

Mimicry in Butterflies: Microscopic Structure

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Abstract. In a study of mimicry, microscopic structures of the scale of butterflies were examined by scanning electron microscopy. These structures were compared between 2 species of butterfly, *Hypolimnas anomala* and *Euploea mulciber*. It is well known that the female of the former species imitates the male of the latter species by use of structural color. In the present study, it was found that the mimic (female *Hypolimnas anomala*) imitates the model (male *Euploea mulciber*) in microscopic structure as well. Furthermore, it was found that the mimic has characteristics of both the male of its own species and the model.

1. Introduction

Mimicry is an interesting phenomenon in which species that are not closely related nevertheless look alike. This accidental resemblance is found throughout nature, and is selected for because it benefits the mimic. The simple genetic explanation of mimicry is that it is a result of mutations and selection pressure. However, mimicry is not a subject so easy to clarify.

Studies of mimicry have generally not progressed beyond the stage of guesswork. This is despite the fact that mimicry has been studied for many years, particularly because it is evidence of natural selection. Various problems inherent in the study of mimicry make experimental verification difficult (Sec. 3). Thus, during the 100 years following discovery of mimicry, not only has little been discovered about mechanisms of mimicry, but little evidence has been found to even verify the function of mimicry.

At present, 140 years after the discovery of mimicry (BATES, 1862), conclusive evidence of the function of mimicry is obtained in studies. However, mimicry (which falls within the biological subject of astringency) is still a mysterious phenomenon. It is difficult to obtain credible answers to the following questions: how mimicry occurs at the genetic level; how mimicry has evolved; why there is no intermediate form between mimic and model; how the present variety of mimicry has been formed.

In this study, I examined the mechanism of mimicry. The development of macroscopic structures involved in mimicry from the molecular or genetic basis of mimicry is a long, complex process. Examination of micron-order structure can help to bridge this large gap. In this paper, I describe an example of the microscopic structure that underlies mimicry, and the way that it is related to structural color.

2. Structural Color

Some species of South American butterfly have brilliant metallic blue wings. The metallic luster of its wings does not fade even after 100 years. This is because the origin of this coloration is not pigment but rather microscopic structure that is not affected by chemical change. The principle of this phenomenon was simply referred to as grating because of its high reflectance. However, its optical characteristics can not be explained by grating. The mystery of the lack of angular dependence of the scattered optical wavelength (it appears blue from wide angle) remained disregarded for many years, except a few researchers (NAGAYAMA, 1999). This mysterious feature was found to be due to a peculiar optical structure. Moreover, it is intimately related to the photonic band, a subject researched by physicists in recent years. Its features were elucidated in recent studies (KINOSHITA *et al.*, 2000, 2002; PARKER *et al.*, 2001).

Structural color is common throughout nature, operating on various principles. Many of these principles have still not been explained. Systematic analysis of this phenomenon is needed, using techniques such as structural model analysis and comparison of spectra. A wide variety of fields of application are relevant to structural color; e.g., most engineering fields in which color is an important factor (like fiber, clothes and paint). Structural color produces color without pigment, it makes tone that is qualitatively impossible by pigment, and it is resistant to discoloration due to chemical change over time.

3. Mimicry and Structural Color

Details of mimicry have been examined in several studies (WICKLER, 1968; EDMUNDS, 1974; UEDA, 1999). A primary focus of mimicry research is Batesian mimicry, in which a species benefits by imitating the appearance of a species that is rarely attacked by predators due to its unpleasant taste. Although Batesian mimicry was discovered in 1862 (BATES, 1862), it remained merely a hypothesis for many years, due to the difficulty of verifying it.

Much of the difficulty in verification of mimicry involves problems with objectivity, which consist of difficulty in designing objective experiments and difficulty related to human perception. Difficulty in designing objective experiments is due to difficulty in preparation of environmental conditions; i.e., natural behavior of an organism can be affected by artificial conditions. For example, in studies of effects of mimicry on predation behavior, conditions can change with time because of learning of a predator. The experimental environment can also disturb the natural behavior of the prey, whether it is the mimic or the model (model is poisonous species imitated by the mimic). It was the work of Brower that first surmounted these difficulties, achieving reappearance of mimicry under experimental conditions for the first time about 100 years after Bates (BROWER, 1958a, b, c). A summary

of recent progress in this field can be found in a paper by UESUGI (1999).

