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Photoreceptor twist: A solution to the false-color problem

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ABSTRACT **In bees and many other insects the majority of photoreceptors are twisted like a corkscrew. Here we show that this structural feature of insect eyes—whose very existence was a source of dispute for several years—is necessary for reliable encoding of information about color. Light reflected from waxy plant surfaces is partially linearly polarized. Moreover, insect photoreceptor membranes are dichroic and thus sensitive to the polarized glare originating from plant surfaces. Taken together, these two phenomena create a serious false-color problem: in the bee's trichromatic color vision system, the color values of a particular part of a plant could be affected not only by the spectral but also by the polarization properties of the reflecting surface. As demonstrated by spectroscopic measurements and optical analyses, the hue of color of a given surface of a plant would change dramatically with the direction of illumination and the bee's line of sight, if the bee possessed straight and thus highly "polarization-sensitive" photoreceptors. However, this false-color problem is overcome completely in photoreceptors that are twisted by exactly the amount we have found to occur in the worker-bee's eye.**

In the mid-1970s a striking discovery stirred up a heated controversy among researchers in insect vision. Except for a small part located at the uppermost dorsal rim of the eye, the retina of the honeybee was found to be composed of photoreceptors that were twisted about their longitudinal axes (1) (Fig. 1). This surprising finding was greeted with disbelief (2). It was considered to be an artifact possibly resulting from mechanical manipulations during the preparation of the eye or from dehydration procedures during the fixation of the retinal tissue. However, as shown by detailed transmission electron microscopy studies (1, 3), the rate and amount of twist remained the same when (*i*) during fixation the tissue was treated with extreme care, (*ii*) fixatives were used that penetrated the cuticle of the head, so that there was no need for cutting the head or eye prior to fixation, and (*iii*) the retina was excised from the eye and manipulated mechanically. We were even able to show how the twist arose during the ontogenetic development of the eye (4, 5). Finally, twisted photoreceptors, or photoreceptors in which the microvilli of the rhabdomeres are not aligned consistently in one particular direction, were found to occur in other species of insect as well—e.g., ants (6), flies (7), crickets (6, 8), beetles (9), and butterflies (10). Thus, photoreceptor twist is now a well-established structural phenomenon in insects. It remains to be elucidated in functional terms.

Owing to the alignment of the absorption vectors of the rhodopsin molecules within the photoreceptor membrane (11), rhabdomeric photoreceptors are dichroic and should thus be highly sensitive to polarized light, if—and only if—their microvilli are all oriented consistently in one particular direction—i.e., if the rhabdomeres are aligned straight along their entire length. According to the hypothesis put forward by Wehner *et al.* in 1975 (1), the twist degrades or even eliminates the polarization sensitivity of the photoreceptors. The crucial test of this hypothesis was provided by intracellular measurements of the polarization sensitivities (PS) of straight and twisted photoreceptors, as they occur in different regions of the worker-bee's retina. The straight photoreceptors located at the dorsal rim of the eye (1, 12) and used exclusively for detecting polarized skylight (13) exhibit high polarization sensitivities (values of PS range between 5 and 10), whereas in the twisted photoreceptors of the remainder of the eye the polarization sensitivity is completely absent (12).

Finally, this raises the question of what the functional significance of the insect's photoreceptor twist might be. The answer lies in a physical phenomenon exhibited by mirror-like surfaces of many inorganic substances such as glass plates or bodies of water and by the surfaces of plants that are coated with waxy and thus shiny epicuticles: incoming unpolarized sunlight gets, at least to a certain degree, linearly polarized whenever it is reflected from such surfaces (14) (see plate II in ref. 15). If the sensors of a color vision system picked up this polarized light, the system would generate "false colors" that could completely obscure the real colors—i.e., the colors defined by the spectral rather than the polarization properties of the object. It is the purpose of this paper first to outline this false-color problem, then to formulate it in quantitative terms, and finally to show how the bee has solved it.

MATERIALS AND METHODS

Computation of Polarization Sensitivities of Straight and Twisted Worker-Bee Rhabdomeres. Following the scheme outlined in ref. 1 we computed the sensitivities of the bee's three spectral types of photoreceptor (λ_{max} = 350, 430, and 530 nm) as a function of wavelength and did so for horizontally and vertically polarized monochromatic light (for microvillar directions present at the distal tips of the photoreceptors see Fig. 2). The computations were done for twisted and untwisted (straight) photoreceptors. The mean rate of twist (10 μm^{-1}) and the lengths of the bee's photoreceptors (200–240 μm) were taken from refs. 1 and 3.

