

Chapter 7

Self-Similarity, Distortion Waves, and the Essence of Morphogenesis: A Generalized View of Color Pattern Formation in Butterfly Wings

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Anyhow, exploring the *consequences* of self-similarity was proving full of extraordinary surprises, helping me to understand the fabric of nature.—Benoit B. Mandelbrot (1983). *The Fractal Geometry of Nature*. Revised edition, Page 423

Abstract The morphology of multicellular organisms can be viewed as structures of three-dimensional bulges and dents of an otherwise nearly two-dimensional epithelial sheet. Morphogenesis is thus a process to stably form those physical distortions over time through differential cellular adhesion, contraction, and aggregation and through cellular changes in size, shape, and number. Such physical distortions may be hierarchically repeated with modifications, which is suggested by self-similar structures in organisms. Butterfly wings are nearly two-dimensional but contain three-dimensional bulges and dents that correspond to organizing centers for color pattern elements. Importantly, an eyespot and its corresponding parafoveal element on a wing, constituting the border symmetry system, are self-similar. From this perspective, I review here the color pattern rules and several formal models that have been proposed, clarifying their relationships with the induction model for positional information. To reinforce the induction model, I propose the distortion hypothesis, in which dynamic epithelial distortion forces at organizing centers, such as the center of a presumptive eyespot, that are produced through changes in cell size spread to surrounding immature cells over distances as morphogenic signals in developing butterfly wings. The physical distortion forces open stretch-activated calcium channels that cause calcium signals in the cell and activate the expression of regulatory genes. These regulatory gene products initiate

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a cascade of structural genes that eventually produce eyespot black rings. Calcium waves also activate a process of genome duplication, resulting in an increase in cell size, as the ploidy hypothesis states. A new distortion of epithelial cells is induced at the center of a presumptive parafoveal element through an increase in cell size, producing self-similarity of the eyespot and the parafoveal element. The self-similar configuration of the border symmetry system further suggests the essence of morphogenesis as the DCG cycle: repeated sequential events of epithelial distortions (D), calcium waves (C), and gene expression changes (G). Future studies should examine these hypotheses and speculations that constitute the induction model in butterfly wings and the generality of the DCG cycle in other organisms.

Keywords Butterfly wing • Color pattern rule • Distortion hypothesis • Eyespot • Induction model • Morphogen • Parafoveal element • Pattern formation • Ploidy hypothesis • Self-similarity

7.1 Introduction

One of the important goals of developmental biology is to understand how morphological structures are produced during development. Morphological structures are usually three-dimensional, but they are initiated as physical changes in a two-dimensional epithelial sheet to create three-dimensional bulges and dents. Developmentally speaking, the origin of morphology in amphibian embryogenesis can be traced back to the blastula stage, which is the stage when a sheet of cells emerges for the first time after fertilization. Subsequently, the plain cellular sheet undergoes dynamic cellular movement for gastrulation and eventually forms an embryo and, later, a complete adult individual. These processes are understood as mechanical changes of the epithelial cells. In this sense, a center of physical distortion forces could correspond to an organizing center. In insects, early embryogenesis is executed in the syncytial blastoderm, which may not be similar to this concept of mechanical changes, but a process of adult tissue formation from imaginal disks in the prepupal and pupal stages involves dynamic physical distortions of the epithelial cells.

In this view, morphogenesis can be considered to be a process of forming physical distortions over time through differential cellular contraction, adhesion between cells, and aggregation among cells and through cellular changes in size, shape, and number. Furthermore, the whole biological structure of a given organism can be viewed as a series of repetitions of epithelial distortions, despite their superficial dissimilarity. This kind of repetition unit may be called the “**morphogenesis unit**.” Therefore, the mechanism employed to produce the morphogenesis unit is the essence of morphogenesis.

This view of morphogenesis has been derived from observations of diverse butterfly wing color patterns and from interpretations of physiologically induced color pattern changes (Otaki 2008a). Butterfly wings are mostly two-dimensional,

but careful examinations reveal that they are indeed three-dimensional (Taira and Otaki 2016), as in other tissues and organs in animals, and therefore likely involve mechanical forces that are generated by cellular changes in size, shape, and number. Butterfly wing disks at the larval and pupal stages are sheets of epithelial cells (more specifically, epidermal cells) that may be ready to accept mechanical changes. Butterfly wings additionally produce three-dimensional microstructures of scales and bristles, the processes of which are interesting but beyond the scope of this paper. In this paper, I endeavor to extract “the essence of morphogenesis” from the color pattern development of the border symmetry system. The border symmetry system is one of the symmetry systems in nymphalid color patterns and consists of border ocelli (eyespot) and parafocal elements (PFEs), which will be explained shortly below.

The repetition unit in biological entities may be identified by seeking homologous structures. **Serial homology** or **modularity** is a popular concept in the field of animal development. A good example of serial homology is serial eyespots on a single wing surface of nymphalid butterflies (Nijhout 1991; Beldade et al. 2002, 2008; Monteiro et al. 2003; Monteiro 2008, 2014). However, in this paper, I focus on **self-similarity**, a concept that is different from serial homology and modularity. Eyespots on a single wing surface are homologous but not self-similar; self-similarity is hierarchical repetition but not parallel repetition.

In the following sections, I first introduce the concept of self-similarity in biological entities using plants as examples. I use plants because they often manifest self-similar structures that are relatively easy to pinpoint, and many of them have been analyzed well mathematically (Mandelbrot 1983; Barnsley et al. 1986; Ball 1999, 2016).

7.2 Self-Similarity in Plants and Animals

In self-similar structures, a large structure contains its own smaller structures, wherein the small ones are nested within the larger one; they are hierarchically produced. In other words, the whole and its partial structures are similar to each other, but they are not necessarily morphologically identical in actual biological systems because of the extreme modifications of the essential process for their morphogenesis. These modifications often make identification of self-similarity difficult in actual biological systems.

One of the most famous self-similar structures in biological entities may be a fern or leaf structure that is produced by fractal branching patterns (Barnsley et al. 1986). Many leaves exhibit clear self-similarity, but the way it manifests is greatly dependent on the plants. A similar leaf branching pattern is also seen in bacterial growth (Ben-Jacob et al. 1994), blood vessels (Family et al. 1989), seaweeds, sponges and corals (Kaandorp and Kübler 2001), and other systems (Ball 1999, 2016), suggesting the universality of the branching fractal structures in biological systems.

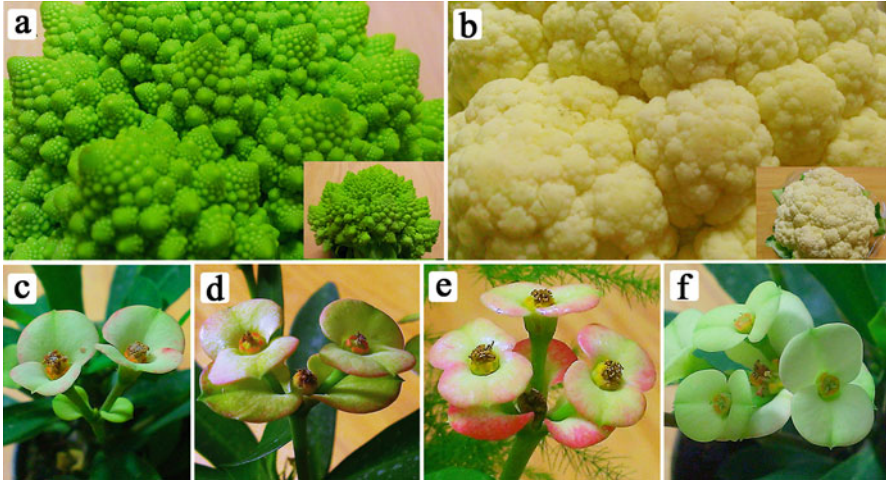


Fig. 7.1 Examples of self-similarity in plants. (a) Buds of cauliflower romanescos. An inset shows the whole structure. (b) Buds of a common cauliflower. An inset shows the whole structure. (c–f) Flowers of the crown of thorns, *Euphorbia milii*

The spiral floret arrangement of cauliflower romanescos (*Brassica oleracea* var. *botrytis*) is another famous example of self-similarity (Fig. 7.1a). A common cauliflower also exhibits self-similarity, but it is less clear (Fig. 7.1b). A similar spiral arrangement can be found in shells (Meinhardt 2009) and other systems (Ball 1999, 2016), suggesting that animals, too, have an ability to produce spiral fractal structures.

A more important and illuminating example salient to a discussion of butterfly color patterns can be found in the flowering pattern of *Euphorbia milii* (Fig. 7.1c–f). A single flower can produce a few smaller flowers from its own flower. This is a nested or hierarchical configuration, and these flowers are self-similar. It appears that this type of self-similarity in a complex biological entity (i.e., a flower in this example) that is not either simple branching or spiral patterns is relatively rare. A potential explanation for this finding is that the original self-similar structures are extensively modified to a degree unnoticeable by human eyes in most biological systems.

These examples in plants, animals, and other organisms demonstrate that organisms have an ability to form self-similar structures. I turn to butterfly wing color patterns from a viewpoint of self-similarity below, but before discussing self-similarity, I first discuss the symmetry in butterfly wing color patterns. Also in the following sections, I propose possible rules for color pattern formation in butterfly wings, which contain my own speculations. I then propose models and hypotheses that incorporate my speculations. For the readers' convenience, I summarize the color pattern rules at the elemental and sub-elemental levels that are discussed below in Table 7.1 and the additional color pattern rules at the scale and cellular levels in Table 7.2. I also summarize the models and hypotheses that are discussed in this paper in Table 7.3.

