to be comparable at the two volcanoes, with the exception of a significantly lower values for selenium from El Chichón.

In summary, during this investigation the sulfur emission from El Chichón, unlike that from other volcanoes at this stage, was primarily in the form of $H_2S$. Of the 29 elements measured in particulate material collected by aircraft from the plume of the volcano, sulfur, chlorine, arsenic, selenium, bromine, antimony, iodine, tungsten, and mercury were found to be enriched relative to bulk pyroclastic material by factors comparable to those determined at Mount St. Helens. Unlike the Mount St. Helens aerosols, however, these samples showed no significant enrichment in zinc or gold. Analysis of elemental composition as a function of particle size revealed that the enriched volatile elements were associated primarily with fine particles and can be expected to have long atmospheric residence times compared with the nonenriched, crustal elements found on larger, more rapidly settling particles. Fine ash injected into the stratosphere by the March eruption was also enriched in the volatile elements relative to ground ash, but these enrichments were significantly smaller than those measured in the quiescent plume samples. These findings support the occurrence of chemical fractionation during eruptions and posteruptive outgassing which is preserved as a signature that can be carried with small aerosols as they are transported over long distances.

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31 May 1983; revised 25 July 1983

# Ultraviolet Visual Pigment in a Vertebrate: A Tetrachromatic Cone System in the Dace

Abstract. *Microspectrophotometric measurements of optically isolated photoreceptors in the Japanese dace, a cyprinid fish, revealed four spectroscopically distinguishable cone pigments and one rod pigment. A visual pigment that absorbs in the near ultraviolet was found in small single cones.*

Sensitivity to ultraviolet (UV) light has been known to exist in ants for about a century and in bees for at least half that long (*1*). More recently, electrophysiological measurements have revealed flies, spiders, and other arthropods to be sensitive to UV light (*2*). A UV-absorbing visual pigment, a retinal-based chromoprotein, whose longest wavelength (alpha band) absorption peaks at about 350 nm, has been extracted from the neuropteran *Ascalaphus macaronius* (*3*). Pigeons (*4*) and hummingbirds (*5*) also respond to light in this spectral region. However, in these vertebrates neither the UV-sensitive receptor nor a UV-absorbing visual pigment has been identified. We now report finding short single cones in an aquatic vertebrate that could mediate UV responses.

Many species of bony fish have well-developed color vision. Among them, members of the cyprinid family (*6*), especially goldfish and carp, have been extensively investigated in vision research. Another cyprinid, the Japanese dace, *Tribolodon hakonensis* (Günther), with the common name "ugui," has been studied recently (*7*). A common fish in Japan, with a well-developed retina and receptors forming a highly regular cone mosaic, ugui has also been studied by electrophysiology, anatomy, and microspectrophotometry (*8*). The horizontal

Table 1. Numerical distribution of ugui photoreceptors as a function of peak absorptions of their visual pigments.

| $\lambda_{max}$ (nm) | Cones | | | | | | Rods | Total |
|---|---|---|---|---|---|---|---|---|
| | Double | | Single | | | | | |
| | Long | Short | Long | Medium | Short 1 | Short 2 | | |
| 600 to 620 | 7 | | 32 | 1 | | | | 40 |
| 570 to 590 | 16 | | 17 | 2 | | | | 35 |
| 520 to 535 | | 17 | 2 | 25 | 1 | | 28 | 73 |
| 500 to 515 | | 11 | 3 | 46 | 1 | | 12 | 73 |
| 405 to 415 | | | | | 13 | 1 | | 14 |
| 350 to 370 | | | | | 1 | 14 | | 15 |
| Total | 23 | 28 | 54 | 74 | 16 | 15 | 40 | 250 |

