# HORMONAL CONTROL OF THE REFLECTING RETINAL PIGMENT IN THE ISOPOD *LIGIA OLFERSI* BRANDT[1]

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The light-adapted and dark-adapted positions of the retinal pigments involved in photomechanical adaptation of compound eyes to changes in illumination have been described for three species of isopods. Whether retinal pigments of isopods are independent effectors or are under nervous or endocrine control has not been determined.

Peabody (1939) was the first investigator to describe migration of retinal pigments in isopods. She used *Idotea balthica* and *Idotea metallica*. In her paper she described only distal and proximal retinal pigment cells. Kleinholz (1961) stated that the fixative she used might have dissolved the reflecting pigment.

Nagano (1949) described the structure of the eye in the isopod *Ligia exotica* including the positions of the retinal pigments in light-adapted and dark-adapted specimens. Of interest herein is his diagram showing the reflecting pigment proximal to the basement membrane in illuminated specimens and distal to the basement membrane in specimens kept in darkness. The objectives of the present investigation were to determine (1) whether the light-adapted and dark-adapted positions of the reflecting pigment in the eyes of *Ligia olfersi* are the same as in *Ligia exotica* and (2) whether migration of the reflecting pigment in *Ligia olfersi* is under hormonal control as in higher crustaceans.

## Materials and Methods

The specimens of *Ligia olfersi* used in this investigation were collected on pilings along the south shore of Lake Pontchartrain in New Orleans, Louisiana. The authors are indebted to Dr. Thomas E. Bowman of the United States National Museum for identifying this species. In the laboratory the *Ligia* were kept in covered aquariums containing damp pieces of paper.

To observe the reflecting pigment, histological sections of the eyes, 10 $\mu$ thick, were prepared. After the appropriate experimental treatment the animals were dropped into boiling water for 15-30 seconds to stop rapidly further migration of the pigment. The heads were then removed and fixed in Carnoy's fluid. Bouin's fluid was unsuitable because it dissolved the reflecting pigment. Paraffin sections were then prepared. As a measure of the position of the reflecting pigment, a reflecting pigment index was devised. This index was the ratio of the mean width of the reflecting pigment in both eyes divided by the distance from the center of the spherical lens to the basement membrane. The latter distance is unaffected by light and darkness. Use of this ratio minimized the effect of size differences among the specimens. The distances were measured with the aid of an ocular micrometer and reflected illumination. The reflecting pigment under such illumination appeared silvery-white. The center of the lens rather than the cornea was chosen as one of the fixed points for measurement because the cornea was frequently torn loose in the histological sections. Each unit of the ocular micrometer at the magnification used corresponded to 10.9 $\mu$. Measurements were made of 20 ommatidia, 10 in each eye. The average ratio for both eyes was then calculated and represented the index of that particular specimen.

Tissue extracts were prepared in the usual manner. The concentrations were two-thirds of a sinus gland, *i.e.* one-third of the isopod's complement, per 0.02 ml physiological saline and one-third of the supraesophageal ganglia in the same volume. The dose injected into each isopod was 0.02 ml.

Student's *t* test was used for determination

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of the level of significance between means. The 5% level was considered the maximum for a significant difference.

## Observations and Results

### *Normal migration of the reflecting retinal pigment in Ligia olfersi*

The object of the first set of experiments was to determine the positions of the reflecting pigment in light-adapted and dark-adapted eyes. Specimens were placed both in a photographic darkroom and in white enameled pans under an incident illumination of 120 ft-c for two hours, at the conclusion of which the isopods were killed.

Inspection of the sectioned eyes revealed that the reflecting pigment always remained distal to the basement membrane (Fig. 1). In dark-adapted eyes this pigment always abutted against the basement membrane, but in about one-half of the light-adapted eyes some of the pigment had migrated a short distance away, about 10 $\mu$, from the basement membrane. Furthermore, measurements of eyes from 15 light-adapted and 11 dark-adapted specimens revealed that the mean width of the reflecting pigment in the light-adapted eyes was 43.9 $\mu$ but in the dark-adapted eyes was 74.5 $\mu$. The difference between the means is statistically highly significant ($p<0.001$). The mean distances from the center of the spherical lens to the basement membrane were 167.3 $\mu$ and 171.1 $\mu$ for light-adapted and dark-adapted eyes respectively. The difference between these means is insignificant statistically. Consequently, the difference between the widths of the reflecting pigment could hardly have been due to size difference alone. The respective reflecting pigment indexes for light-adapted and dark-adapted eyes were 0.262 and 0.435 (Fig. 2A, B). The difference between these means is also highly significant statistically ($p<0.001$). Accord-

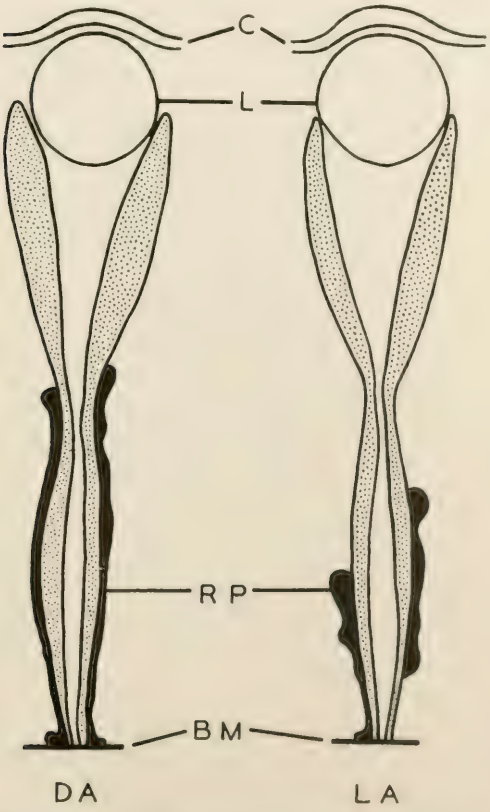


Figure 1. Diagrammatic representation of ommatidia from dark-adapted (DA) and light-adapted (LA) eyes. BM, basement membrane; C, cornea; L, lens; RP, reflecting pigment.