Table 7.1 Color pattern rules at the elemental and sub-elemental levels

1. Symmetry rule (color symmetry rule)	Pigment distribution is symmetric in a given system or element
2. Core-paracore rule	A unit of a symmetry system is composed of a single core element and a pair of paracore element
3. Self-similarity rule (nesting rule)	An eyespot and its accompanying parafocal element are self-similar
4. Binary rule (binary color rule)	Eyespot (and other elements) is depicted in dark color against light background color
5. Imaginary ring rule	An eyespot has a vanishingly weak light ring outside the outermost dark ring
6. Inside-wide rule	In a full eyespot, the inner dark core ring (disk) is larger in width than the outer dark ring
7. Uncoupling rule	Sub-elements of an eyespot can be uncoupled from the rest of the eyespot
8. Midline rule	Center of a natural eyespot is placed at the midline of a wing compartment

Table 7.2 Color pattern rules at the scale and cellular levels

1. One-cell one-scale rule	A single scale cell produces a single scale throughout a butterfly wing
2. Color-size correlation rule for scales	Scales of elements (dark-colored scales) are larger than scales of background nearby (light-colored scales)
3. Central maxima rule for elemental scale size	Scales at the center of an element have the largest size in that element
4. Size-ploidy correlation rule for scales and cells	Scale size is correlated with the degree of ploidy of scale cells
5. Distortion rule for organizing centers	Organizing centers are physically distorted as bulges and dents that are reflected in pupal cuticle spots

7.3 Part I: Color Pattern Rules

7.3.1 *Symmetry in Butterfly Wing Color Patterns*

Highly diverse butterfly wing color patterns are thought to have been derived from a basic overall wing color pattern called the **nymphalid ground plan**. The nymphalid ground plan is a sketch of a general color pattern that was obtained by inductive reasoning from observations of many actual butterflies. This pattern was independently proposed by Schwanwitsch (1924) and Süffert (1927). Based on these two original schemes, a modern version was proposed by Nijhout (1991, 2001), and a few minor revisions were introduced by Otaki (2012a).

The nymphalid ground plan is composed of color pattern “**elements**,” which are placed on a “**background**” (Fig. 7.2). The important point is that the elements are symmetrically arranged regarding pigment composition (i.e., coloration) (Nijhout

Table 7.3 Models and hypothesis for color pattern formation

1. Concentration gradient model (gradient model)	The classical model based on diffusive morphogen gradient that is released from organizing center. Thresholds are inherently set in signal-receiving cells
2. Transient models (collective)	Models that have been proposed transiently and withdrawn readily to investigate the simplest models for color pattern determination, including the two sub-step model and the multiple morphogen model
3. Adopted models (collective)	Models that have been proposed fragmentally but adopted to be synthesized as the induction model. Adopted models are the wave model, the two-morphogen model, and the heterochronic uncoupling model
4. Threshold change model	The most popular model that could explain color pattern modifications induced by physical damage and by pharmacological or temperature treatment
5. Induction model	An alternative model that proposes a sequential release of wavelike morphogenic signals from organizing center and dynamic interactions between signals
6. Rolling-ball model	A way of signal dispersion in the induction model, mainly based on the results of pharmacological modifications of parafocal elements and eyespots
7. Signal settlement mechanisms	A ways of signal settlement in the induction model. Three mechanisms are proposed: time-out mechanism, spontaneous velocity-loss mechanism, and repulsive velocity-loss mechanism. The latter has two sub-mechanisms: self-repulsive and nonself-repulsive velocity-loss mechanisms
8. Ploidy hypothesis	The hypothesis that morphogenic signal for color patterns is a ploidy signal. Scale color is determined as a result of cell size and the degree of ploidy
9. Physical distortion hypothesis	The hypothesis that morphogenic signal for color patterns is physical distortions of epithelial sheet
10. DCG cycle	The essence of morphogenesis in the revised version of the induction model, producing self-similar structures. D, distortion waves; C, calcium waves; G, gene expression

1994); this principle may be called the **symmetry rule** or, more accurately, **color symmetry rule**. In contrast, elemental shape is often very asymmetric. It has been believed that elemental symmetry in coloration comes from circular arrangements of morphogenic signals (signals that function as morphogens) from the organizing center located at the center of a prospective element.

There are three major symmetry systems (the basal, central, and border symmetry systems) and two peripheral systems (the wing root and marginal band systems) on the wings of nymphalid butterflies (Nijhout 1991, 2001; Otaki 2009, 2012a; Taira et al. 2015), although Martin and Reed (2014) stated reasonably that the basal

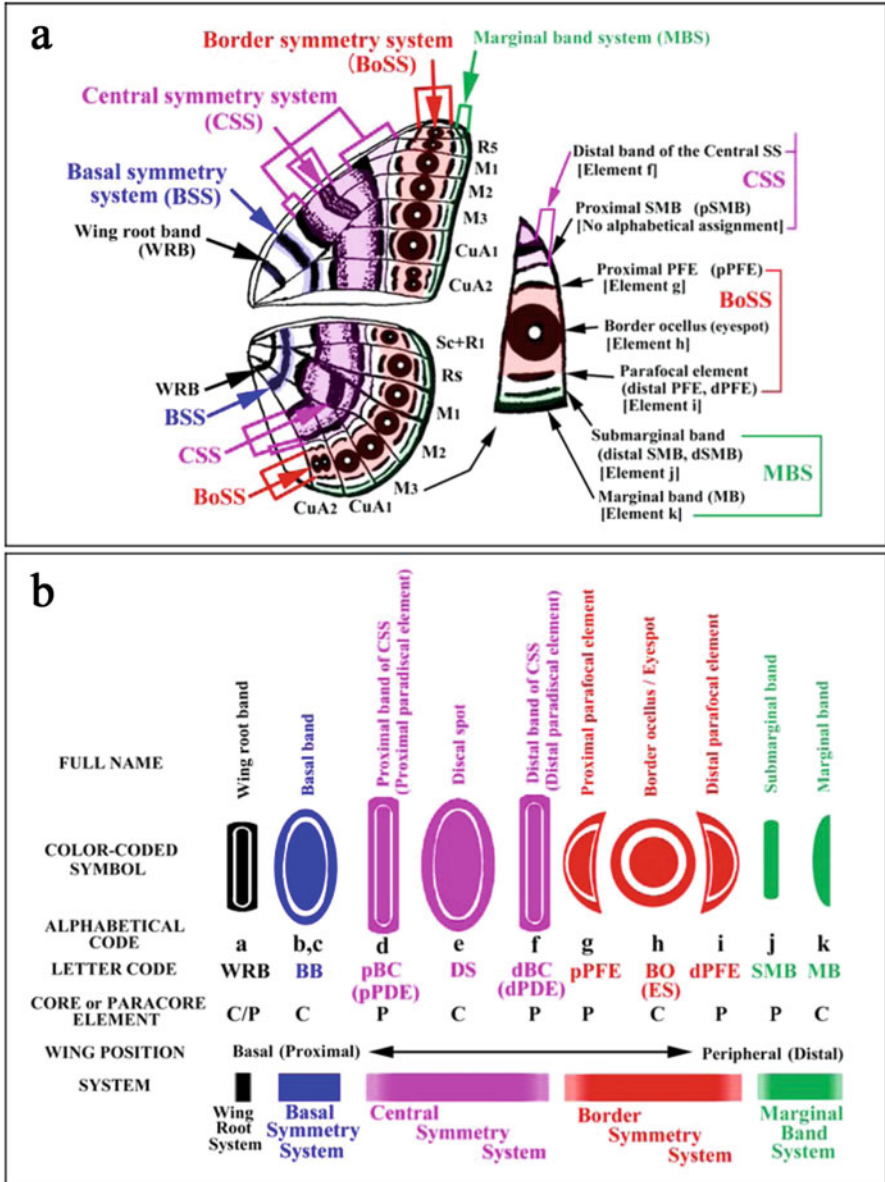


Fig. 7.2 The nymphalid ground plan. Reproduced and modified from Otaki (2012a) and Taira et al. (2015). (a) A standard scheme. In this scheme, dSMB is a part of the distal band of the central symmetry system (dBC) and thus may be omitted from the ground plan. Elements are aligned from the basal (*left*) to the peripheral (*right*)

symmetry system may be associated with the central symmetry system. The two peripheral systems are also likely symmetric, but simply because they are placed at the wing margins, only a portion of them are expressed on a wing. It is likely that all five systems share the same developmental mechanism. In other words, they can be considered to have been derived from modifications of the basic “**ground pattern**” for a single symmetry system. In this sense, they are homologous. Importantly, it is reasonable to assume that each unit of a symmetry system is primarily organized by a single organizing center during development.

7.3.2 *The Core-Paracore Rule and Self-Similarity Rule*

Because eyespots and PFEs belong to the border symmetry system (Otaki 2009; Dhungel and Otaki 2009), it is likely that the unit of color pattern (or the basic “ground pattern”) in any symmetry system of the nymphalid ground plan is composed of the **core element** and a pair of **paracore elements** (Otaki 2012a), which may be dubbed the **core-paracore rule**. The single elemental system containing the core and paracore elements is symmetric, and a single core element is symmetric regarding pigment composition. Likewise, a single paracore element is symmetric. Importantly, the pigment composition of a paracore element is often similar to that of a corresponding core element. Thus, the core-paracore rule may be elaborated as the **self-similarity rule (the nesting rule)**. Based on the core-paracore rule and the self-similarity rule, the diversity of the symmetry system can be understood as various modifications of the basic process of elemental formation (Fig. 7.3).

7.3.3 *The Border Symmetry System and Its Self-Similarity*

To understand the core-paracore relationship, I hereafter mainly focus on the border symmetry system in nymphalid butterfly wings. The core and paracore elements in this system are border ocelli (BOs or eyespots) and PFEs, respectively. PFEs are often found on the distal side of eyespots (dPFEs), and those on the proximal sides (pPFEs) are less frequent (Otaki 2009). When it is simply known as a parafocal element, dPFE is meant.

Examples of the border symmetry system are shown here. In *Argyreus hyperbius*, BOs and PFEs are both beige in color, although they have different shapes (Fig. 7.4a, left). In contrast, the submarginal bands are differently colored. This coloration pattern probably arises because BOs and PFEs belong to the same system, and they are different from submarginal bands, which belong to the marginal band system (Taira and Otaki 2016). In *Vanessa indica*, BOs and PFEs are similar both in coloration and shape (Fig. 7.4a, middle). In *Araschnia burejana*, PFEs are elongated oval rings with or without blue filling inside (Fig. 7.4a, right).