Measurement of the State of Polarization of Light Reflected from Plant Surfaces. The incident Leitz MPV-2 microspectrophotometer used in former studies (16, 17) was equipped with a prism in the back focal plane to allow for oblique epi-illumination. As the object (a small piece of a leaf or a flower petal of a particular species of plant) was mounted on a goniometer stage, different combinations of specular and diffuse reflectance could be measured for various tilt angles of the reflecting object. We computed the specular and diffuse reflectance spectra based on measurements of the object, tilted (*i*) so that all specular reflections from the narrow illuminating beam were collected by the microscope objective and (*ii*) so that only diffuse reflections were collected. We define K = 1 as the ratio of specular/diffuse

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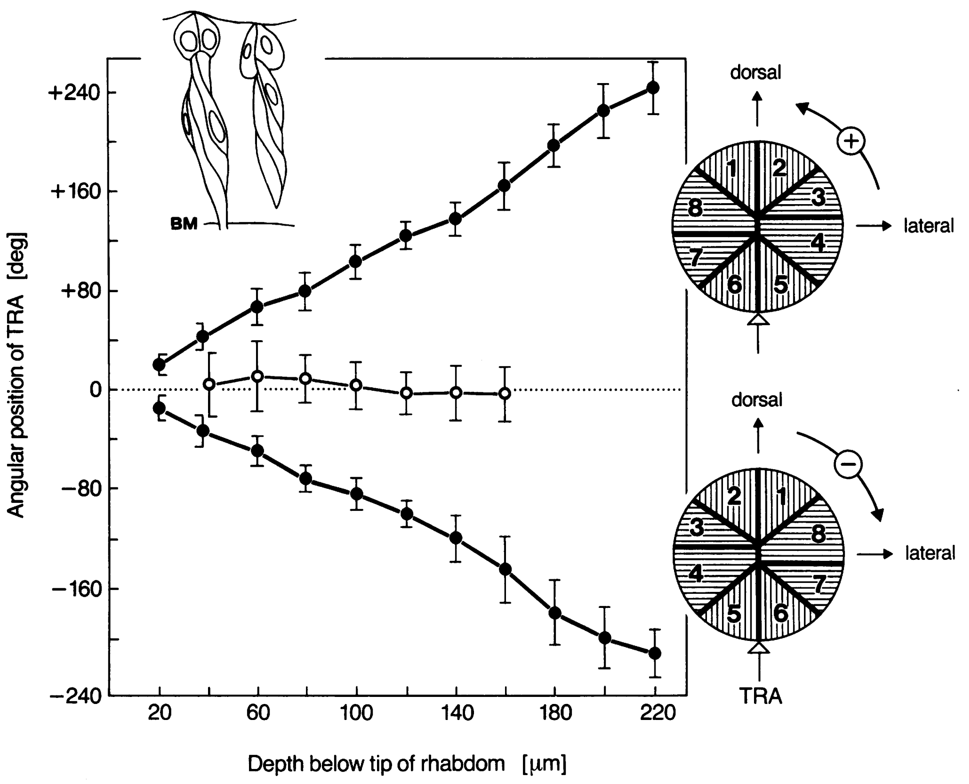

FIG. 1. Rate and amount of twist in worker-bee photoreceptors. The filled symbols characterize two types of rhabdoms that occur within the bee retina (− or + as indicated at the right), depending whether the twist is clockwise or counterclockwise. The open symbols characterize rhabdoms from the POL area at the uppermost dorsal rim of the eye that exhibit no twist. Those rhabdoms are used exclusively for polarization vision. The *Inset* depicts two ommatidia of an early pupal stage of development, in which the rhabdoms have not yet formed. Even so, the bundles of photoreceptor cells are already twisted; BM, basement membrane of compound eye; TRA, transverse axis of rhabdom.

reflectances corresponding to condition 1 and K = 0 corresponding to condition 2.

The most extensive studies were done on leaves and petals of *Taraxacum officinale* (Compositae), *Prunus spinosa* (Rosaceae), *Laurus nobilis* (Lauraceae), and *Poa pratensis* (Gramineae). Using these reflectance spectra and the spectral sensitivity functions of the three spectral types of worker-bee photoreceptors, either twisted or straight, we computed tri-stimulus values (18, 19) for each plant surface under various conditions of illumination. Finally, the tri-stimulus values were plotted within equilateral color triangles referring to color vision systems composed of either twisted or untwisted (straight) photoreceptors.

