Hindsight of Butterflies

KENTARO ARIKAWA

Butterflies sense light with their genitalia. Four photoreceptor cells in the genitalia mediate this photosensitivity. Such photoreceptors, which exist in body parts other than eyes, are collectively called extraocular photoreceptors. Extraocular photoreceptors have been found in various groups of animals, both vertebrates and invertebrates (Yoshida 1979). One of the most extensively studied cases is the photoreceptor cells in the pineal gland of the vertebrate brain: The pineal photoreceptors receive light to entrain animals' daily activity.

In arthropods, extraocular photoreceptors are roughly divided into two types, according to their general location. The first type is found in the central nervous system. A classic example is the crayfish caudal photoreceptor, a photoreceptive interneuron in the abdominal nervous system, which mediates an escape response upon light stimulation of the abdomen (Wilkens 1988). The second type is found outside the central nervous system as sensory neurons, with the photoreceptive site located on the periphery of the animals. The existence of the peripheral type of photoreceptor had long been indicated in certain scorpions (Zwicky 1968), but the first conclusively documented case was that of the butterfly genital photoreceptors (Arikawa et al. 1980).

In this article, I give an overview of the studies that my colleagues and I have conducted on the genital photoreceptor system of the butterfly. After describing how this unique photoreceptive system was discovered, I then describe the response characteristics and the anatomy of the photoreceptor cells and conclude with a discussion of how butterflies use the genital photoreceptors.

THE *PAPILIO* BUTTERFLY HAS LIGHT SENSITIVITY IN THE GENITALIA, WHICH APPEARS TO BE CRUCIAL FOR REPRODUCTIVE BEHAVIOR

Discovery of the genital photoreceptors

The genital photoreceptor of butterflies was discovered accidentally in the Japanese yellow swallowtail butterfly, *Papilio xuthus* (Arikawa et al. 1980). As a graduate student studying the neuronal mechanism of host-plant selection by female *Papilio*, I was analyzing input–output relations between neurons in the abdominal nervous system. In the course of analyzing mechanoreceptive inputs from the ovipositor, I encountered a sensory neuron that was actively producing spikes (action potentials) in the nerve bundle entering the ovipositor. The neuron was active even when the mechanoreceptive hairs on the ovipositor were not stimulated. This unexpected neuronal activity obstructed the analysis I intended to perform, so I turned off the illumination for the microscope. Strangely enough, this action caused the spike activity to cease. I first thought that I mistakenly unplugged a wire, and, to fix it, I again turned on the light. Surprisingly, the spikes immediately returned.

Kentaro Arikawa (e-mail: arikawa@yokohama-cu.ac.jp) is a professor in the Graduate School of Integrated Science, Yokohama City University, Kanazawa-ku, Yokohama 236-0027, Japan.