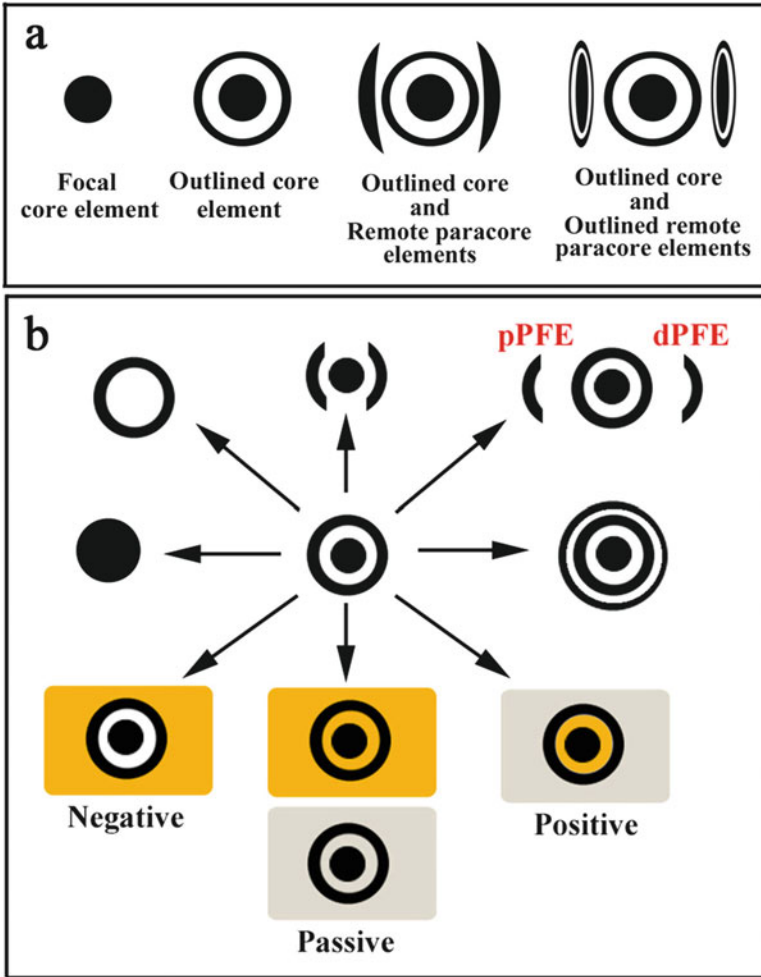


Fig. 7.3 Morphological transformation of color patterns of the border symmetry system. Reproduced from Otaki (2012a). (a) Stepwise changes from the simplest black dot (left) to the complicated self-similar pattern (right). (b) Diverse transformation of a standard eyespot to various eyespots. Coloration of the inner light ring in a negative, passive, or positive fashion also contributes to eyespot diversity.

This configuration of the border symmetry system appears to be typical in nymphalid butterflies.

Self-similarity between BOs and PFEs is not always clear in the cases above, but in *Tarattia lysanias*, the outer ring of a BO is isolated from the inner black disk, which is similar in shape to a PFE (Fig. 7.4b, left). The inner black disk of a BO is also divided into two rods in *Symbrenthia leoparda*, making a distinction in shape between BO and PFE difficult (Fig. 7.4b, right). Rod-shaped BOs and eyespot-

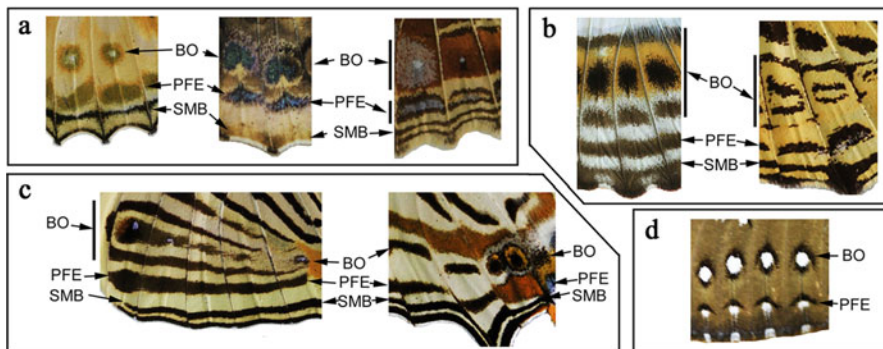


Fig. 7.4 Examples of the border symmetry system in nymphalid butterflies. BO, border ocellus; PFE, parafoveal element; SMB, submarginal band. (a) *Argyreus hyperbius* (left), *Vanessa indica* (middle), and *Araschnia burejana* (right). (b) *Tarattia lysanias* (left) and *Symbrenthia leopard* (right). (c) *Colobura dirce* (left) and *Cyrestis camillus* (right). (d) *Hamanumida daedalus*

shaped BOs coexist on the identical wing surface in *Colobura dirce* and *Cyrestis camillus* (Fig. 7.4c). I believe, therefore, that a PFE is equivalent to an eyespot ring (Dhungel and Otaki 2009; Otaki 2009).

Another intriguing case is found in *Hamanumida daedalus*, where both BOs and PFEs are circular (not rod-shaped) and are similar to each other (Fig. 7.4d). This case strongly argues for self-similarity between BOs and PFEs.

7.3.4 Eyespot Pattern Rules: The Binary Rule and Inside-Wide Rule

To developmentally understand the symmetry rule, the core-paracore rule, and the self-similarity rule discussed above, additional rules regarding nymphalid butterfly color patterns will be discussed here.

An eyespot (BO) is composed of its parts, which may be called sub-elements. Typically, from the center to the peripheral regions, an eyespot is composed of a white dot, a dark (usually black) inner ring (disk), a light ring, and the outermost dark ring. Often, the light ring is variously colored, and the white dot may be absent. Additional rings may exist. The overall shape also varies from a near-true circle to extreme elongation such as rods and lines. Despite these diverse cases, the simplest eyespot is composed of two dark rings (inner and outer dark rings) and one light ring between them. Importantly, the light ring is similar or even identical to the background in coloration. That is, an eyespot is depicted in a dark color against a light background. This is called the **binary rule (binary color rule)** (Otaki 2011a). The binary rule can be revealed when BOs are expressed as rods or lines. *Symbrenthia leopard* (Fig. 7.4b, right) and *Colobura dirce* (Fig. 7.4c, left) illustrate this point: the light rings are continuous with the background, and they are colored

without any distinction from the background. The binary rule also implies that the outer dark ring (including PFE) is remotely located from the inner dark ring (disk). This means that morphogenic signals for the outer ring and PFE can travel long distances from the center of the symmetry system.

However, it is also true that in many eyespots, a light ring is not completely identical to the background but may be variously colored. I consider the light ring coloration to be evolutionary modifications. Because it is sandwiched by two dark regions, the light region has to have means to inhibit the invasion of black pigmentation during development. That is, I believe that the inhibitory signal is upregulated in the light ring. This inhibitory signal might have linked to pigment synthesis pathways later in evolution. The inhibitory signal also exists in the background region in contact with the outermost dark ring. This region often shows a vanishingly weak “light ring,” which is called the imaginary ring (Otaki 2011b). This pattern may be dubbed the **imaginary ring rule**.

In nymphalid eyespots, the dark inner ring is almost always larger than the outer rings in width. This is called the **inside-wide rule** (Otaki 2012b). A “typical” eyespot without distortion that illustrates the inside-wide rule well can be found frequently in Satyrinae (Fig. 7.5a). Non-Satyrinae eyespots are likely more diverse but still largely follow the inside-wide rule (Fig. 7.5b). However, an exception to this inside-wide rule is small “immature” eyespots (Otaki 2011b), which were probably still developing when the signaling and reception steps were terminated (see below for the four-step process). Alternatively, inhibitory signals were upregulated earlier in the immature eyespot than in the mature eyespot (see

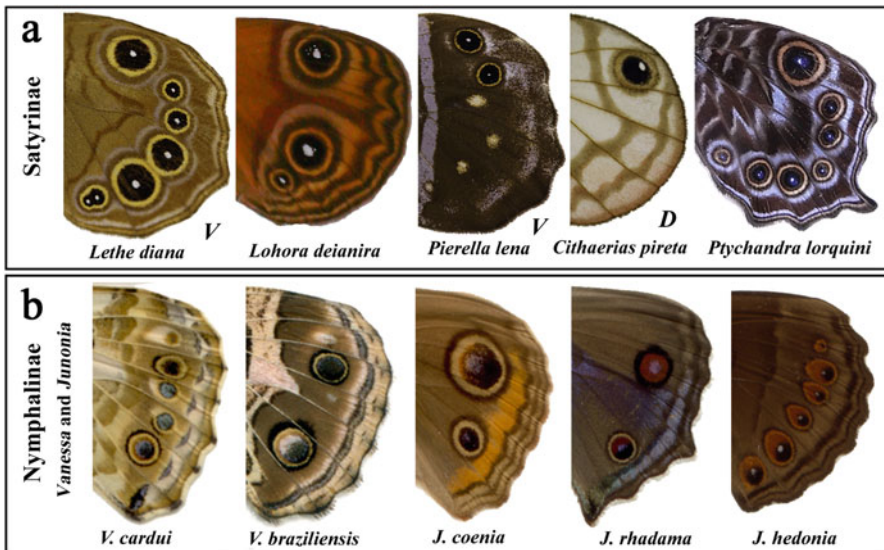


Fig. 7.5 Examples of nymphalid butterfly eyespots. (a) Satyrinae. (b) Nymphalinae

below for the induction model). These immature eyespots can be found among consecutive eyespots on a wing in many species (Fig. 7.5a, b).

