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Studies on Light Sensitivity in Butterflies

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We focus our attention to two separate topics on butterflies' light sensitivity: color vision and a unique photoreceptive system located on the genitalia. I will give a brief overview of both topics.

Color Vision

Study on insect color vision is pioneered by Karl von Frisch. Frisch demonstrated that honeybees have color vision when searching for food: honeybees can even see UV as a separate color, but instead they don't discriminate red from gray. Since the impact of Frisch's work was so strong that similar study in other insects had become less intense. Probably therefore, it had mistakenly become more or less a general understanding that all insects are not able to discriminate red. However, many butterflies collect nectar from red flowers, which honeybees seldom visit. Do butterflies see colors, including red? First convincing demonstration of red sensitive photoreceptors in the butterfly compound eye appeared in 1983 (Matic, J Comp Physiol A 152:169-182, 1983), which directly and strongly stimulated us to initiate the study of the compound eye of the Japanese yellow swallowtail butterfly, *Papilio xuthus*.

By combining electrophysiological, histological, and molecular biological methods, we analyzed cellular organization of the *Papilio* retina (Arikawa, 2003). We so far identified six classes of spectral receptors (UV, violet, blue, green, red, and broadband receptors) there (Arikawa *et al*., 1987; Arikawa *et al*., 2003). We then localized these receptors in the ommatidia, the building blocks of the compound eye, each containing nine photoreceptor cells. Quite unexpectedly, we found that the ommatidia are divided into three types in terms of the spectral receptor classes they contain. The three spectrally heterogeneous ommatidia distribute rather randomly over the compound eye (Arikawa and Stavenga, 1997).

In the course of the physiological and anatomical studies of the *Papilio* retina, we found several interesting phenomena. For example, the broadband receptors coexpress green absorbing and red absorbing visual pigments both contribute to generate receptor potential (Arikawa *et al*., 2003; Kitamoto *et al*., 1998). This indicates that a dogma in vision physiology, which is that a photoreceptor cell express a single type of visual pigment, needs adjustment. We also found a reverse case: violet receptors express UV absorbing visual pigment that is functional also in UV receptors (Kitamoto *et al*., 2000). What constructs the violet spectral sensitivity from UV visual pigment is 3-hydroxyretinol that is somehow accumulated in the distal portion of the ommatidia. 3-hydroxyretinol there absorbs UV and thus acts as an UV absorbing filter (Arikawa *et al*., 1999). An extreme case was found in the eye of the small white butterfly, *Pieris rapae*, where green, red, and deep-red receptors all share a green-absorbing visual pigment. Red and deep-red pigments around the rhabdom act as spectral filters that produce distinct spectral sensitivities from a single visual pigment (Wakakuwa *et al*., 2004).

Do butterflies really see colors? To answer the question we established a protocol of behavioral experiments. We trained butterflies to take sucrose solution on a paper patch of certain color in a small cage. We let the trained butterflies select the training color among different colors or different shades of grays, and demonstrated that foraging *Papilio* have real color vision when searching for food (Kinoshita *et al*., 1999), and have even color constancy (Kinoshita and Arikawa, 2000). Another behavioral experiment where we use monochromatic stimuli has suggested that the *Papilio* color vision system is tetrachromatic based on the UV, blue, green, and red receptors.

Genital photoreceptors

I was a first year master's student when I accidentally noticed that *Papilio* butterflies have photoreceptive organ on their genitalia (Arikawa *et al*., 1980). Since then I have been studying on this subject intermittently. We so far found that the photoreceptors are UV sensitive and that the photoreceptors are similar to those of primitive photoreceptors found in the earthworm skin (Miyako *et al*., 1993, 1995). Such photoreceptors exist in all butterfly species we tested, but not found in other insects including moths (Arikawa and Aoki, 1982).

We studied the biological function of these photoreceptors by analyzing behavior of photoreceptor-ablated individuals. Photoreceptor-ablated males could not achieve copulation although they actively tried to mate. Males probably confirm the correct coupling with females optically. If the genitalia are not correctly aligned, the coupling would be incomplete, leaving some space through which light can enter, so that the photoreceptor response continues. In such a case, the male releases the coupling and tries to copulate again (Arikawa *et al.*, 1996). Females use the genital photoreceptors when laying eggs. Photoreceptors are located on the ovipositor, which is exposed to bright light only when the females attach an egg on the leaf surface. Even though the photoreceptors were ablated, females could find leaves for laying eggs, did extend the ovipositor and tried to put eggs on the leave. However, eggs are never delivered, and the females give up eventually. This indicates that the females probably confirm that the ovipositor is sufficiently extruded and is ready to lay eggs by the light sense of the ovipositor (Arikawa and Takagi, 2001).

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