As mentioned above, there is also the difficult problem of human perception. Mimicry by prey involves already 3 organisms: model, mimic and predator. In addition to this complexity, the addition of the human observer means that there are actually 4 organisms involved. In other words, there are important differences between human perception of the prey and the predator's perception of the prey. For example, unlike human vision, the vision of birds (the main predators of insects) is composed of 4 primary colors. Moreover, birds can also perceive ultraviolet light, which insects can also perceive. Therefore, birds may perceive patterns that are different from those that a human being perceives. Consequently, in order to judge whether a mimic looks like a model, we must "ask the birds". For instance, although the case of industrial melanism of the peppered moth (*Biston betularia*) is used in textbooks as a good example of natural selection, the possibility of misinterpreting such phenomena was recently pointed out (MAJERUS, 1998; SARGENT *et al.*, 1998; SHIBATANI, 1999).

Due to the above considerations, in the present study, I investigated mimicry on the basis of microscopic structure, not macroscopic visual patterns. There are some butterfly species that mimic structural color, and comparison of structures involved in this form of mimicry at the microscopic level has the following advantages over macroscopic study:

- 1) Objectivity of observation

Differences in perception between humans and birds are excluded when the microscopic structures that determine optical properties are compared.

- 2) Examination of the underlying mechanism

We can determine the basis of the resemblance between the mimic and model (whether they have the same structure at the microscopic level, or whether the same effect is produced by different structures).

4. Experiments and Results

I examined 2 species that have structural color: the poisonous model *Euploea mulciber* (hereafter referred to as *Euploea*), and the nonpoisonous mimic *Hypolimnias anomala* (hereafter referred to as *Hypolimnias*). These species, which are well known as an example of Batesian mimicry, are distributed together in Southeast Asia. Because *Euploea* belongs to the family Danaidae and *Hypolimnias* belongs to the family Nymphalidae, they are not considered closely related (their taxonomic separation is above the level of genus). As shown macroscopically in Fig. 1, the female *Hypolimnias* is considered to be the mimic of the male *Euploea*.

A recent study of mimicry suggests that the reason why generally mimicry appears only in females is because the benefit due to mimicry is larger in females than in males (OHSAKI, 1995). It is thought that the reason why structural coloration is conspicuous only in the male in the model species (*Euploea*) is that it functions in displaying to the female, as in the case of the peacock's tail.

In the present study, microscopic structure was observed by scanning electron microscopy (SEM). Male *Euploea* was compared with male *Hypolimnias*, because their macroscopic appearance differs greatly, and because the structure of their scale can be used to confirm distinction between them. In general, the structure of scale is useful for



Fig. 1. Left: *Euploea mulciber* (poisonous). Right: *Hypolimnas anomala* (nonpoisonous). Upper, female; lower, male. Upper right, mimic; lower left, model.

taxonomic classification when distinction by normal way is difficult because of small differences in copulatory organs or visual patterns (SAIGUSA, 1961; WARREN, 1961; KATAOKA, 1997). For reference, rough characteristics of the microscopic structure of scale are shown in Fig. 2 (GHIRADELLA and RADIGAN, 1976).

I found clear differences in structural characteristics between the 2 species (Fig. 3). In the male *Euploea* (model), the ridge has a shelf-like structure composed of multiple layers of stick-shaped units. In the male *Hypolimnas*, the ridge is not composed of multiple layers, but has the appearance of a wall composed of wide triangular units. These 2 structures are hereafter referred to as multi-layer type and wall type, respectively. The layered structure of the multi-layer type corresponds to the fragmentary multiple layer responsible for the structural color (KINOSHITA *et al.*, 2000).

Generally, the structure of scale consists of 2 kinds of units that alternate along a line (Fig. 2). Because they form 2 layers, the upper and lower scales are referred to as cover scales and basal scales, respectively. The roles of these layers are discussed in a report by YOSHIDA and AOKI (1989). In both *Euploea* and *Hypolimnas*, there was no great difference in microscopic structure between the cover scales and basal scales. That is, both the cover and basal scales were of the multi-layer type for *Euploea*, and were both of the wall type for *Hypolimnas*.