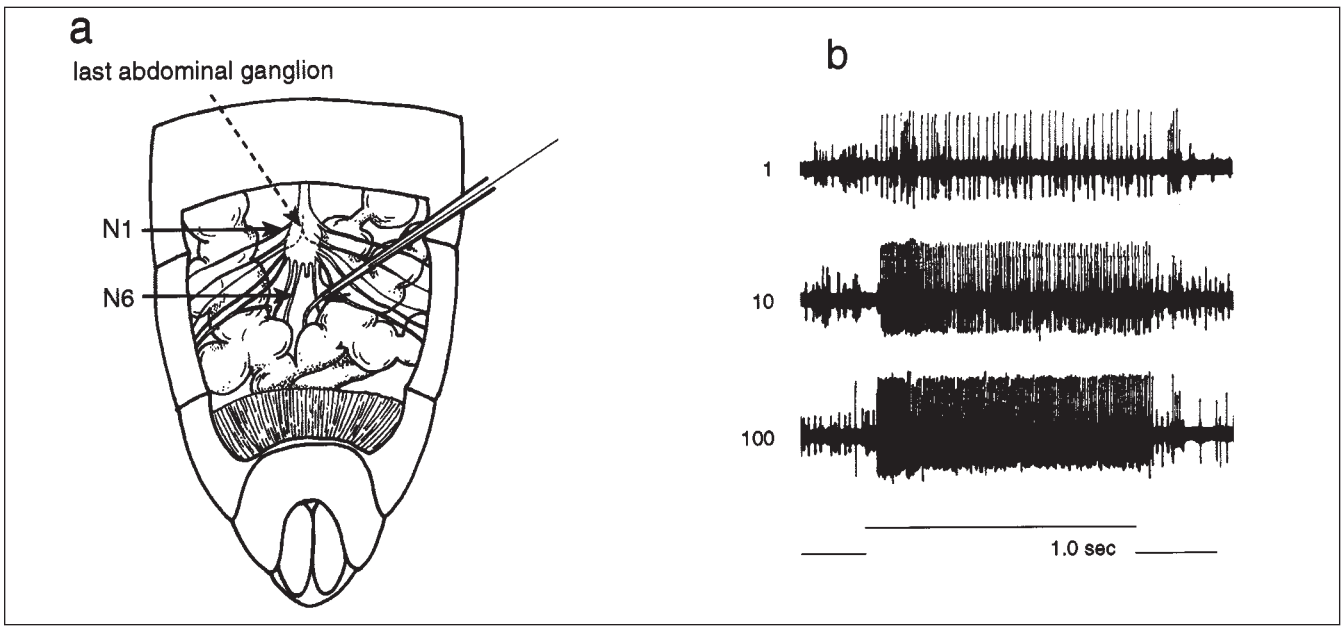

Figure 1. Photoreceptor responses. (a) A schematic drawing of the abdominal nervous system of a female, fixed ventral side up. The last abdominal ganglion bears six pairs of lateral nerve bundles numbered N1 to N6 from the anterior side. The drawing also shows a suction electrode pulling up the distal cut end of an N6 to record the nervous activity. (b) Example of the sustained train of spikes of the photoreceptor recorded by the suction electrode in response to 1-second light flashes of different intensities. Numbers on the left indicate relative intensities of stimulation.

Rather excited by the phenomenon, I cut off the butterfly's head, removed its thorax, and sectioned its abdomen. Some hours later, the remaining tiny piece of cuticle, with a stump of nerve bundle at the tip of the electrode, still produced spikes in response to light flashes (Figure 1). This finding clearly indicated that *Papilio* has "hindsight" mediated by "eyes" on the genitalia. What is this hindsight for? To answer this question, I began to study the genital photoreceptive system, putting aside the original subject, the analysis of oviposition behavior. Interestingly, it is now apparent that the genital photoreceptors control oviposition in females.

Because I was studying oviposition, I first observed the genital photoreceptor responses in a female. Soon I found similar effects in males. In both sexes, six pairs of lateral nerve bundles (numbered N1 to N6 from the most anterior pair) enter the last abdominal ganglion (Figure 1a). I found that the two most posterior pairs, N5 and N6, contain axons that respond to light stimulation of the genitalia in both sexes. Later I investigated other lepidopteran species and determined that genital photoreceptors exist in all butterfly species tested (16 species from 7 families), including skippers (Hesperiidae, considered to be close to moths). However, they do not appear to exist in moths (6 species from 3 families), either in diurnal or nocturnal species (Arikawa and Aoki 1982). Moreover, larvae of *Papilio xuthus* (and presumably other butterfly species) do not have the genital photoreceptor system. The photoreceptor system develops in the late pupal stage (Miyako et al. 1995), which suggests that it is used for adult-specific behaviors.

Response characteristics. The genital photoreceptors produce a sustained train of spikes in response to a light flash (Figure 1b). The maximum firing rate of the photoreceptor is about 300 spikes per second (Arikawa et al. 1997). Using spike frequency as a measure of the response, I was able to determine the spectral sensitivity of the photoreceptors. All four photoreceptor cells, in both males and females, are highly sensitive to light in the ultraviolet to blue (340–460 nm) wavelength region (Arikawa and Aoki 1982). This sensitivity range is probably suitable for detecting contrast in bright sunshine, an important ability because butterflies mate in sunshine.

The precise location of the photoreceptive sites was also studied electrophysiologically. I localized the photoreceptive sites by scanning a small spot of light while recording the photoreceptor response from the nerve. Two pairs of photoreceptive sites (P1 and P2) were found in both sexes (indicated by large arrows in Figure 2). The nerve N6 contains the axon originating from the P1 site, whereas the N5 contains the axon from the P2 site.

In males, the P1 exists in the scaphium, the pale-brown sclerotization of the dorsal surface of the anal tube. An uncolored patch of cuticle marks the inner margin of the scaphium (Figure 3a). When the uncolored region is covered by opaque material, the photoreceptor response disappears, indicating that the photosensitive structure is located immediately beneath or at least very close to this region. The second pair, the P2, lies slightly ventral to the penis. The P2 region is also covered by transparent cuticle.