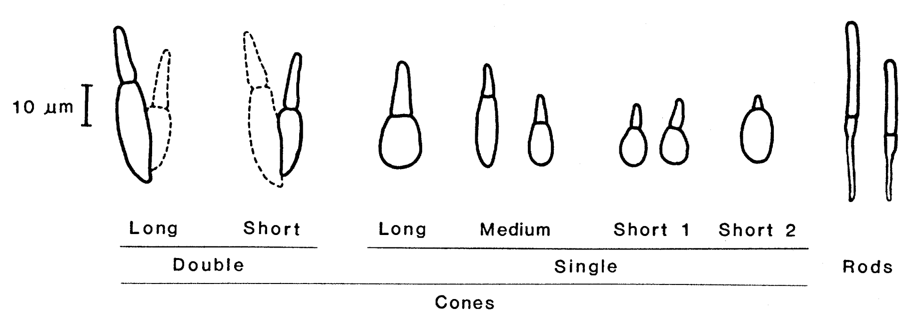


Fig. 1. Distal elements of ugui photoreceptors (devoid of myoid, nucleus, and synaptic processes). Morphology was determined at a magnification of ×1000; cell contours were traced from photomicrographs.

cells that respond differentially to different wavelengths (chromaticity type) in ugui were much more sensitive to blue light (down to 400 nm) than those in carp. The preliminary microspectrophotometric measurement of ugui photoreceptors, however, yielded an apparent lack of difference in visual pigments with respect to carp. Thus the high blue sensitivity in ugui could not be explained.

In this study, aimed at resolving this discrepancy (*9, 10*), we found that ugui photoreceptors resemble in many ways those of other cyprinids (*6*). In addition to numerous rods, the retina of ugui has long, medium, and short single cones and unequal double cones (Fig. 1). The outer segments (which contain the visual pigment) vary in size; the diameter ranges from 1.5 to 2 μm in small cones and rods to 3 to 4 μm in the other cones, and length ranges from 4 or 5 to between 15 and 20 μm. The red-absorbing pigment ($\lambda_{max}$ indicating the wavelength of peak absorption) is found in the longer member of the double cones and in the long single cones (Table 1). The shorter member of the double and the medium-sized single cones absorb green. In these features the similarity to goldfish is pronounced (*11*). However, the ugui has short single cones of two types. Although they overlap in size, the larger (type 1) usually bears a violet-absorbing pigment, whereas the smaller and more tapered (type 2) contains a UV-absorbing visual pigment.

The absorbance (A) and linear dichroism (LD) spectra of the five visual pigments in ugui are shown in Fig. 2. Several single-cell, multiscan measurements (each of which caused negligible bleaching) were averaged to produce each spectrum (*12*). The red-absorbing pigment, found in long double and single cones, peaked in the range of 570 to 620 nm. The rod pigment absorbed green and peaked in the range of 510 to 535 nm. The short double and medium single cones also contained a somewhat bluer green–absorbing pigment, with a $\lambda_{max}$ of 500 to 530 nm. The spectra of the short single cones contained enough scatter that specific peak values could not be determined; for the type 1 cones, the peak lay between 405 and 415 nm, and for the type 2 cones, between 350 and 370 nm. Exogenous 11-*cis* retinal added to the suspension of retinal fragments exposed to light and kept in the dark for at least 1 hour (*13*) established that the ugui uses a mixed-chromophore system. When regenerated, the long single and double cones, the medium single and short double cones, and the rods yielded spectra with $\lambda_{max}$ values of about 570, 505, and 510 nm, respectively. Since pure retinal (vitamin $A_1$ aldehyde) pigments have a shorter $\lambda_{max}$ than any mixture and the corresponding dehydro-pigments (based on vitamin $A_2$ aldehyde), it seems probable that in vivo outer segments contain their mixtures. Although mixed chromophore visual systems in fishes are not rare, the variability of chromophore mixing in ugui was surprising, for we obtained nearly all possible $\lambda_{max}$ values in each range from among the same group of specimens, kept under identical conditions. We have not yet detected visual pigment regeneration in short single cones. One unsolved difficulty is the extensive overlap between the spectra of the newly acquired "free" chromophore and of the coexisting chromoprotein.