The behavior of PFEs is worth mentioning. A PFE becomes larger when it is displaced toward the corresponding eyespot by pharmacological treatment (Otaki 2008a, 2012b), which follows the inside-wide rule. However, the PFE is sometimes larger than the entire BO, contrary to the inside-wide rule (if it is considered to be a part of an eyespot system as discussed above), as seen in *Argyreus hyperbius* (Fig. 7.4a, left). This exception probably occurs because, once moved away from the core element, the PFE behaves independently as a source of morphogenic signal, as the self-similarity rule suggests.

7.3.5 Eyespot Pattern Rules: The Uncoupling Rule and Midline Rule

An analysis of diverse eyespots indicates that the dark inner ring and the outer ring are not always placed on a single symmetry axis (Otaki 2011b). They appear to be independent of each other to some extent. This conclusion is also supported by physical damage experiments in which the outer ring enlarges and the inner ring diminishes in size in a single eyespot in response to damage (Otaki 2011c). Similarly, an eyespot white spot (“focus”) behaves independently from the rest of the eyespot (eyespot body) (Iwata and Otaki 2016a). The uncoupling of the white spot is probably somewhat surprising for those who are not familiar with the genus *Calisto*, which has a white spot not at the center but outside an eyespot (Fig. 7.6). This type of semi-independent behavior of sub-elements is dubbed the **uncoupling rule**. The uncoupling behavior of sub-elements has been suggested in Nijhout (1990), Monteiro (2008), and Iwata and Otaki (2016a, b).

Despite the uncoupling, elemental centers are primarily located on the midline of a compartment (one of the Nijhout’s design principles for formal models described in Nijhout (1990)); this may be called the **midline rule**. In contrast, damage-



Fig. 7.6 Eyespots of *Calisto tasajera*. Reproduced and modified from Iwata and Otaki (2016a). (a) Ventral side of the whole wings. (b, c) Ventral hindwing eyespots of two different individuals. White spots are often located outside of the main eyespots in this species

induced elements can emerge at the non-midline (Otaki 2011c). Because the midline is defined by the wing veins, there is no doubt that the wing veins and compartments play critical roles in determining the location of a given element, as elaborated in Nijhout (1978, 1990, 1991).

7.4 Part II: Formal Models toward the Induction Model

7.4.1 *Four Steps for Color Pattern Formation as a Starting Frame*

It is first important to recognize as a starting frame that there are four sequential steps of color pattern formation: signaling, reception, interpretation, and expression (Otaki 2008a, 2012b). The signaling step was executed by organizing cells, whereas the other three steps were executed by immature scale cells that receive positional information. Most models, including the induction model below, focus on the signaling step and do not pay much attention to the latter three steps. However, the diversity of actual butterfly color patterns may be realized by changes in any single step, at least theoretically.

7.4.2 *Gradient Model for Positional Information*

The **concentration gradient model** for positional information is probably still the most popular model to explain butterfly eyespot formation (Nijhout 1978, 1980a, 1981, 1990, 1991; French and Brakefield 1992, 1995; Brakefield and French 1995; Monteiro et al. 2001). However, the gradient model cannot easily explain the pattern rules discussed above. Furthermore, it is difficult to explain the additional features of diverse color patterns in actual butterfly wings such as multiple dark rings and differences between small and large eyespots that have drastically different morphology in adjacent compartments using this model (Otaki 2011a, b). Additionally, this model cannot explain dynamic signal interactions (Otaki 2011a, b, c). Time series of color deposition in pupal wings have revealed that red color for an eyespot light ring that develops earlier is “overwritten” by black color that develops later and that a given black area develops as a fusion of patchy black islands (Iwata et al. 2014). These ontogenic observations are not compatible with the gradient model.

However, these facts do not completely deny the usefulness of the gradient model. I believe that a concentration gradient of signaling molecules may play an important role in finalizing the expression of genes for pigment synthesis in a relatively short range (e.g., within a given eyespot ring) (see below). In this sense, gene expression changes may be a result (not a cause) of upstream long-range signals from the center of a prospective eyespot.

7.4.3 *Transient Models for TS-Type Modifications and Parafocal Elements*

Although I mentioned that the conventional gradient model was not satisfactory, I did not immediately reach this conclusion; I devised a few models before the induction model. I here collectively call them **transient models** because they were transiently proposed and readily discarded. Nonetheless, these models are important to determining the simplest (most parsimonious) model that reasonably explains experimentally induced and naturally occurring eyespots and PFEs. The inclusion of PFEs in the process of making a formal model is critical because both eyespots and PFEs belong to the same symmetry system.

To explain the PFE formation in eyespot-forming and eyespot-less compartments based on the gradient model, the two sub-step model for eyespots and PFEs has been proposed (Otaki 2008a). In this model, a diffusive gradient is first formed to determine the location of PFEs in both eyespot-forming and eyespot-less compartments. After the determination of the PFE location by the periphery of the gradient, the gradient entirely disappears quickly and does not form an eyespot in an eyespot-less compartment. Note that the presence and absence of an eyespot cannot be attributed to threshold differences between the two compartments because they have the same threshold levels if the thresholds exist at all, as shown in an eyespot that occupies two or more compartments (Otaki 2011b). This two sub-step model should also mean that the reception step first takes a snapshot of the PFE, and after the disappearance of the eyespot signal, another snapshot should be taken. This model is too awkward to be accurate, but it hints at the importance of uncoupling the behavior of the PFE from the eyespot proper.

Multiple morphogens (and multiple receptors) for PFEs and eyespots may also save the gradient model. In this multiple morphogen model, there are a few different chemicals that act as morphogens. This model explains a difference between the eyespot-forming and eyespot-less compartments. That is, a morphogen for a PFE is secreted in both compartments, but a morphogen for an eyespot is not secreted in an eyespot-less compartment. However, considering that the PFE is equivalent to the outer eyespot ring belonging to the border symmetry system, multiple morphogen factors are not likely. The introduction of multiple factors in a model can produce all-around models but violates the parsimony of model construction.

Despite these efforts, it is better to abandon the idea of the gradient model, considering its difficulty in explaining the color pattern rules and other points discussed in the previous section. An alternative model is the wave model, in which the signal is transmitted as a series of waves (Otaki 2008a). In this context, the two sub-step model discussed above may be modified to support the wave model, in which the first morphogen for a PFE is released as the first wave and the second morphogen is released as the second wave for the eyespot (more precisely, as the second wave for the eyespot outer ring and then as the third wave for the eyespot inner ring) (Otaki 2008a; Dhungel and Otaki 2009). In this two-morphogen

model (wave model), two (or three) morphogens are identical in chemical (or physical) qualities (and therefore different from multiple chemical factors) but are released heterochronically as a train of pulses, being consistent with the heterochronic uncoupling model for TS-type changes (see below). These two models (the wave model and the two-morphogen model) have not been discarded. Rather, they have been adopted, together with the heterochronic model below, and synthesized as the induction model. They may collectively be called the **adopted models**.

There is a weakness in this wave model (Otaki 2008a). Focal damage produces a smaller-than-usual eyespot, indicating the source dependence of the signal. In general, wave signals are not source dependent, theoretically. However, the results of damage experiments can be explained well by the revised version of the induction model (see below).

7.4.4 Heterochronic Uncoupling Model for TS-Type Changes

I have examined the color pattern modifications induced by temperature shock or pharmacological treatments (collectively called the TS-type modifications) (Hiyama et al. 2012; Otaki and Yamamoto 2004a, b; Otaki et al. 2005b, 2006, 2010; Otaki 2007, 2008b, c; Mahdi et al. 2010, 2011) (Fig. 7.7a). It is worth noting that temperature treatments (Nijhout 1984) and pharmacological treatments (Otaki 1998, 2008a; Serfas and Carroll 2005) are the only means that can efficiently create

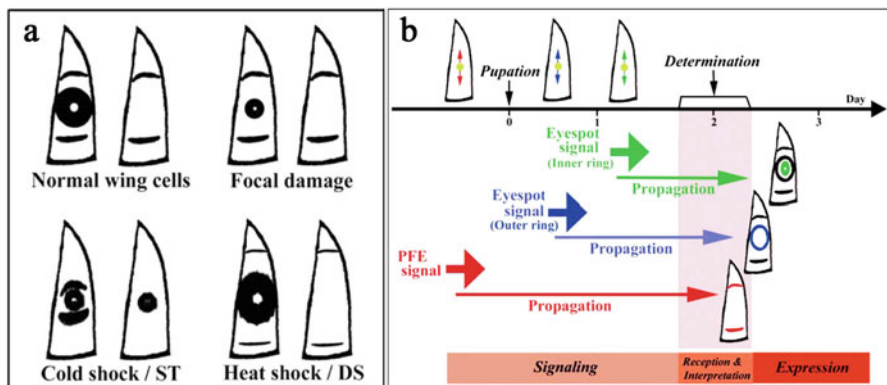


Fig. 7.7 Effects of physiological treatments on eyespot and parafocal element. Reproduced and modified from (Otaki 2011a). (a) Modification patterns of various treatments. Two wing compartments (one with an eyespot and a parafocal element and the other with a parafocal element only). ST and DS indicate treatment with sodium tungstate and dextran sulfate, respectively. (b) Interpretation of the modifications. Signals are released in the order parafocal element, eyespot outer ring, and eyespot inner ring

this “artificial rearrangement of elements” or “elemental transformation,” which is reminiscent of evolutionary trial and error to invent new color patterns based on the nymphalid ground plan. These color pattern modifications are evolutionarily and physiologically relevant (Hiyama et al. 2012; Otaki and Yamamoto 2004a, b; Otaki et al. 2005b, 2006, 2010; Otaki 2007, 2008b, c; Mahdi et al. 2010, 2011), justifying their use as an important method to construct a formal model. The **threshold change model** is the most popular interpretation of the TS-type modifications (Otaki 1998, 2008a; Serfas and Carroll 2005) as well as of physically induced modifications (Nijhout 1980a, 1985; French and Brakefield 1992, 1995; Brakefield and French 1995). However, the TS-type modifications cannot be reproduced by simple threshold changes, as not only relative locations but also the size and colors of the elements are changed. For example, modifications of PFE in an eyespot-less compartment often produce eyespot-like spots (Otaki 2008a).