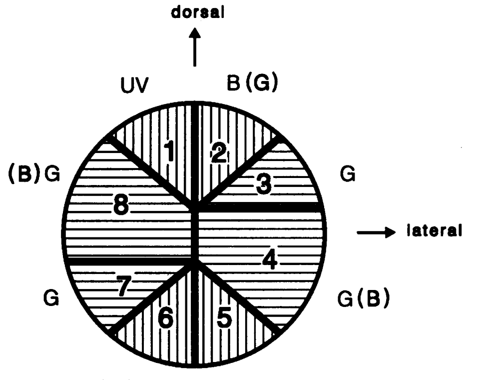

FIG. 2. Schematic cross section of the distal tip of a rhabdom of the worker-bee compound eye. The eight rhabdomeres of which the rhabdom is composed are denoted by numerals 1–8. The hatching within a rhabdomere indicates the microvillar orientation, which is also the direction of maximal polarization sensitivity. UV, B, and G and UV, (B), and (G) indicate two possible assignments for the spectral sensitivity functions of the eight photoreceptor cells—i.e., maximally sensitive in the ultraviolet, blue, and green spectral regions, respectively. According to optophysiological measurements the assignment UV, B, G rather than UV, (B), (G) is realized in the worker-bee eye.

RESULTS

The main result of the present account can be illustrated best by comparing Fig. 3 *a* and *b* on the one hand and Fig. 3*c* on the other. The data points shown in Fig. 3 represent the spectral loci of a leaf of *T. officinale* illuminated under various conditions and seen by sets of straight or twisted photoreceptors, respectively. The plotted lines represent loci of varying ratios of specular to diffuse reflection (K values). They all radiate out from a spectral locus (depicted by the encircled point) that indicates the diffuse component of leaf reflection and thus characterizes the hue of color a bee would perceive of a *Taraxacum* leaf in unpolarized light. Increasing distances from this diffuse-reflection locus mark increasing ratios of specular to diffuse reflection. Along the plotted lines these ratios K vary over 4 orders of magnitude ($0.01 \leq K \leq 10.0$). In these examples the specular (i.e., strongly directional surface) reflections are assumed to be completely polarized. Thus, increasing values of K mean that the light reflected from the leaf is increasingly polarized. The influence the direction of polarization ($\phi_{\max}$) has on the position of the spectral locus is depicted by the family of lines plotted for $\phi_{\max}$ = 0, 10, 20, 30, 45, 60, 70, 80, and 90°.

In a color vision system equipped with untwisted photoreceptors the spectral loci of a *Taraxacum* leaf cover a wide range in tri-stimulus space (Fig. 3 *a* and *b*). Take, for example, the case of Fig. 3*a* and $\phi_{\max}$ = 900. As the relative amount of the specular component of reflection increases, the

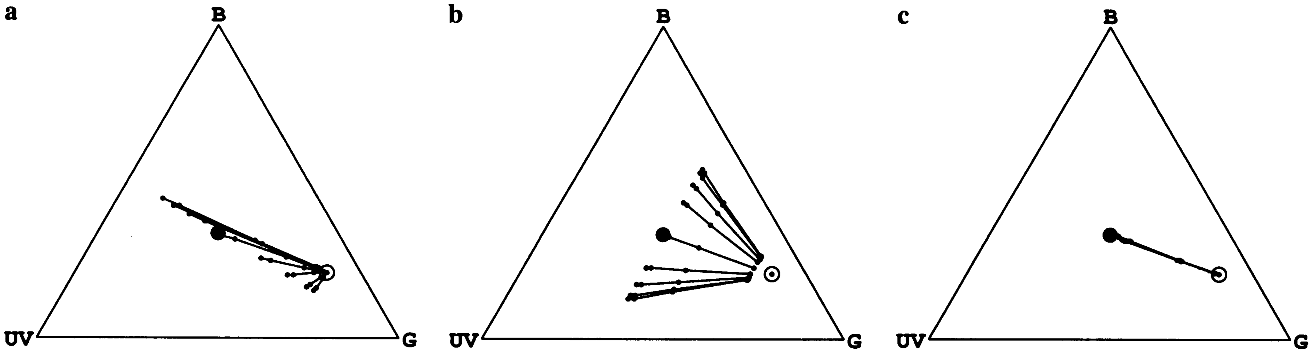

FIG. 3. Polarizational false-color loci for the two types of rhabdoms described in the legend to Fig. 1. The large filled circle (●) marks the white point. The encircled point is the chromaticity of diffuse reflections from a *Taraxacum* leaf. Loci of fixed polarization angle and varying K are plotted to explore the range of false colors as the fraction of polarized specular reflections ranges from very small (points near the open circle) to very large (points near the white point). Loci for nine angles are shown. The rhabdoms of *a* and *c* have the microvilli of B receptors parallel to those of UV receptors, whereas in *b* they are perpendicular (compare Fig. 2). The rhabdoms of *a* and *b* are straight, whereas that of *c* is twisted. Polarizational false colors are eliminated by the twist.

spectral locus of the leaf moves toward the white point W and then even beyond W in the region of the complementary color. Translated into the human color vision system this phenomenon means that an object that appears, say, yellow when seen in unpolarized light changes its color to even blue, when the value of K varies due to changes in the conditions of illumination. Fig. 3 *a* and *b* are based on different geometrical arrangements of the three spectral types of photoreceptor as they have been proposed for the retina of the honeybee in refs. 20 and 21, respectively.

