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### Polarization Reflecting Iridophores in the Arms of the Squid *Loligo pealeii*

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Distinct polarization body patterns have been recorded in cephalopods. In cuttlefish (*Sepia officinalis*) and squid (*Loligo pealeii*) these patterns are postulated to constitute a discrete communication channel that may be “hidden” from some of their predators (1, 2). In squid, the patterns of polarization are most prominent as long, narrow stripes along the arms (3). Examination of the skin of *L. pealeii* has now revealed very localized rows of iridophore cells that are reflecting and polarizing incident light and thus producing these patterns. Topical application of acetylcholine (ACh) to the arms of *L. pealeii* induced a change in the polarization reflection, as in other squid species (4). Moreover, silver staining and acetylcholinesterase histochemistry suggest that these iridophores are under direct neural control, unlike any known cephalopod iridophore.

Reflection and polarization of incident light by squid iridophores is accomplished by layers of intracellular platelets that are positioned parallel to each other (5). The spectrum (color) of the reflection can change from red/pink to blue and depends upon the distance between platelets, the orientation of the platelets, and the direction of viewing (6, 7). In squid dermis, iridophores have been found heretofore only beneath the layer of chromatophores (4). Iridophores are found in many parts of squid skin, but in most species they are especially abundant on the mantle. Because the polarization patterns in *Loligo pealeii* are created within very localized areas on the arms (Fig. 1B), we examined the skin in those areas and looked for structures that could potentially reflect light to produce polarization patterns.

For *in vitro* examination, pieces of fresh skin containing the polarizing sections were stretched to original size onto a paraffin-coated petri dish filled with chilled filtered seawater. The tissue was then examined with a Zeiss SVII dissecting microscope equipped with a polarization indifferent digital camera, under depolarized epi-illumination, and with a rotating linear polarizing filter (Polaroid HN38S) installed in the outgoing light path. Three

consecutive images were then taken with the filter set at preset angles (arbitrarily defined as 0°, 45°, and 90°). The images were then analyzed with custom-made software, and the polarization characteristics of the reflected light were determined.

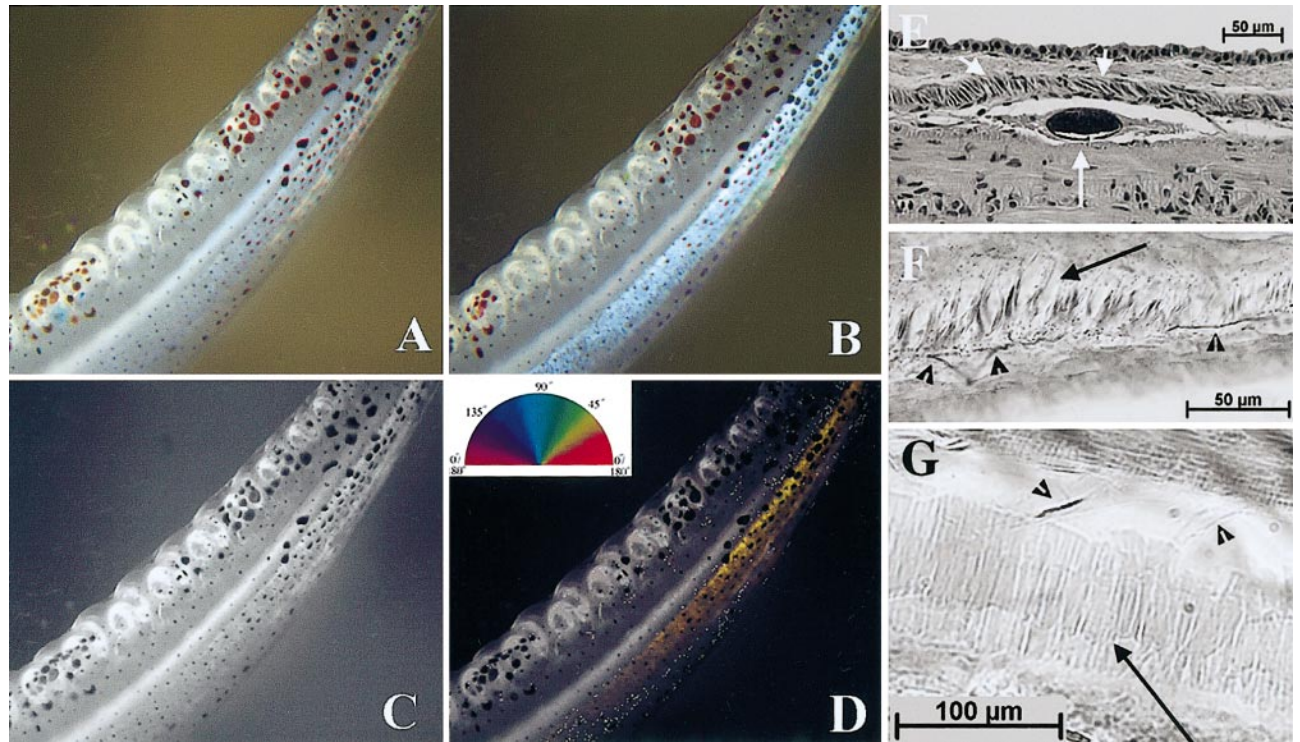
For morphology, arms were preserved in 10% formalin in buffered seawater for 3 d and, after washing, they were cut, mounted, and transferred to 70% ETOH. Samples were then sectioned at 40–200  $\mu\text{m}$  intervals and stained with Mayer’s hematoxylin and eosin, Masson’s trichrome, and silver (Holmes’ silver nitrate method). Sectioning and processing arm tissue for acetylcholinesterase histochemistry was done according to the method of Mesulam and Van Hoesen (8), using the acetylthiocholine medium specified by Geneser-Jensen and Blackstad (9). Images were then observed with a Zeiss Axioplan microscope equipped with an internal scaling and calibration system.