The primary objective in this study was the identification of the UV-absorbing substance found in the outer segments of the short single type 2 cones. Transverse linear dichroism of the alpha band seems to be a definitive test for a native visual pigment. Although retinal and retinol (and their dehydro-analogs) all absorb in the near UV when present in bleached cells (and thus absorb UV light), they show no preference for linearly polarized light; or, as is most often the case, they preferentially absorb light with axial polarization (negative LD). In contrast, unexposed rod and cone outer segments (in lateral view) always prefer to absorb light with transverse (to the cell axis) polarization (positive LD) in the main absorption band of their visual pigment (Fig. 2). A quantitative measure of this property is the dichroic ratio, which is commonly defined as the ratio of peak absorbances measured with linearly polarized light (*14*). The ratio of absorbance at transverse polarization to that obtained with axially polarized light falls between 1 and 5 for all vertebrate photoreceptors investigated so far and between 1 and 3 for goldfish cones (*10*). Applying a similar determination to the spectra of Fig. 2 yields dichroic ratios of 1.8 (long single), 3.2 (rods), 2.5 (medium single), 2.2 (type 1), and 2.0 (type 2). Thus, the ugui photoreceptors, including

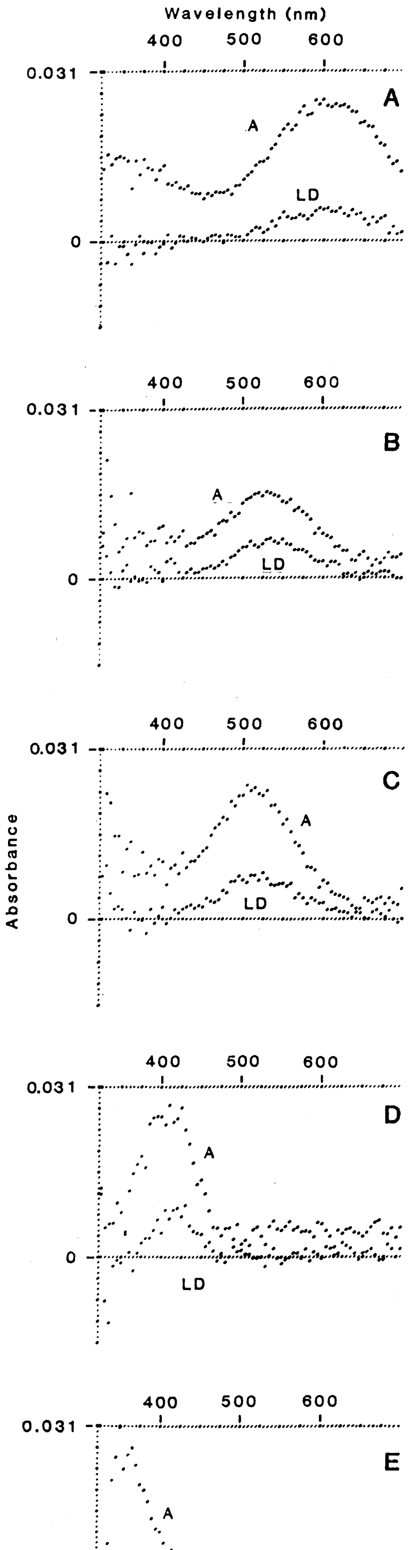


Fig. 2. Absorbance (A) and linear dichroism (LD) spectra of ugui photoreceptors. The LD trace ordinates are relative. Transverse dichroism is positive; axial dichroism is negative. Each absorbance curve is plotted on an absolute scale with divisions of approximately $10^{-3}$ absorbance unit. (A) Average spectra gathered in 64 scans in wavelength from seven long single cones (600 to 620 nm). (B) Average curves gathered in 32 spectral scans from four isolated rods. (C) Average responses gathered in 32 scans from four medium-sized single cones. (D) Spectra averaged from 32 scans of three short single (type 1) cones. (E) Average spectra gathered in 40 scans from three short single (type 2) cones. The arrow points to the peak (440 nm) of a small negative LD band of a transient photoproduct (caused by inadvertent bleaching).

the UV-sensitive type 2 cone, have linear dichroism similar to that of other vertebrates.