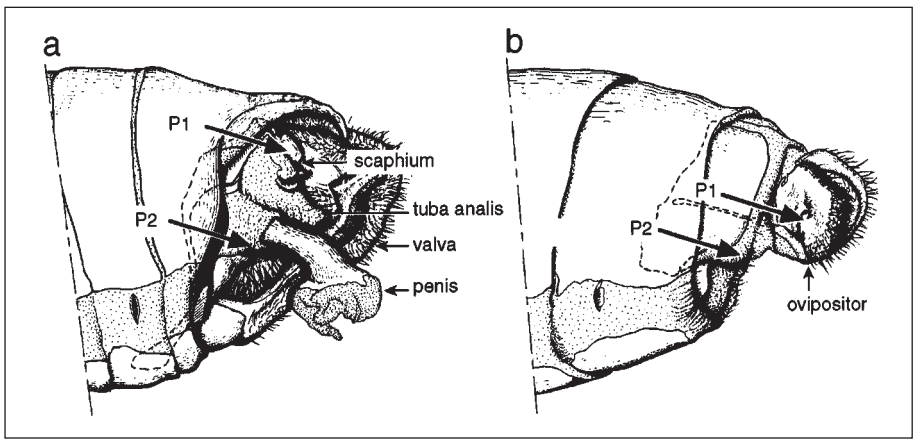

Figure 2. Left side view of the abdominal tip of both sexes. Location of two pairs of genital photoreceptors, P1 and P2, is indicated by arrows; (a) male and (b) female.

In females, the P1 is found on the lateral side of the ovipositor. The ovipositor is a dark-brown, hairy pair of lobes between which the anus and the oviduct open to the exterior. On the lateral side of each lobe is a characteristic concave structure with an inner surface that is uncolored and free of hairs (Figure 3b). The photoreceptor response disappears when the uncolored cuticle of the concave region is shielded, indicating that the photoreceptive organelle exists around this region. The P2 region, which is slightly ventral to the ovipositor, is covered by yellow, hairy scales but is not colored.

Structure. To investigate the structure of the photoreceptor cell in the genitalia, I studied the internal structure of the P1 photoreceptive sites (i.e., the scaphium of the male and the ovipositor of the female) by light and by electron microscopy. Both the scaphium and the ovipositor bear many hairs (Figure 3). Each of these mechanoreceptive hairs has a small sensory neuron at the base. Among the numerous cell bodies of the small mechanosensory neurons, there is a large (about 30×40 μm) ovoid structure containing the cell body of a sensory neuron. The cell body in the ovoid structure tapers to form an axon, which extends from the N6 into the last abdominal ganglion, where it branches and terminates (Arikawa and Aoki 1982).

Electron microscopy demonstrates that about 30% of the cross-sectional area of the ovoid structure is occupied by an organelle that resembles a phaosome (from Greek *phaos* meaning light and *some* meaning body), a structure first found in a photoreceptor cell in earthworm skin (Figure 4; Roelich et al. 1970). The *Papilio* phaosome consists of two components: (1) membrane-enclosed, electron-lucent areas and (2) irregularly packed tubules of membranes.

Serial sections revealed that the electron-lucent components are obliquely sectioned profiles of processes that protrude from the distal side of the photoreceptor cell body. The tip of these distal processes bears tubular membranes. The tubular membranes repeatedly bifurcate to form finer tubules with a diameter between 0.1 and 0.3 μm, which is much larger than the diameter of the rhabdomeral microvilli of the compound eye photoreceptors (about 0.07 μm) (Miyako et al. 1993). The difference in size is important: Photoreceptor cells evolved to enhance the efficiency of photon capture by increasing the surface area of membranes to embed photoreceptive pigments, thus the larger the membrane, the better the photoreception. Thick tubules with their irregular organization are found in some rather primitive photoreceptor cells (Eakin 1982), such as those of the genital photoreceptor cells of the butterfly. Whether photoreceptive pigments are embedded in the tubular membranes of butterfly genital photoreceptor cells—and, if so, how—remains for further investigation.

Function of genital photoreceptors

Males use the genital photoreceptors to achieve correct coupling, whereas females use them to confirm whether the ovipositor is properly pushed out upon oviposition.

Copulation in males. To investigate the biological function of the genital photoreceptors, we first observed the genitalia of an intact male under a dissecting microscope.