In sharp contrast to what occurs in a trichromatic set of straight photoreceptors, in a set of twisted photoreceptors the hue of color does not change with either K or $\phi_{\max}$. The polarizational angle $\phi_{\max}$ has no influence at all on the hue (dominant wavelength) of a given object, and variations in K alter merely the saturation of the color but leave the hue of color unaffected (Fig. 3*c*): as K increases, the tri-stimulus position of the object moves from the diffuse-reflection locus (⊙) to the white locus (●) but does not deviate from the line connecting the two points, nor does it ever overshoot the white locus.

DISCUSSION

Light reflected from the surfaces of plants is partially linearly polarized. The reader can easily be convinced of this phenomenon by looking through a rotating polarizer at a meadow or the edge of a wood. A dramatic lightening and darkening occurs as the polarizer rotates. The degree and direction of polarization depend on the microtexture of the plant surface, on the direction of the incoming light (direct sunlight or scattered skylight), and on how the reflecting surface is tilted with respect to the direction of illumination and the line of sight.

As insect photoreceptors are dichroic and thus potentially sensitive to the polarized light reflected from the insect's terrestrial environment, a serious false-color problem arises: how can a color vision system avoid being contaminated by polarized light and thus signaling false colors? As shown in the present account, this problem is potentially severe. If the bee's color vision system were equipped with polarization-sensitive input channels—i.e., with dichroic photoreceptors in which all microvilli are parallel—a particular piece of vegetation would give rise, in tri-stimulus space, to a wide range of spectral loci. At any one time, the spectral locus of the object would depend on the conditions of illumination and the bee's direction of view. This means that for the bee the hue of a given part of a plant would change, whenever an approaching bee changed its direction of flight and thus its direction of view—a completely unwanted phenomenon. For example, when zigzagging over a meadow with all its differently inclined surfaces of leaves, the bee would experience pointillistic fireworks of false colors that would make it difficult to impossible to detect the real colors of the flowers. In some cases, the false colors can even be complementary to, and more saturated than, the true colors of the leaves.

The bee's solution to the false-color problem is to get rid of the polarization sensitivity of its eyes by twisting its photoreceptors. As demonstrated by the present investigation, the amount of rhabdomeric twist observed in bees completely suffices for eliminating the polarization sensitivity of the photoreceptors. Hence, the photoreceptor twist protects the bee's color vision system from getting contaminated by false colors.

Furthermore, our measurements and computations of polarizational false colors show that the false-color problem is much more serious for leaves than for the petals of flowers. In most cases, the petals are roughened by all sorts of microsculptures—e.g., nipples, knobs, and ridges—which themselves are again covered by yet smaller microstructures (22). In a Mandelbrotian world this is a vivid demonstration of the fractal geometry of Nature. The optical consequences of surface microsculpturing are that more of the incident light enters the petal, much less light is specularly reflected, and the small amount that is specularly reflected is sprayed into a wide range of angles.

Seen from this point of view, the photoreceptor twist is a necessary requirement mainly for recognizing and localizing, rather than finally identifying, the real colors of the flowers against a broad background of false-color signals, within which the color signals of the flowers would otherwise get completely obscured.

Finally, one wonders why insects have designed dichroic photoreceptors in the first place, when they later try hard to get rid of the polarization sensitivity caused by the dichroism of their photoreceptor cells. The most likely answer is the following. Apart from the fact that rolled-up photoreceptor membranes are intrinsically dichroic to a small degree, even if the molecules are not aligned (23), small rhabdomeric photoreceptors catch more quanta, even of unpolarized light, if the absorption vectors of the light-sensitive molecules are arranged within the microvillar membrane in an ordered rather than random way (24). Consequently, let us end by entertaining the following hypothesis. Photoreceptor cells with microvilli are an ancient evolutionary innovation, predating insects. Like other arthropods with compound eyes, insects stack their photoreceptor microvilli in long rhabdomeres to increase further the absorbance of visual pigment that is presented to the incoming light. As a second step, bees have imparted twist to rhabdoms in the region of the eye involved in color vision to abolish the polarization sensitivity that had resulted as a side effect of the increase in light sensitivity. Hence, the photoreceptor twist enables the insect to keep its color vision system clean of any contamination caused by the ubiquitous presence of polarized light. It is only in a specialized region of the eye that the polarization sensitivity is retained and exploited for skylight navigation (1, 13).

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