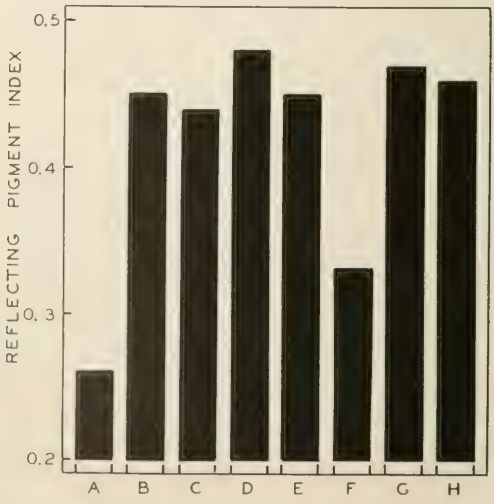


Figure 2. Mean reflecting pigment indexes of (A) light-adapted specimens, (B) dark-adapted specimens, (C) dark-adapted specimens that had received physiological saline, (D) dark-adapted specimens that had received an extract of sinus glands, (E) dark-adapted specimens that had received an extract of supraesophageal ganglia, (F) light-adapted specimens that had received physiological saline, (G) light-adapted specimens that had received an extract of sinus glands, and (H) light-adapted specimens that had received an extract of supraesophageal ganglia.

ing to these reflecting pigment indexes the reflecting pigment is 1.66 times wider in dark-adapted than in light-adapted eyes.

### *Effect of extracts of sinus glands and supraesophageal ganglia on the reflecting pigment*

The object of this experiment was to determine whether migration of the reflecting pigment might be controlled by a principle in the sinus glands or supraesophageal ganglia. Extracts were injected into specimens maintained in the darkroom and into specimens in white containers under an illumination of 120 ft-c. The injections in the darkroom were performed with the aid of a dim, red photographic lamp. Forty-five minutes after injection of the extracts the isopods were sacrificed. The experiments with illuminated specimens were performed three times, with isopods in the darkroom two times. Nine illuminated specimens were injected with extract of supraesophageal ganglia, 10 illuminated isopods with sinus gland extract. Six isopods were injected with each extract in the darkroom. Control specimens received saline alone.

The extracts had no effect on the isopods kept in darkness (Fig. 2C, D, E). However, these extracts caused a dark-adaptational response in the light-adapted specimens (Fig. 2F, G, H). The saline caused a slight but statistically insignificant dark-adaptational response in the light-adapted controls. However, the dark-adaptation shown by the illuminated isopods injected with tissue extracts was equal to that of specimens kept in the darkroom for two hours. These responses were statistically highly significant; $p<0.001$ for the sinus gland extracts and $p<0.01$ for the extracts of supraesophageal ganglia.

## Discussion

The description of the positions of the reflecting pigment in light-adapted and dark-adapted specimens of *Ligia olfersi* presented above does not agree with the description of the same phenomenon presented by Nagano (1949) for *Ligia exotica.* He reported that the reflecting pigment lay distal to the basement membrane in darkness and migrated proximal to the basement membrane in light. Herein, however, we noted that this pigment always remained distal to the basement membrane. Elongation of the reflecting pigment in dim light would render this pigment more capable of reflecting light onto the retinula cells, thereby increasing the visual efficiency of the eye in dim light.

Kleinholz (1936) has shown that eyestalk extracts will cause light-adaptation of the reflecting pigment in the prawn *Palaemonetes vulgaris.* Nagano (1947) found the same response in the shrimp *Paratya compressa.* With *Ligia olfersi,* however, the response of the reflecting pigment to extracts of sinus glands and supraesophageal ganglia was dark-adaptation. These observations constitute the first report of (1) a reflecting pigment dark-adapting principle among crustaceans and (2) a retinal pigment activator in isopods. Perhaps further investigation will show that the principle occurs among higher crustaceans as well as in the isopod *Ligia olfersi.*

## Summary and Conclusions

1. The reflecting retinal pigment of the isopod *Ligia olfersi* migrates in response to light and darkness.

2. The reflecting pigment always remains distal to the basement membrane. The width of the reflecting pigment is greater in dark-adapted than in light-adapted eyes.

3. The sinus gland and supraesophageal ganglia contain a principle that causes dark-adaptation of the reflecting pigment.

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ABSTRACT

The reflecting retinal pigment of the isopod *Ligia olfersi* migrates in response to light and darkness. The pigment, occupying a wider area in dark-adapted than light-adapted eyes, always remains distal to the basement membrane. Extracts of the sinus glands and supraesophageal ganglia cause a dark-adaptational response of the reflecting pigment. This paper represents the first report of endocrine regulation of a retinal pigment in isopods. The reflecting pigment is involved in photomechanical adaptation of the eye in response to changes in illumination.