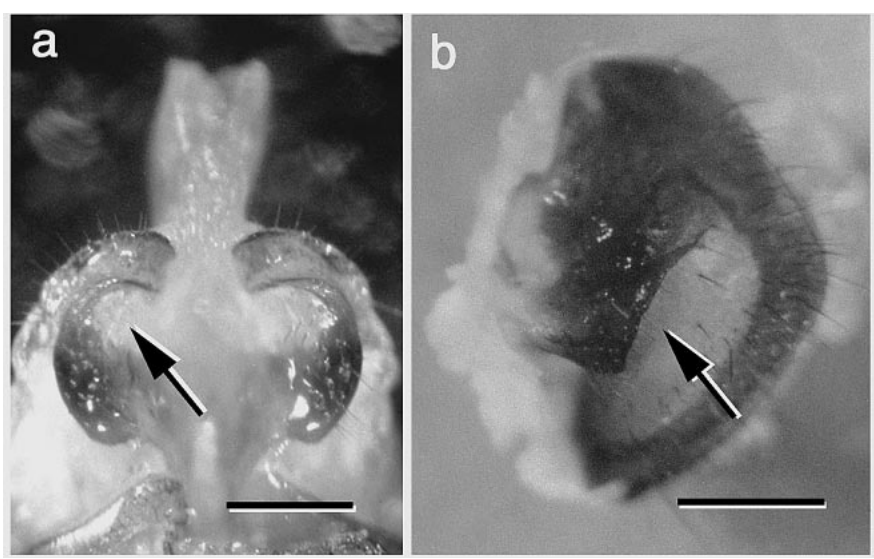

Figure 3. Transparent patch of cuticle (arrows) on the scaphium (a) and on the ovipositor (b). Scales equal to 500 μm.

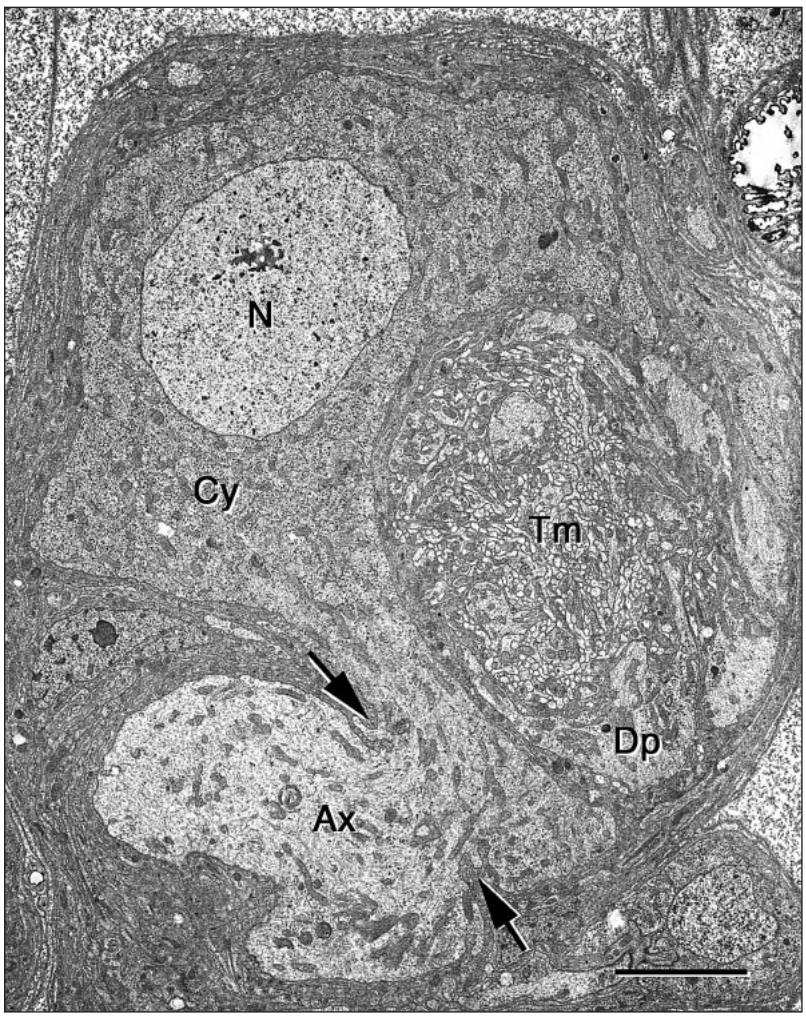

Figure 4. The ovoid structure that contains the cell body of the sensory neuron in the female P1. The structure also contains a phaosome that consists of some distal processes (Dp) and tubular membranes (Tm). An axon (Ax) extends from the cell body. The axon hillock is marked by arrows. Cy, cell body cytoplasm; N, nucleus of the sensory neuron; n, nucleus of a glial cell. Scale equal to 5 µm.

In response to photostimulation of the genitalia, the male widely opened the valva, a pair of lobes for supporting female genitalia during copulation (Figure 5). The valva closed when the light was turned off. Illumination with red light did not evoke valva opening. Because photoreceptors' spectral sensitivity is virtually nonexistent in red light (Arikawa and Aoki 1982), the valva-opening response is most likely mediated by genital photoreceptor input (Arikawa 1993). That finding motivated us to further investigate the possible involvement of genital photoreceptors in mating behavior (Arikawa et al. 1996, 1997).