Because the TS-type modifications are interpreted as a series of possible color pattern snapshots during development, the modifications are likely consequences of a delay of the signaling step (slow signal propagation) or an acceleration of the reception step (Otaki 2008a). That is, temperature shock and pharmacological treatments introduce a time difference between the signaling and reception steps, leading to the heterochronic uncoupling model for TS-type changes. This model simply notes that the TS-type modifications are products of snapshots of propagating signals, which is part of the basis of the induction model (Fig. 7.7b).

7.5 Part III: Induction Model

7.5.1 *An Overview*

To be consistent with the color pattern rules discussed in Part I above and to reflect a few relevant models discussed in Part II, an integrated model is required. To this end, I have proposed the **induction model** (Otaki 2011a, 2012b). This model is largely based on the “movement” of PFEs and eyespots by tungstate injection and other physiological treatments (Fig. 7.7a). In other words, the induction model is not based on the putative diffusive molecule, which is in contrast to the gradient model.

The physiological modifications can be interpreted as follows, which is indeed a simplified version of the induction model to explain a determination process of the border symmetry system (Fig. 7.7b). Signals for PFEs, the outer ring, and the inner ring are released independently in this order with defined intervals, and each signal propagates independently. These signals are simultaneously received by immature scale cells at the reception step.

7.5.2 *Early and Late Stages*

The induction model can be separated into many steps but roughly into two stages: the early and late stages (Fig. 7.8a). The early stage is the primary signal expansion and settlement. The late stage is the induction of activating signals (and their self-enhancement) and inhibitory signals and their stabilizing interactions. The late stage of the induction model employs the concept of “the short-range activation and long-range lateral inhibition” (Fig. 7.8b), which is the core concept of the reaction-diffusion model (Gierer and Meinhardt 1972; Meinhardt and Gierer 1974, 2000; Meinhardt 1982). In the induction model, the dark and light areas in an eyespot correspond to the areas of activator and inhibitor signals, respectively.

In contrast, the early stage does not follow the reaction-diffusion mechanism because the method of signal propagation is different; the signal is thought to be propagated according to the **rolling-ball model** (Otaki 2012b). The signal behaves like numerous minute balls rolling on a board of even friction (constant deceleration) (Fig. 7.9a). This behavior is described by classical mechanics. The propagation is thus determined by the initial velocity of each minute unit signal. In addition, the interval of signal release determines the overall shape of an eyespot. The signals propagate slowly and gradually slow down. These properties of signals satisfy the binary rule and the inside-wide rule and produce natural and experimentally induced eyespots and PFEs. These properties also satisfy the uncoupling and heterochronic nature of the signal. It is also possible to simulate morphological differences between small and large eyespots (Fig. 7.9b).

7.5.3 *Settlement Mechanisms*

In the induction model, there are different modes of **signal settlement** that are proposed (Otaki 2012b). First, a snapshot of propagating signals may be taken by the transition from the signaling to reception steps (the time-out mechanism). Second, propagating signals stop when velocity is lost spontaneously because of low initial velocity (spontaneous velocity-loss mechanism) and when the propagation is blocked by an inhibitory signal nearby (repulsive velocity-loss mechanism). The repulsion comes not only from a nearby element (non-self-repulsive velocity-loss mechanism) but also from the signals for the imaginary ring (the outermost inhibitory ring that is not well expressed) that are induced by the outermost dark ring (self-repulsive velocity-loss mechanism). In this sense, the speed and level of the inhibitory signal induction primarily determine the final size of an eyespot. The self-repulsive mechanism thus ensures autonomous determination of an eyespot.

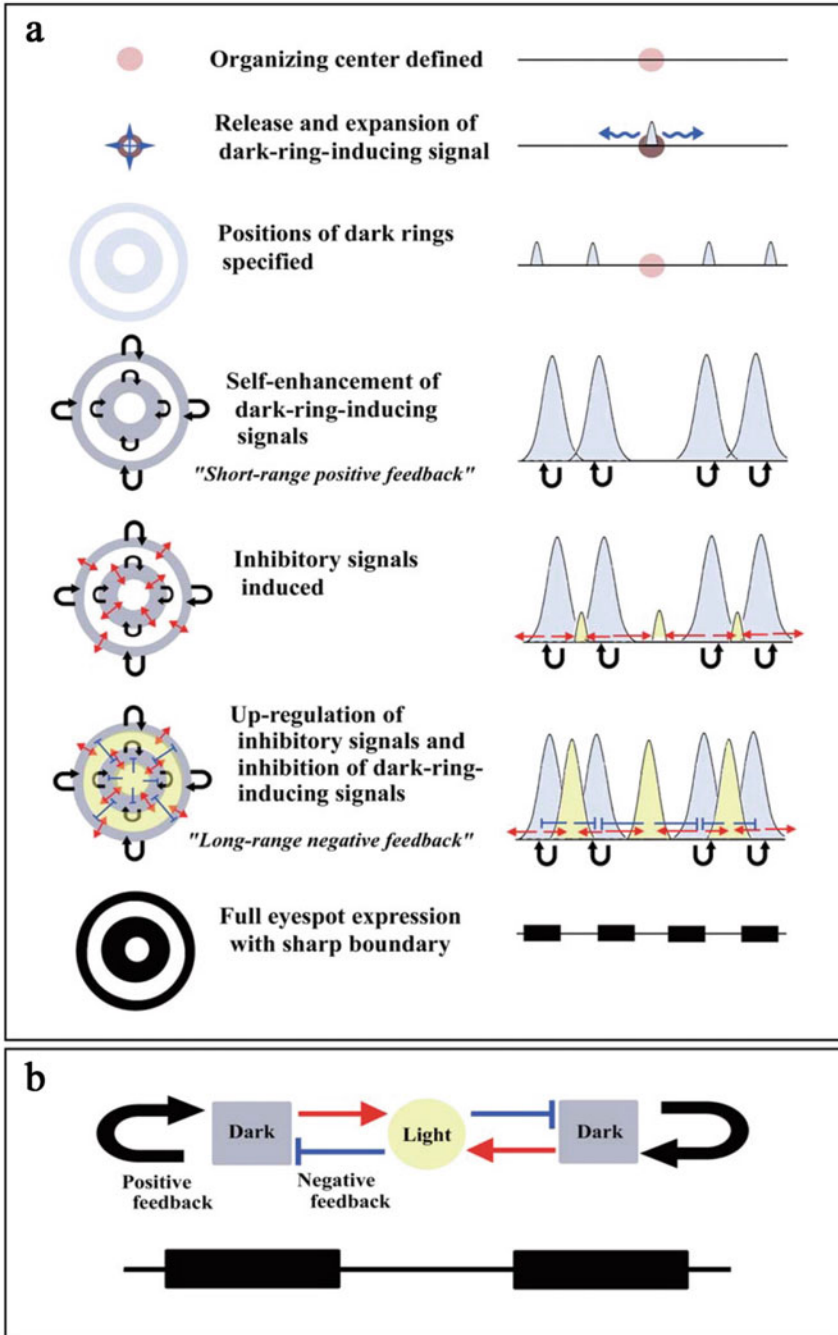


Fig. 7.8 Induction model for positional information. Reproduced from Otaki (2011a). (a) Sequential steps of eyespot formation. (b) Short-range activation and long-range inhibition in the late stage of the induction model

