and intraspecific competition, and so on. The matter of utmost concern for a butterfly would be to survive in a given environment, and most of its characteristics including wing color pattern would have evolved to fit or adapt to the surrounding environment. This means that such characteristics including wing color pattern represent some kinds of strategies for survival, that must be ultimately related to changes in

the fitness in the population biology. Here, the word “fitness” roughly means the number of offspring per adult in the next generation, a useful quantity to discuss temporal changes in population of an organism.

The problem is to find the way how to integrate key aspects. As an example, I would like to introduce a theoretical research by Ohsaki (1995), who succeeded to link the biochemical aspect to mimicry of a female-limited mimetic species *P. polytes*, whose females mimic the wing-color patterning of the unpalatable *P. aristrochiae* by means of the chemical pigment carotenoid. It is known that the physiological life span of the mimic f. *polytes* is shorter than the non-mimic f. *cyrus*. In general, wing color patterns have specific biological functions, such as mutual recognition, protection from predators, and mimicry, which play important roles in butterfly survival. On the other hand, chemical pigments of wing colors are synthesized in cells by biochemical reaction networks. By use of the cost-benefit theory, Ohsaki linked the chemical pigment to mimicry, and he concluded that owing to their consumption of the chemical, the physiological life span of mimetic females is shorter than that of non-mimetic females. This conclusion may indicate a reduction in the fitness of the mimetic females. This example shows that changes in wing color patterning could affect the fitness or the future population of a butterfly species, and at the same time, this shows a deep linkage of a biochemical process to a different ecological event, mimicry.

## CONCLUSION AND DISCUSSIONS

In this article, I briefly reviewed the progress of mathematical models for formation and diversification of color pattern in butterfly wings. I also reviewed another kind of mathematical model for cellular pattern formation of scale cells as the basic process of pattern formation in butterfly wings, even though both color pattern and cellular pattern are not directly related with each other. The mathematical models are all constructed on the basis of experimental data, which involve evidences in both macro- and micro-level scales, depending on pattern forming phenomena.

Until today, genetic or molecular level researches have focused mainly on the local pattern such as eyespot pattern, and on the other hand, there exist few researches on the global pattern. With respect to mathematical modeling, the situation is almost the same as that in experimental researches. It is generally recognized that in order to understand evolution and diversity in biological phenomena including the butterfly wing pattern, we have to investigate non-model organisms such as insects including butterfly from both experimental and mathematical modeling aspects.

There exist discussions on the general concept and methodology for the treatment of morphological phenomena and evolution in biology and paleontology such as “morphodynamics” (Seilacher, 1991). Regarding butterfly wing patterns, I have stressed the importance of integration of different aspects such as gene and environment for full understanding of the diversity in wing color patterns (Sekimura, 2013). A hint to go ahead is for us to simply remember that the butterfly wing is an organ of a living organism, a butterfly surviving in a variety of surrounded environments. Different aspects on the color pattern such as gene and environment are from characteristics of a single living organism, a butterfly, and

they should be unified into one comprehensive feature of the butterfly after integration process. However, it is not always clear how to pursue the integration process at the moment.

I think that at least we should do the process of linking one aspect to another. I would like to show the reader an example, even though the animal in question is not butterfly, but fish. This is an experiment-based theoretical paper (Asai et al., 1999), in which the analysis has been done by using reaction-diffusion equations, that is, an activator-inhibitor system. Parameters such as reaction rates and diffusion constants in the equations originate from basic biochemical reaction networks and random mobilities of chemicals in cells. Authors succeeded to link parameter values in the activator-inhibitor system to activities of the *leopard* gene, which controls changes in Zebrafish skin color patterns. This is a good case study to connect a biochemical process directly to genetic activity.

We are at the first stage in the course of an integrative approach to the analysis of pattern and diversity in butterfly wings. We could connect a few of all the different aspects that are necessary to catch a full understanding of evolution and diversity in wing patterns. All of these step by step processes will be integrated into a comprehensive and realistic feature of wing patterns under the concept of survival strategies of a beautiful creature, butterfly. Some mathematical models have contributed to our understanding of the problem so far and, in the future, they will continue to play an important role in the analysis of pattern formation and the integration process of different aspects in question.

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