We therefore set up a series of behavioral experiments. We first observed the mating behavior of intact *Papilio xuthus* in an outdoor cage. We positioned a virgin female in the cage by attaching the dorsal cuticle of the thorax with beeswax to the bottom end of an insect pin and by fixing the upper end of the pin to a horizontal bar. By releasing an intact male in the cage and observing its actions, we determined that mating behavior consists of at least six steps (Figure 6a; Arikawa et al. 1997). First, a male approaches a female (step 1). The male then gets into the venter-to-venter position with the female, opening the valva (step 2). The male then searches for the female's genitalia by touching her abdomen with his genitalia exposed between the fully opened valva (step 3), firmly clasping her genitalia by using the superuncus and scaphium (see Figure 6b, 6c). When the male has clasped the female's genitalia correctly (step 4), he inserts the penis, ejaculates, and then plugs the vagina with his own secretion while keeping the end-to-end copulatory posture (step 5), after which the pair finally separates (step 6, not shown). Under this experimental condition, 66% of the tested males copulated with the virgin females hanging in the cage (66% copulation success rate).

To see whether and how the genital photoreceptors are involved in the mating behavior, we ablated the photoreceptor input in both males and females and observed their behavior. We ablated the P1s by gently rubbing the P1 site with a fine heat probe or by painting the site with black mascara. As a control, we used transparent mascara.

Figure 7 shows the results. After treating the P1s of males, we let them mate with pinned intact females. The copulation success rate (black

Figure 5. Genitalia of a coupling pair as seen from the ventral side. The male (below) supports the genitalia of the female (above), using the bilateral valva.

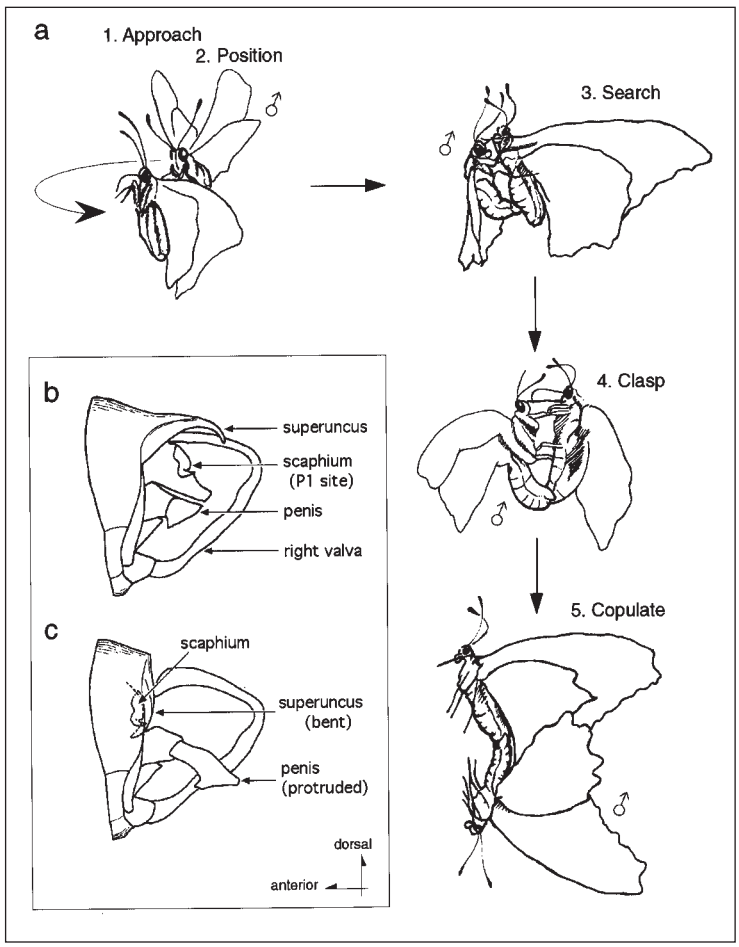

Figure 6. Mating behavior of* Papilio xuthus.** ***(a) Sequence of mating behavior observed in the outdoor cage. The male was freely flying, whereas the female was mounted in the cage but could still flap her wings. (b) Left side view of male genitalia, not copulating. Left valva was removed for clarity. (c) Genitalia of a copulating male (step 5). The female genitalia are omitted for clarity.

areas) of the P1–heat-ablated males decreased significantly, to 28%. The decrease was observed also in the P1–black-painted males (23%). During the mating trial, the P1 ablated males spent a long time in the searching stage (step 3) (gray areas in Figure 7), repeatedly clasping and releasing female genitalia. The P1–clear-painted males mated at the normal rate (67%). These results indicate that the males somehow use the light signal from the genitalia for copulation. The P1 ablation might have reduced the motivation to mate, we conjectured, but the fraction of individuals that did not perform mating behavior, which was about 25% in intact males, remained constant in all the treatments. Clearly, most of the P1-ablated males did want to mate, but they simply could not. By contrast to males, P1 ablation in females did not have any effect on the copulation success rate.