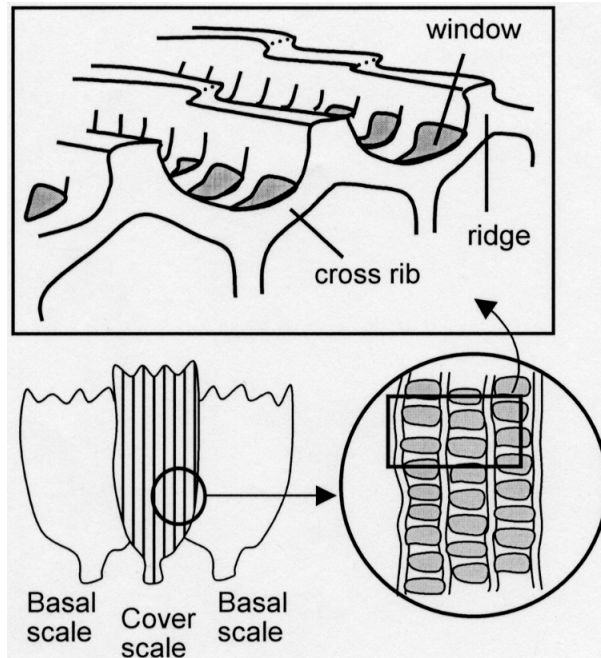


Fig. 2. Structure of scale.

Before showing the most important result about the female *Hypolimnas* (mimic), it is worth reconfirming the interest: since this mimicry is based on resemblance of structural color, it is to be expected that there is similarity in microscopic structure between the model and the mimic. However, there are developmental difficulties in achieving this similarity. There are usually clear differences in scale structure between butterfly species that are so distantly related. So, it is possible that the mimic uses structural color based on principles other than those used by the model, or that it imitates the model using means other than structural color?

The results of SEM observation of the female *Hypolimnas* are shown in Fig. 4. The images clearly show that the cover scale of the mimic closely resembles the multi-layer structure of the model. However, the basal scale of the mimic is almost identical to that of the male of the mimic species: a wall-type structure consisting of triangular units.

5. Summary

In the present study, I found that the cover scales of the mimic and the model have the same multi-layer type structure, and that the basal scales of the male and female of the mimic species have the same wall type structure. Thus, it appears that the structure of this mimic's cover scales has been shaped to conform to that of its model for the imitation effect (with exceeding a distinction of taxonomic family), whereas the structure of the mimic's

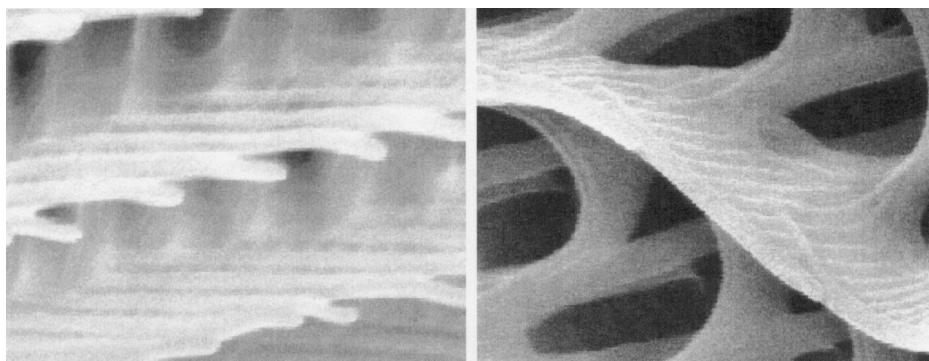


Fig. 3. Comparison of magnified images of scale between males of the 2 species. Left: male *Euploea* ($\times 60\,000$). Right: male *Hypolimnas* ($\times 50\,000$).



Fig. 4. Magnified images of scale of mimic (female *Hypolimnas*). Left: cover scale ($\times 60\,000$). Right: basal scale ($\times 60\,000$).

basal scales has remained relatively unaffected. In future studies, I will investigate the generality of this phenomenon.

The study of mimicry involves many phenomena in addition to visual mimicry. Chemical mimicry may be more common than visual mimicry because essence of mimicry is use of signals between individuals. Recent investigation of mimicry of sounds and chemical signals has produced general idea of molecular mimicry and stimulated research in this field (NAKAMURA *et al.*, 2000). The present findings appear to be an example of microscopic structural mimicry (the term “microscopic mimicry” could be confused with chemical mimicry). However, it was not the purpose of the present study to establish a new type of mimicry, but rather to explore methods for increasing objectivity and elucidating mechanisms in the study of mimicry.

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