Another property of the type 2 outer segment is its sensitivity to actinic "bleaching" lights. Both the alpha-band A and LD peaks diminish in size in response to such lights. The resulting bleaching difference spectrum has a transient new peak at about 440 nm with a corresponding negative LD (arrow in Fig. 2E). Therefore, when the light-absorbing substance in type 2 cells is bleached, the resulting photoproducts share the dichroic characteristics of photoproducts formed in the other cones of ugui and other vertebrates.

Although the wavelength ranges and cell distribution in Table 1 may imply more, there are probably only five opsins present in ugui photoreceptors. Most of the observed $\lambda_{max}$ variations could be simulated by the use of various proportions of the two known chromophores and five opsins. The much narrower ranges and shorter $\lambda_{max}$ values found in the regeneration experiments support this conclusion. The larger numbers of long-single than long-double and short-double than medium-single cones found at longer wavelengths probably have no significance. Although much is yet to be discovered, the high blue sensitivity in physiological responses can now be explained, at least qualitatively, from spectral determinations. Whether ugui can perceive UV light and, if so, what benefit it derives from that ability are not known. Ugui, however, has been reported to be a fast and powerful swimmer that tends to feed at dusk and that sights its prey of aquatic and terrestrial insects from below, catching them in an upward move at the surface of the water (*15*). Broader spectral coverage may be helpful in detecting insects against sky light as background illumination, or it may be used in detecting special markings for identifying conspecifics.

Not only ugui, but also the roaches (*16*), goldfish (*17*), pigeons (*4*), hummingbirds (*5*), and chickens (*18*) seem good candidates for having UV-absorbing cones. Even the human retina may have UV receptors, in view of the high UV sensitivity of the aphakic human observer (*1*, *19*). Although the yellow lenses of adults normally block the penetration of UV light to the depth of the retina (thus apparently rendering UV receptors useless), such receptors may nevertheless be present either vestigially or to serve some function in ontogenetic development.

The tetrachromatic cone system (or pentachromatic eye) of ugui does not contradict current color vision theories (*20*). Combining Young's three-receptor theory with Hering's opponent color theory, Svaetichin concluded, "Three is the minimum number of receptors on which the Hering opponent system can be based; four would also do, but nature designs economically. This is the only magic of the number three in vision. The finding of a fourth cone in the periphery of the human retina would not at all reduce the value of Young's idea" (*21*). Depending on nature's original intent, tetra or even higher chromaticity may be economical (*22*). Since ugui is an ordinary fish and not apparently a singular creation of nature, we expect other animals to be similarly endowed.

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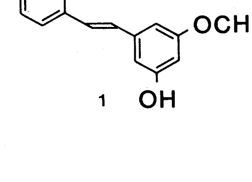
29 March 1983; accepted 4 August 1983

# Pinosylvin Methyl Ether Deters Snowshoe Hare Feeding on Green Alder

Abstract. *Pinosylvin methyl ether (PME), a toxic phenol, is a potent deterrent to showshoe hare feeding on green alder. Concentrations of PME found in green alder parts can account for the low palatability of winter-dormant foliar buds and staminate catkins but cannot affect internode palatability. The lack of a PME-related defense system in internodes suggests that green alder has at least a two-level defense system: defense of growth stages and defense of parts within growth stages.*

Herbivores do not feed on all parts of a plant; they usually eat specific parts (*1*). For example, when feeding upon winter-dormant green alder (*Alnus crispa*), snowshoe hares (*Lepus americanus*) eat internodes and reject foliar buds and staminate catkins (Fig. 1). Foliar buds and catkins contain high concentrations of nutrients and nonstructural carbohydrates and low concentrations of fiber and methanol-soluble phenolic constituents as compared to internodes (Table 1). Thus factors other than these constituents must influence snowshoe hare preferences for green alder parts. We



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