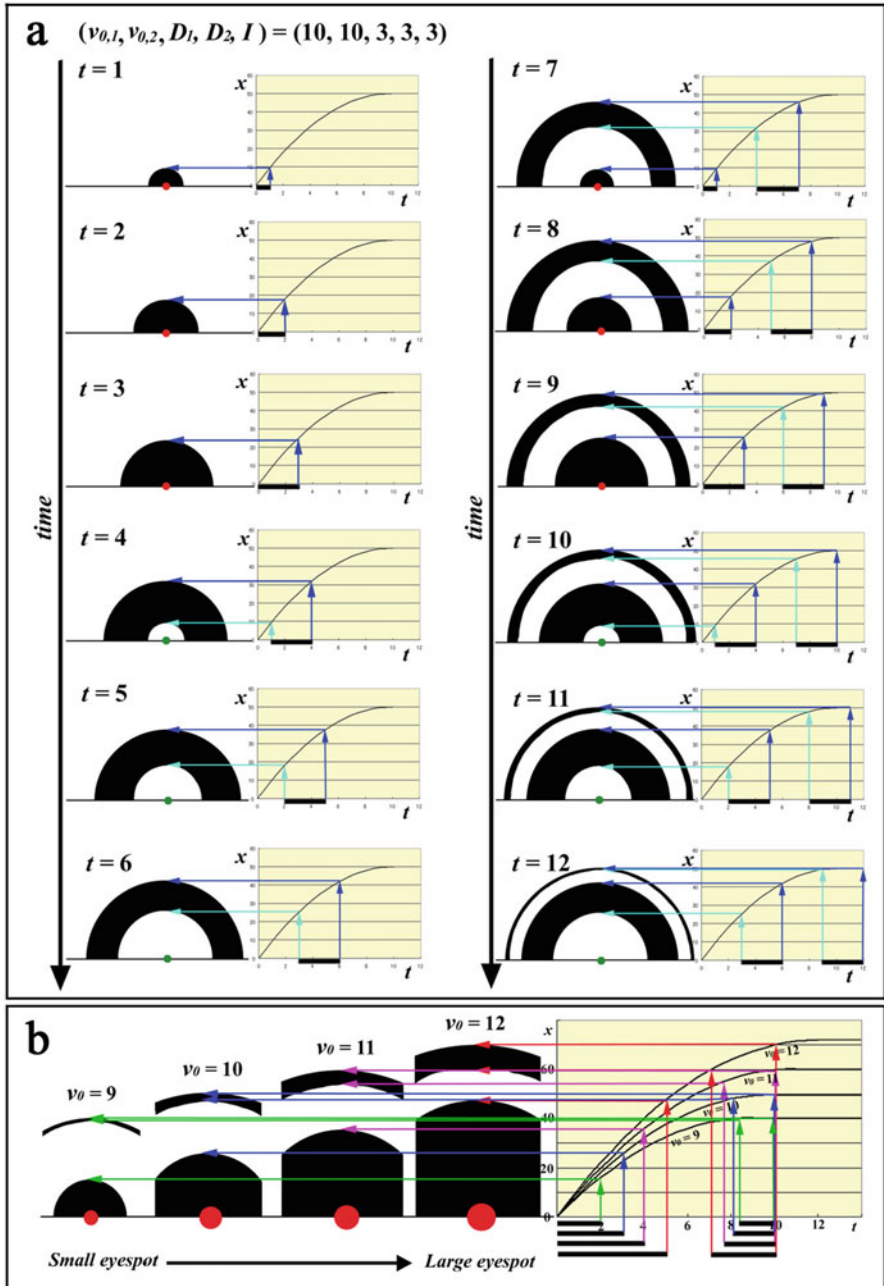


Fig. 7.9 Simulation of eyespot formation based on the rolling-ball model. Reproduced from Otaki (2012b). (a) Time course of developmental signals for a typical eyespot. The signals follow the curve shown on the right side of each time point. Initial velocity (v_0) and signal duration (D) are set for two black rings together with their signal interval (I). (b) Effect of various initial velocities (v_0). Various eyespots are produced

7.5.4 *Mechanisms for Self-Similarity*

There should be a mechanism that produces self-similar structures, which is based on the following mechanism: highly enhanced activating (black-inducing) signals in the late stage would signify a new organizing center. This mechanism can be explained by the ploidy hypothesis (Iwata and Otaki 2016b), which states that the morphogenic signal for color patterns is indeed a ploidy signal that induces polyploidization and cellular size increase (see below), together with the physical distortion hypothesis, which states that cellular and epithelial distortions act as morphogenic signals (see below). The origin of distortions can be considered as organizing centers. Importantly, the self-similarity of eyespots and PFEs argues for the repulsive velocity-loss mechanism and against the time-out mechanism because the signal dynamics should still persist after the possible time-out for the primary organizing centers for eyespots, when the secondary organizing centers for parafocal elements are determined and become activated. That is, the time-out mechanism cannot explain the heterochronic behaviors of the primary and secondary signal dynamics.

7.5.5 *Reality Check*

Is there any signal that can follow the rolling-ball model in biological systems? In the mesoscopic world (not microscopic world explained by quantum physics nor macroscopic world explained by classical mechanics) of cells and molecules in water, Brownian motion and non-covalent molecular interactions prohibit the rolling-ball-like behavior of a molecule. In contrast, mechanical force can be transmitted easily via an epithelial sheet if epithelial cells are connected firmly but flexibly. That is, epithelial distortions may show rolling-ball-like behavior and act as morphogenic signals from organizing centers. In Part IV below, I review evidence for the ploidy hypothesis and the distortion hypothesis.

7.6 **Part IV: Ploidy, Calcium Waves, and Physical Distortions**

7.6.1 *Scale Size of Elements*

At the cellular level, one cell builds one scale (Nijhout 1991), which may be dubbed the **one-cell one-scale rule**. Therefore, any morphological features of scales directly indicate the developmental status of the scale-building cells (or simply scale cells). Scale size distribution is graded from the basal to peripheral areas of a wing in butterflies and moths (Kristensen and Simonsen 2003; Simonsen and

Kristensen 2003). Similar size gradation has been found in the background scales in *Junonia orithya*, *J. almana*, *Vanessa indica*, and *V. cardui* (Kusaba and Otaki 2009; Dhungel and Otaki 2013; Iwata and Otaki 2016b).

What about the size of scales that constitute elements? In *J. orithya* and *J. almana*, the scale size of an element is larger than that of its surrounding background (Kusaba and Otaki 2009; Iwata and Otaki 2016b) (Fig. 7.10). In this sense, scale color and size are reasonably correlated, which can be called the **color-size correlation rule for scales**. This rule may sound trivial but is indeed important as a clue to understanding the possible nature of morphogenic signals for color patterns (see below). Furthermore, the largest scales in an element are found roughly at the center of an element (Kusaba and Otaki 2009; Iwata and Otaki 2016b). This may be called the **central maxima rule for elemental scale size**. It is important to recognize that scale size changes suddenly at the boundary between the inner black ring of an eyespot and a yellow ring. There are similar abrupt changes at the outer ring boundary and the PFE boundary. These abrupt size changes may reflect the independence of black areas (the binary rule and the uncoupling rule) rather than gradual changes of positional information.

Additionally, scales of different colors differ in their structure, such as overall scale shape and scale ultrastructure (Gilbert et al. 1988; Nijhout 1991; Janssen et al. 2001). Our laboratory also obtained similar results using *Junonia* and other butterflies (Kusaba and Otaki 2009; Iwata and Otaki unpublished data; Kazama et al. 2017).

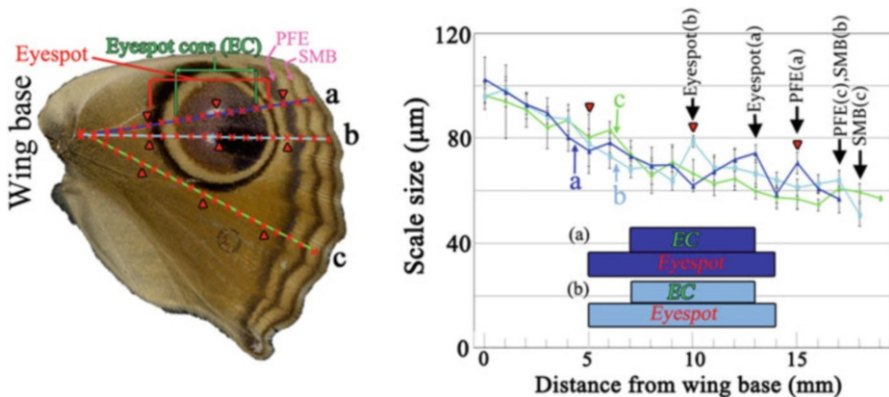


Fig. 7.10 Scale size distribution on a wing of *Junonia almana*. Reproduced from Iwata and Otaki (2016b). A dorsal forewing was examined along lines *a*, *b*, and *c* in 1.0 mm intervals (left). Results are shown in the graph (right). Along line *b*, scale size peaked at the center of the eyespot. Along line *a*, the peak was located at the distal edge of the eyespot core and also at the center of the parafoveal element (PFE). Line *c* did not show conspicuous peaks. All lines showed the size decrease from the basal to the peripheral areas except at the elemental positions

7.6.2 Ploidy Hypothesis

According to Henke (1946) and Henke and Pohley (1952), scale size reflects the degrees of ploidy of the cell in moths (Sonhdi 1963; Cho and Nijhout 2013). This size-ploidy relationship, or the **size-ploidy correlation rule for scales and cells**, is probably applicable to butterflies. This leads us to propose the **ploidy hypothesis** (Fig. 7.11a) (Dhungel and Otaki 2013; Iwata and Otaki 2016a, b). This hypothesis states that morphogenic signals induce polyploidization of signal-receiving cells. The higher the ploidy level, the larger the cell. The larger the cell, the larger the scale it can produce. Simply because a high ploidy level means high numbers of genes for pigment synthesis enzymes, the concentration of pigment in the scales can

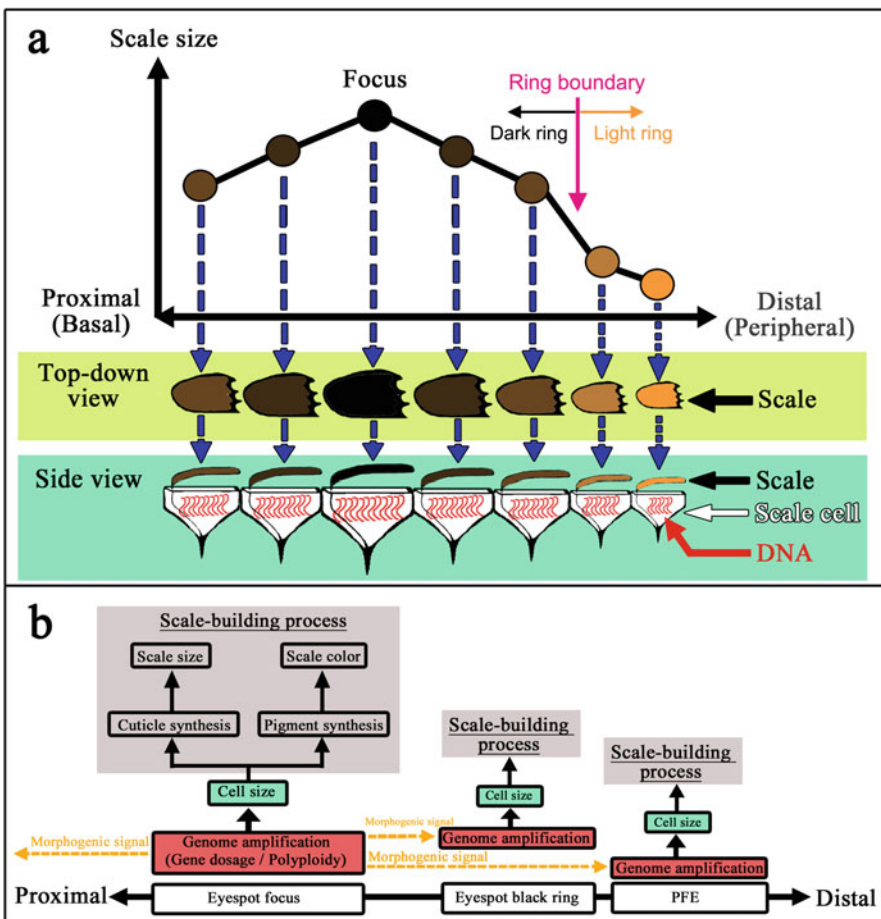


Fig. 7.11 Ploidy hypothesis. Reproduced from Iwata and Otaki (2016b). (a) Scale size distribution and its relationship with cell size. (b) A hypothetical determination process for scale color and size based on the induction model

change their coloration. Alternatively, gene dosage determines which pigment to be synthesized. In this way, the level of morphogenic signals indirectly determines the levels of pigment chemicals in a scale through the regulation of polyploidization or gene dosage. The ploidy hypothesis is an important component of the induction model (Fig. 7.11b).