To address the manner in which the P1s control mating behavior, we recorded the P1 response in the males during mating (Arikawa et al. 1997). First, we determined the relative positions of the genitalia of mating butterflies at each behavioral step by observation. The positions were then mimicked by mechanically opening and closing the valva and by placing an isolated female abdomen in various locations near the male, which was fixed on the stage for electrophysiological recording (Arikawa and Miyako-Shimazaki 1996). For the males that copulated, we recorded responses during clasp and copulation (steps 4 and 5). At step 2, when the male fully opens the valva, the P1 response increases to about 100 spikes per second at 2000 lux. The response decreases by half while the male is searching for the female's genitalia (step 3). The response decreases further to about 25 spikes per second at step 4, when the male correctly clasps the female's genitalia with the superuncus and scaphium.

We hypothesize that the large decrease in P1 response informs the male that the female's vagina is correctly positioned for penis insertion. The male P1 is located on the scaphium, which is used to clasp the female's genitalia together with the bent superuncus (Figure 6c). Given their location during copulation, the P1s probably are not exposed to light when the mates couple properly. If the genitalia were misaligned, some space would be available through which light could enter, so that the P1 response would continue, in which case the male would release the clasp and return to the search step. The males with the P1 painted black never experienced such a drop in response because there was little P1 activity to begin with. Those animals continued the genitalia search even when the female's genitalia were correctly aligned, until they finally gave up.

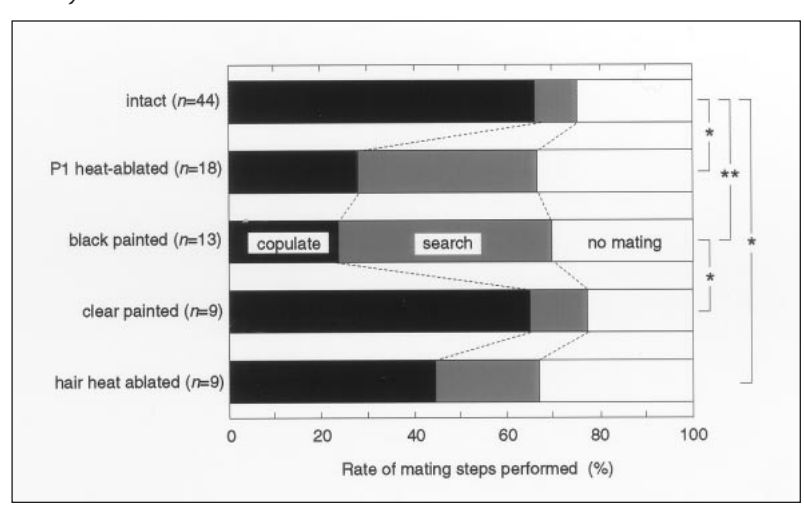

Figure 7. Effect of P1 ablation on reproductive behavior of male* Papilio xuthus.** ***The Mann-Whitney* U *test was used to analyze statistical difference in copulation success rate between treatments (*, P < 0.05; **, P < 0.01).

The scaphium bears not only photoreceptors but also mechanoreceptive hairs. To determine the contribution of the mechanoreceptors in mating, we heat ablated the mechanoreceptors on the scaphium of males and observed their mating behavior. The copulation success rate of the mechanoreceptor-ablated males decreased to 44%, indicating the necessity of a mechanical sense for achieving copulation (Figure 7). This finding may explain why one-third of P1 heat-ablated males can still copulate: Males can locate the females' genitalia by using the mechanical sense. In addition, the other pair of genital photoreceptors, the P2s, may also contribute to mating behavior, although this possibility has not yet been tested.

Oviposition in females. It is not surprising that P1 ablation in females did not have any effect on the copulation success rate because the P1s of females are located on the lateral side of the ovipositor, which is usually covered with hairy scales at the abdominal tip. Coupling does not affect the amount of light reaching the ovipositor.

Instead, the females' photoreceptors may be involved in oviposition behavior. On stimulation by light of the abdominal tip, females sometimes push out the ovipositor. A similar movement of the ovipositor is observed when the females are laying eggs. The females of *Papilio* lay eggs on citrus leaves, the food of the larvae, after they confirm the taste of the leaf by using contact chemoreceptors on their forelegs. If they determine that the leaf is of the correct species, they curl the abdomen, push out the ovipositor, touch the leaf surface with it, and then deposit an egg on the leaf.

The mechanical sense organs on the ovipositor play an important role in oviposition control in lepidopteran insects (Yamaoka et al. 1971). In fact, when we heat ablated the mechanoreceptors on the ovipositor of a female *Papilio* that was actively laying eggs, the female could no longer lay eggs (Figure 8). The mechanoreceptor-ablated females push the ovipositor strongly against the leaf to locate the egg-laying site, but they do not deposit an egg, suggesting that the mechanical input from the ovipositor informs females that the leaf is there.