Strong partially linearly polarized reflection could be identified in specific lines along the animals’ arms (Fig. 1A–D) and was often associated with physical colors such as blue or pink. Microscopic examination of skin tissue at these locations revealed the existence of a new type of iridophore. These reflecting cells were located in very narrow areas of the skin,  $60 \pm 26 \mu\text{m}$  ( $n = 24$ ) underneath the skin surface, and organized as long stripes. Cell length was  $267 \pm 131 \mu\text{m}$  ( $n = 22$ ), and cell width was  $14.8 \pm 7.2 \mu\text{m}$  ( $n = 26$ ). These long stripes of iridophores are consistent with the red and highly polarizing iridophores reported by Mäthger and Denton (7), but the squid arm iridophores are much narrower. Unlike other squid iridophores, which are found beneath the chromatophore layer, these cells were situated above the chromatophores (Fig. 1E). Platelets [ $1.8 \pm 1.2 \mu\text{m}$  wide and  $14.1 \pm 6.4 \mu\text{m}$  long ( $n = 138$ )] were set in an angle inside the cell and were organized parallel to each other with a variability of  $7.9^\circ \pm 4.0^\circ$  ( $n = 25$ ). Inter-platelet space was  $1.2 \pm 0.7 \mu\text{m}$  ( $n = 150$ ), providing for an average density of  $30.8 \pm 9.0$  platelets per 100  $\mu\text{m}$ .

Previous studies have never furnished evidence of innervated squid iridophores (4). This is surprising considering the speed with which changes in color—even iridescent color—occur in cephalopods. Hanlon *et al.* (4) found that iridophores in the squids *Lolliguncula brevis* and *Loligo plei* became iridescent when treated with ACh, but no nerve fibers were found adjacent to or

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**Figure 1.** An arm of a squid as seen in normal light (A); through a linear polarizer set at  $45^\circ$  to the orientation of maximal polarized reflection (B); as a black and white image of A, which is presumably what a color-blind, polarization-insensitive predator would see (C); and when % polarization is coded into saturation (the color image), and orientation of polarization is encoded into hue (the scale)—note that the polarization reflection is very localized into a specific stripe along the arm (D). Light microscopy of cross sections in squid arms with H&E staining (E) shows iridophores (short arrows) above the chromatophores (long arrow), which is a novel arrangement. In (F), a DIC image of a silver-stained section which indicates potential nerve fibers (short arrows) immediately adjacent to or on an iridophore (long arrow). In (G), acetylcholinesterase staining (short arrows) adjacent to an iridophore cell (long arrow) also indicates potential locations of innervation.

near the iridophore cells. They therefore surmised that ACh would diffuse to the iridophore cell surfaces, would bind to ACh receptors there and thus would induce an ultrastructural change in the platelets to produce iridescence.

However, polarization patterns on cephalopods change in just a second or two, suggesting neural rather than hormonal control. Topical application of  $10^{-3}$  M ACh to isolated skin patches induced polarization reflections. Silver staining revealed nerve fibers in very close proximity to the iridophores (Fig. 1F), suggesting that these cells may be innervated directly. Finally, staining for acetylcholinesterase revealed specific active areas at the attachment of potential nerve fibers to the iridophores (Fig. 1G).

Our results present quite a different cellular structure—and potential control mechanism—in which a polarization pattern is produced in the arms of squid. The control of these structures, and the significance of polarization patterns to squids, remain to be investigated.

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