The recent discovery that a cell cycle regulator, *cortex*, plays a role in the darkening of the wings in butterflies and moths (Nadeau et al. 2016; van't Hof et al. 2016) may be a surprise for many biologists, but this discovery fits well with the ploidy hypothesis, although it is not discussed in these papers. This cell cycle regulator may control a process of polyploidization of immature scale cells, which determines the final coloration of scales according to the ploidy hypothesis.

7.6.3 *Calcium Waves*

Recently, spontaneous long-range calcium waves have been discovered in the developing pupal wings *in vivo* (Ohno and Otaki 2015b). Calcium waves have been found to be released from the prospective eyespot centers and from damage sites (Fig. 7.12), although wave origins are not restricted to known elemental centers. At least four different types of waves are observed: expanding ring or traveling line, wandering line or point, oscillating area, and traveling oscillating area. Color patterns are disrupted by the injection of thapsigargin, a well-characterized inhibitor of Ca^{2+} -ATPase in the endoplasmic reticulum. For example, fuzzy boundaries of pattern elements have been reported in thapsigargin-treated individuals (Otaki et al. 2005b; Ohno and Otaki 2015b). I speculate that the calcium waves act as the activator in the late stage of the induction model, but calcium waves are not morphogenic signals themselves. Morphogenic signals are likely to be physical distortions (see below), and calcium waves may be released from these distortion waves.

7.6.4 *Physical Distortion Hypothesis*

What are the morphogenic signals? Despite the prediction of the rolling-ball model, it is difficult to imagine numerous minute “balls” rolling out from the center of a prospective eyespot. A hint comes from a study on pupal cuticle spots and their associated structures. Remarkably, organizing centers are often marked inherently as pupal cuticle focal spots in butterflies (Nijhout 1980a, b, 1990, 1991; Otaki et al. 2005a; Taira and Otaki 2016) (Fig. 7.13). This feature is especially notable in *Junonia* butterflies, but it is widely seen in many nymphalid butterflies that have eyespots or black spots (Otaki et al. 2005a). In addition, some cuticle focal spots are accompanied by cuticle marks. These spots and marks are likely produced by organizing cells for adult eyespots. The epithelial distortion structures of the

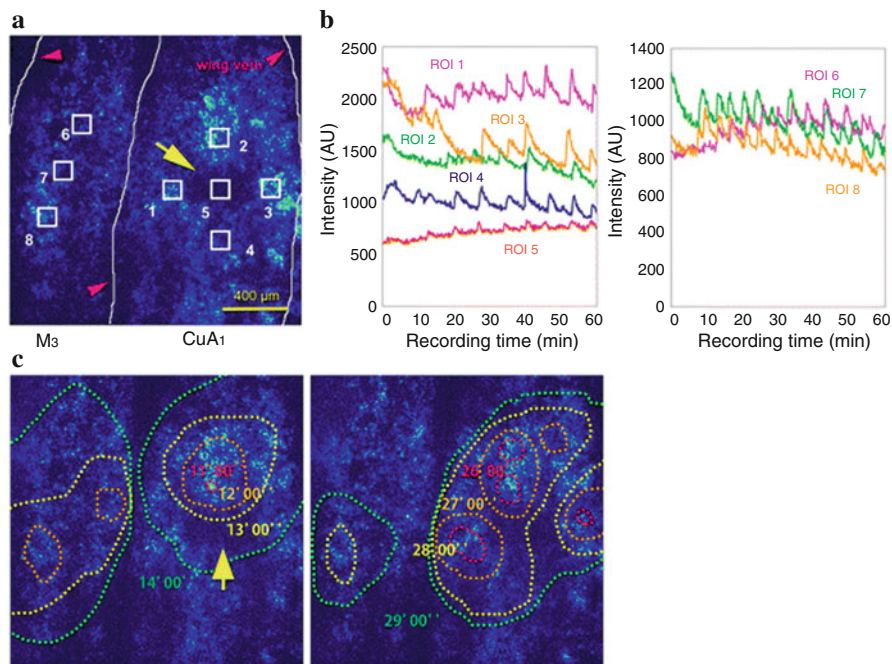


Fig. 7.12 Spontaneous calcium waves from the prospective organizing center for eyespot. Reproduced and modified from Ohno and Otaki (2015b). (a) Calcium signals (*blue*) in the M_3 and CuA_1 compartments. ROIs 1–8 were examined for intensity changes in the following panels. The *yellow arrow* indicates the prospective eyespot (also in c). The *red arrowheads* indicate the wing veins. (b) Fluorescence intensity changes of Fluo-4 in ROIs. (c) Propagating calcium signals around the prospective eyespot area. Panels in a and c show a single identical visual field at different time points. The shape of a wave at a given time point (in min) is depicted by a *dotted circle*

prospective elements have also been confirmed by *in vivo* imaging of the living tissue (Ohno and Otaki 2015a; Iwasaki et al. 2017). The association of the organizing centers with distortion structures may be called the **distortion rule for organizing centers**.

It is likely that the cellular volume increase or change in shape at the particular position results in the formation of the pupal cuticle spot as a by-product. The cellular changes would cause epithelial distortions, which could expand as a series of waves. The slow contraction of the wing tissue during the early pupal stage revealed by time-lapse movies (Iwata et al. 2014) probably helps to expand the distortion waves. That is, the **physical distortion hypothesis** states that morphogenic signals are physical distortions of an epithelial sheet. The distortion hypothesis thus states that morphogenic signals cannot be reduced to a substance. Rather, these signals are a wave, i.e., a physical phase change of a medium (the epithelial sheet). To realize this signaling system, the epithelial sheet has to have a tension or at least cellular connections in some way, which is likely the case (Ohno and Otaki

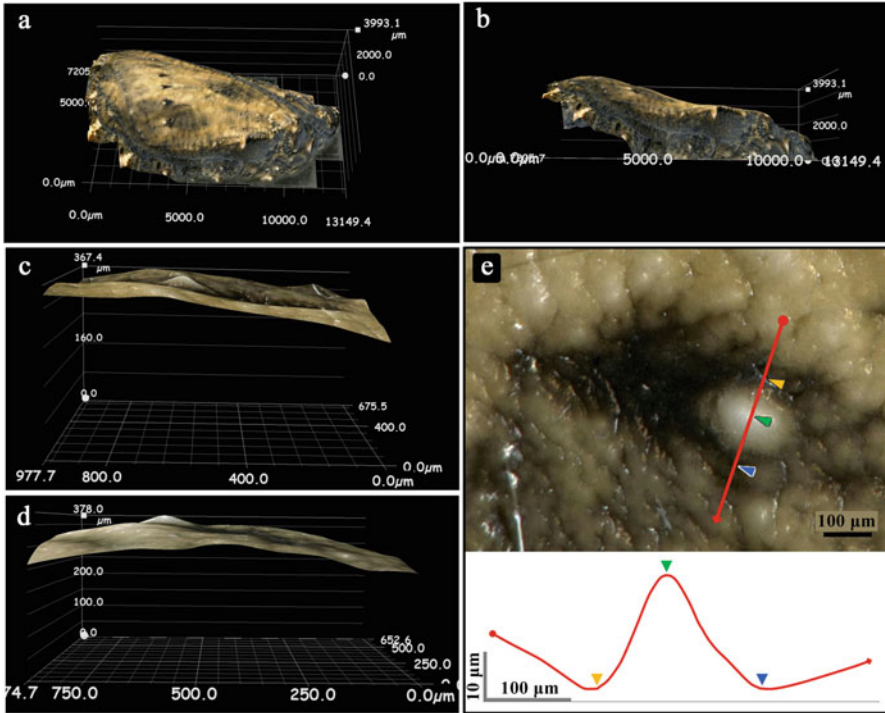


Fig. 7.13 Pupal focal cuticle spot of *Junonia orithya* based on three-dimensional reconstruction. Reproduced from Taira and Otaki (2016). (a) Top-down view of the entire left forewing surface. (b) Side view. (c, d) A pupal cuticle spot. (e) High-magnification image of a pupal cuticle spot and its cross-sectional height. Colored arrowheads in the image indicate the site of measurement in the graph

2015a). A physical distortion could open stretch-activated calcium channels, as in other systems (Lee et al. 1999; Tracey et al. 2003; O’Neil and Heller 2005; Hillyard et al. 2010). Epithelial cells that have received enough calcium ions inside could duplicate their genome and differentiate into scale cells that harbor specified cell size and specified scale size. In this time series, gene expression changes are downstream (not upstream) events; in other words, these changes are not a cause but a result of morphogenic signal propagation.

The distortion hypothesis states that mechanical disturbance of an epithelial sheet functions as morphogenic signals. This idea may sound unfamiliar to biologists, but this should not be a reason to reject this model as long as the model is consistent with experimental and observational results. Fortunately, mechanobiology is an expanding interdisciplinary field between biology and physics (Iskratsch et al. 2014). Changes in the mechanical property of a cellular sheet may be caused by physical damage and subsequent wound-healing processes (Antunes et al. 2013) and by cell death (Teng and Toyama 2011; Toyama et al. 2008) in addition to cellular size and shape changes.