Heat ablation of the P1s of females disrupts oviposition behavior, too. Once an intact female curls her abdomen to lay an egg on the leaf, she deposits an egg at the success rate of about 80% (i.e., 8 eggs are deposited per 10 abdomen curls). However, the success rate drops significantly, to only a few percentage points, if the P1s are heat ablated (Figure 8). Like the mechanoreceptor-ablated females, the P1-ablated females strongly push the leaf with the ovipositor but do not lay eggs.

It is somewhat strange that the P1-ablated females touch the leaf with the ovipositor and still do not lay eggs, because their mechanoreceptors are intact. Presumably, the mechanical input from the ovipositor is effective only when the P1s are active. When not laying eggs, the ovipositor is covered by hairy scales and hidden in the abdominal tip. The ovipositor must be sufficiently pushed out on oviposition, or the deposited egg will not be properly attached to the leaf surface. Electrophysiologically, the P1 hardly responds to light when the ovipositor is not pushed out from the abdominal tip. Once pushed out, the P1 activity increases significantly. Therefore, the female uses the change in the P1 activity as a measure of the ovipositor position: If the P1 receives light, it means that the ovipositor itself is exposed to light (i.e., the ovipositor is sufficiently pushed out). Then the female is ready to accept the mechanical input as the indicator of the leaf location. In this context, the female P1s are used to detect the position of the butterfly's own ovipositor.

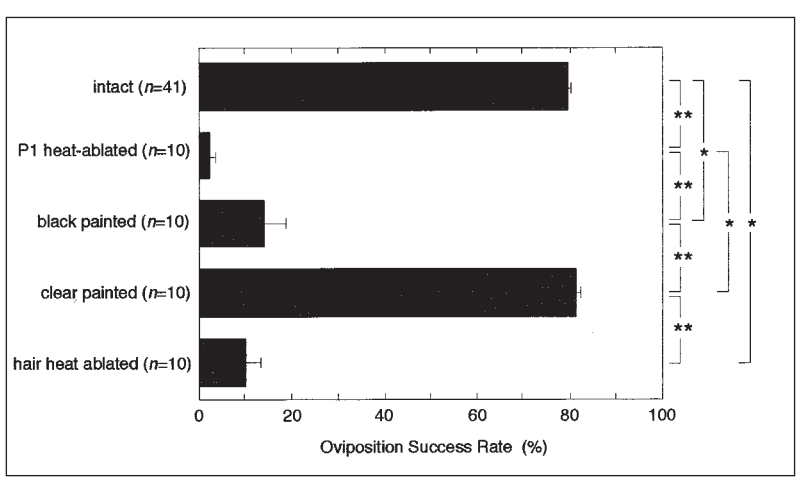

Figure 8. Effect of P1 ablation on oviposition success rate of* Papilio xuthus*. Oviposition success rate was measured by counting the number of eggs per total number of abdomen curling responses. The Mann-Whitney* U *test was used to analyze statistical difference in oviposition success rate between treatments (*, P < 0.05; **, P < 0.01).

Concluding remarks

Extraocular photoreceptors are known in many animals, and the butterfly genital photoreceptor system is one of the best understood of all such cases. In addition to understanding much about the structure and response characteristics of the photoreceptor cells, we also have a good idea of what *Papilio* use hindsight for.

Males use the P1s to confirm correct coupling. The P1 system would detect any slight gap between the genitalia of the male and female produced by incomplete coupling. Detection of such a gap can be achieved by mechanical means. In fact, some P1-ablated males did complete coupling, probably by using mechanoreceptive hairs to overcome the loss of the photoreceptors. Nevertheless, based on our observations, we believe that photoreceptors are probably more sensitive than mechanoreceptors for confirming coupling.

In females, P1s are used to determine whether the ovipositor is sufficiently pushed out from the abdominal tip on oviposition. Monitoring the relative position of the ovipositor can, of course, be achieved also with a mechanical sense that somehow measures contraction of ovipositor muscles. However, the use of an optical system is probably more reliable. If some material, such as the animal's own excrement or detached scales, covers the tip of the abdomen, the egg cannot be properly delivered or attached to the leaf surface. If the ovipositor position is monitored only mechanically, the butterfly would be unable to detect any material covering the ovipositor. If the ovipositor is exposed to light, however—which means that the ovipositor is not shielded—then the butterfly can reliably conclude that the ovipositor is ready for proper oviposition.

Moreover, from a neurobiological point of view, some interesting phenomena observed in the P1 system may lead us to analyze brain architecture and function in general. One phenomenon concerns lateral asymmetry of the brain. In the behavioral experiments described above, we always ablated the P1s bilaterally. However, it turns out that the ablation of just the left P1 of males significantly reduced the copulation success rate, whereas the ablation of the right P1 did not have significant effect; hence, there is lateral asymmetry in the P1 function (Sato et al. 1997). We have not noticed any difference in the structure and sensitivity between the left and right photoreceptors; thus the lateral asymmetry most likely originates in the asymmetry in the central nervous system—in this case, most likely in the last abdominal ganglion.

Lateral asymmetry of the brain has long been recognized and extensively studied in higher animals, including humans, but the neuronal basis of this lateral asymmetry is not yet understood, mainly because of the enormous complexity of the brain. The last abdominal ganglion of the butterfly is small and rather simple in cellular organization, however, and this system may therefore provide a unique opportunity for studying the cellular basis of brain asymmetry.

The simplicity of the last abdominal ganglion is advantageous also for the basic study of integration of sensory signals. In females, we showed that two different sensory modalities are involved in a behavior: Both mechanical and light senses are crucial for proper oviposition. Because important sensory organs such as compound eyes and antennae are located in the head, different sets of sensory information are generally integrated in the brain of the butterfly, which is simple compared with mammalian brains but complex enough to permit analysis of the neuronal basis of behavior. In the case of butterfly oviposition, both mechanoreceptors and photoreceptors project their axons into the last abdominal ganglion, where they terminate. Analyzing how light and mechanical information are integrated in the last abdominal ganglion would be a good starting point for better understanding sensory integration in general.

Acknowledgments

My thanks to Kiyoshi Aoki, Eisuke Eguchi, Yumiko Miyako-Shimazaki, Daisuke Suyama, Takanori Fujii, Miho Sato, and Nobuhiro Takagi for scientific contributions to the study, and to Doekele Stavenga for a critical reading of the manuscript. I also thank five anonymous reviewers for valuable comments. This work was supported by research grants from the Ministry of Education, Science, and Culture of Japan; the Whitehall Foundation; the Uehara Memorial Foundation; the Sumitomo Foundation; the Novartis Foundation; and the Kanagawa Academy of Science and Technology.

References cited

Arikawa K. 1993. Valva-opening response induced by the light stimulation of the genital photoreceptors of male butterflies. Naturwiss 80: 326–328.

Arikawa K, Aoki K. 1982. Response characteristics and occurrence of extraocular photoreceptors on lepidopteran genitalia. Journal of Comparative Physiology A 148: 483–489.

Arikawa K, Miyako-Shimazaki Y. 1996. Combination of physiological and anatomical methods for studying extraocular photoreceptors on the genitalia of the butterfly, *Papilio xuthus*. Journal of Neuroscience Methods 69: 75–82.

Arikawa K, Eguchi E, Yoshida A, Aoki K. 1980. Multiple extraocular photoreceptive areas on genitalia of butterfly, *Papilio xuthus*. Nature 288: 700–702.

Arikawa K, Suyama D, Fujii T. 1996. Light on butterfly mating. Nature 382: 119.

———. 1997. Hindsight by genitalia: Photo-guided copulation in butterflies. Journal of Comparative Physiology A 180: 295–299.

Eakin RM. 1982. Continuity and diversity in photoreceptors. Pages 91–105 in Westfall JA, ed. Visual Cells in Evolution. New York: Raven Press.

Miyako Y, Arikawa K, Eguchi E. 1993. Ultrastructure of the extraocular photoreceptor in the genitalia of a butterfly, *Papilio xuthus*. Journal of Comparative Neurology 327: 458–468.

———. 1995. Morphogenesis of the photoreceptive site and development of the electrical responses in the butterfly genital photoreceptors during the pupal period. Journal of Comparative Neurology 363: 296–306.

Roelich P, Aros B, Viragh S. 1970. Fine structure of photoreceptor cells in the earthworm, *Lumbricus terrestris*. Z Zellforsch 104: 345–357.

Sato M, Fujii T, Arikawa K. 1997. Lateral asymmetry of the genital photoreceptor function on the mating behavior of the butterfly *Papilio xuthus*. Zoological Science 14S: 106.

Wilkens LA. 1988. The crayfish caudal photoreceptor: Advances and questions after the first half century. Comparative Biochemistry and Physiology 91C: 61–68.

Yamaoka K, Hoshino M, Hirao T. 1971. Role of sensory hairs on the anal paillae in oviposition behaviour of *Bombyx mori*. Journal of Insect Physiology 17: 897–911.

Yoshida M. 1979. Extraocular photoreception. Pages 582–640 in Autrum H, ed. Handbook of Sensory Physiology. New York: Springer-Verlag.

Zwicky KT. 1968. A light response in the tail of *Urodacus*, a scorpion. Life Science 7: 257–262.