7.6.5 *Damage-Induced Ectopic Elements*

Physical damage at the prospective eyespot center immediately after pupation has been shown to reduce or eliminate eyespots, but damage at the prospective background induces ectopic elements in butterfly wings (Nijhout 1985; Brakefield and French 1995; French and Brakefield 1992, 1995; Otaki et al. 2005a, b; Otaki 2011c). Ectopic eyespots are most likely by-products of a wound-healing process. I believe that physical damage elicits physical distortions of the epithelial sheet. Interestingly, the genes expressed are similar in normal development and in the healing process (Monteiro et al. 2006). Likewise, physical damage elicits calcium waves in normal development and in the healing process (Ohno and Otaki 2015b). Thus, the wound-healing process and the normal process of color pattern development would share similar mechanisms not only at the phenotypic level but also at the molecular level.

If the putative morphogen from a natural organizing center is a specific substance, it is difficult to imagine that physical damage confers an ability in immature epithelial cells to synthesize that specific substance. Probably partly for this reason, it is often interpreted that physical damage (and also pharmacological treatments) increases or decreases the “preset” threshold levels of signal-receiving immature scale cells in the conventional gradient model (Nijhout 1985; Brakefield and French 1995; French and Brakefield 1992, 1995; Otaki et al. 2005a, b; Otaki 2011c). Although it is entirely possible that this interpretation explains many damage-induced effects, dynamic interactions between two adjacent eyespots during development, shown in *J. almana*, suggest that a simple change in threshold levels is not realistic; when one eyespot becomes smaller as a result of damage, the other eyespot becomes larger (Otaki 2011c). It should also be noted that a possible mechanism of how damage lowers threshold, if this is the case, has never been well explained.

7.6.6 *Focal Damage*

What will occur when physical damage at the eyespot focal site is elicited? At the early stage of pupae, a smaller-than-normal eyespot is produced. Interestingly, late damage produces a larger-than-normal eyespot. The late damage result is explained by the addition of a new signal because this is similar to the fact that background damage produces a new signal for an eyespot or a black spot. The early damage result is explained as the damage of the signal-producing cells, resulting in the low-level signal. However, this result may indicate the source dependence of the signal, whereas wave signals are supposed to be source independent.

Considering the physical distortion hypothesis, the focal damage during the signal release may simply relax the distortion of the epithelial sheet. As a result, a distortion wave cannot go away. It may even go back to the original state. In contrast, at the later stage, epithelial distortions may have already been relaxed and

the signal is ready to settle. Thus, the late focal damage may recreate the distortion, such as the background damage, resulting in a larger-than-normal eyespot.

7.7 Part V: Generalization and Essence

7.7.1 Reinforced Version of the Induction Model

To summarize, the reinforced version of the induction model for eyespot development is explained below (Fig. 7.14). This scheme includes many speculations to bridge the fragmented knowledge of the butterfly wing system. For simplicity, the development of a simple black disk (i.e., black spot) is delineated first below (Fig. 7.14a).

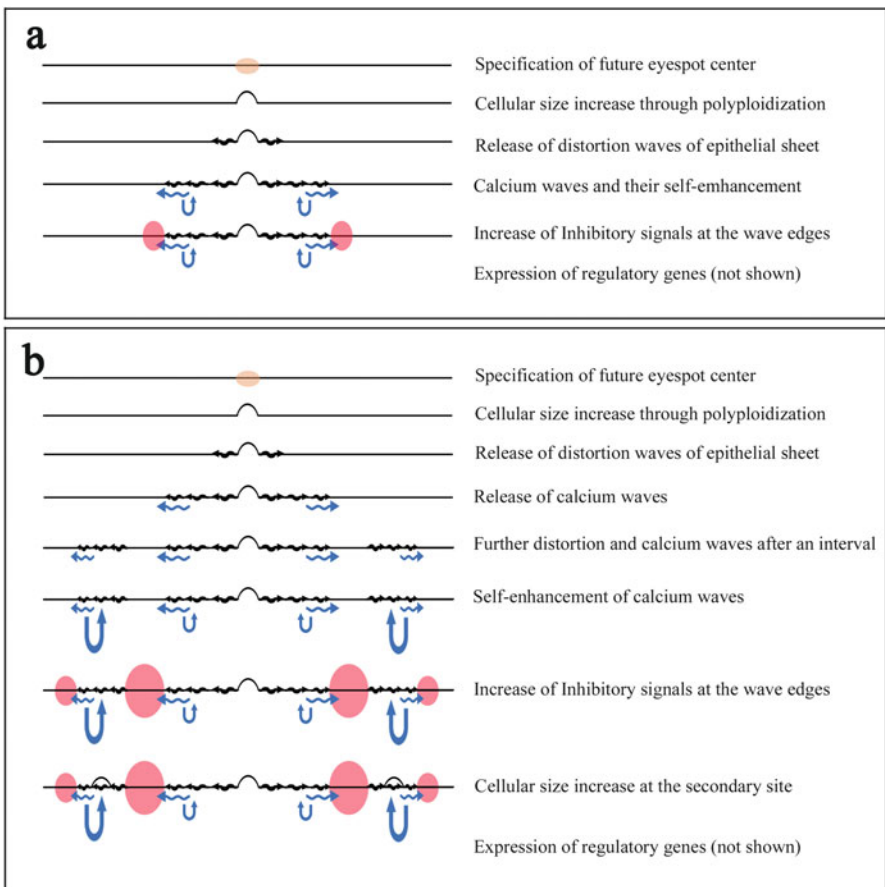


Fig. 7.14 Reinforced induction model. Time series of events from the top to the bottom. (a) Black spot formation. (b) Eyespot formation

In the beginning, a future eyespot center (organizing center) is first specified. Physical distortion of the epithelial sheet is formed due to cellular size changes and deformations. These cellular changes would cause distortion waves that propagate radially to surrounding cells, according to the rolling-ball model. The propagating waves are “translated” into chemical signals, i.e., calcium waves, possibly through a stretch-activated calcium ion channel on the membrane, acting as an activator in a reaction-diffusion model, as traveling calcium waves have been detected (Ohno and Otaki 2015b). As the physical distortions and their associated calcium signals propagate, calcium signals may be enhanced by themselves as oscillations, as oscillating calcium waves have also been detected (Ohno and Otaki 2015b). Calcium oscillations induce unknown inhibitory signals in cells located in the periphery of the oscillations. The induced inhibitory signals inhibit further propagation of the original calcium signals, finalizing the position and shape of the black spot. Calcium oscillations stimulate cells to undergo genome amplification and to express a set of regulatory genes such as *Wnt*-family genes (Monteiro et al. 2006; Martin and Reed 2014), *spalt* and *Distal-less* (Monteiro et al. 2013; Adhikari and Otaki 2016; Dhungel et al. 2016; Zhang and Reed 2016). Alternatively, calcium oscillations may be stabilized by the *Wnt*/ Ca^{2+} transduction pathway that involves intracellular calcium release (Kühl et al. 2000; Kohn and Moon 2005). Cellular size increases in the prospective black ring according to the genome size or ploidy level. This process may be regulated by the *cortex* gene, which has been identified recently (Nadeau et al. 2016; van’t Hof et al. 2016). The final cellular size or the degrees of polyploidy then determine a repertoire of pigment synthesis genes to be expressed.

When an eyespot is produced, the scheme is more complicated (Fig. 7.14b). A released distortion wave does not readily induce calcium waves, but it progresses for some time. In the meantime, the distortion wave for the outer black ring is terminated, but after an interval, a new distortion wave for the inner black ring is released. At this point, calcium wave induction and its self-enhancement occur, and inhibitory signals are produced at the wave edges, which finalize the position of the black rings. Genome amplification and the expression of regulatory genes follow. Cellular size increases at the prospective black rings according to the number of genomes in a cell. Where the calcium oscillations by self-enhancement are highly active, the high degree of cellular size increase occurs, resulting in the formation of a secondary organizing center, which is often seen in PFEs. This second round of color pattern determination ensures self-similarity between the eyespot and PFE.

In this series of events, the three most important events are distortion waves (D), calcium waves (C), and gene expression changes (G), which may be called the **DCG cycle**. This series of events repeats twice to create the self-similarity between the eyespot and PFE.

7.7.2 *Generalization to Other Systems*

Thus far, I have discussed the nymphalid wing color pattern system. The applicability of the information above to other butterfly systems has not been examined, but the lycaenid system is probably similar because the symmetry rule and the core-paracore rule hold true, at least in the lycaenid central symmetry system (Iwata et al. 2013, 2015). The fish skin system is different from the butterfly wing system in that epidermal cells in fish can move in response to surrounding cells, whereas butterfly cells cannot move. Nonetheless, the inductive nature of different colors based on short-range activation and long-range inhibition is likely shared in fish and butterflies; both systems can produce ectopic patterns associated with calcium waves after physical damage (Ohno and Otaki 2012).

Morphogenesis is three-dimensionally dynamic in any developmental system, but a good example of three-dimensional dynamism of the epithelial sheet is the morphogenetic furrow in the *Drosophila* retina (Greenwood and Struhl 1999; Schlichting and Dahmann 2008; Sato et al. 2013). The furrow is a physical distortion of the imaginal eye disk. This epithelial fold moves, and its movement coincides with cellular differentiation. The furrow may physically elicit the expression of morphogenetic genes such as *hedgehog* and *decapentaplegic* if the furrow is not a physical by-product of cellular differentiation.

7.7.3 *DCG Cycle for Self-Similarity and Its Implications*

Nearly two-dimensional butterfly wing color patterns can be viewed, somewhat ironically, as a developmental and evolutionary application of three-dimensional bulges and dents that are used in general morphogenesis. To achieve self-similar structures, organisms evolve to transmit a signal from the primary to secondary organizing centers through distortion waves of the epithelial sheet. This mechanical lateral signaling mechanism can cover a long distance with simplicity. Thus, it may be a very early evolutionary innovation. Evolution of the signal translator, mechanosensory calcium channels, might have followed, together with several genes that stabilize calcium oscillations and inhibition. In conclusion, the DCG cycle for self-similar structures has deep implications for biological evolution and development.

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