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SPECIFICITY OF THE HOST-INDUCED NEGATIVE PHOTOTAXIS OF THE SYMBIOTIC WATER MITE, *UNIONICOLA FORMOSA*[1]

HERNANDO A. DEL PORTILLO[2] AND RONALD V. DIMOCK, JR.[3]

Department of Biology, Wake Forest University, Winston-Salem, NC 27109

Abstract

The water mite *Unionicola formosa* (Acarina: Unionicolidae) exhibits positive phototaxis when free of any chemical influence of its molluscan host, *Anodonta imbecilis*. When mites are exposed either to water from the host's mantle cavity or to an homogenate of host mantle tissue, the sign of their phototaxis reverses to negative. The behavioral threshold concentration of mantle homogenate that induces negative phototaxis to monochromatic light is approximately 0.7 μg protein/ml. Negative phototaxis does not occur in the presence of any chemical influence of the bivalves *Elliptio complanata* or *Anodonta cataracta*, neither of which species harbors this symbiont at the study site. A component of mantle tissue from *Anodonta imbecilis* that elicits the negative phototaxis is heat labile, sensitive to trypsin, and has a molecular weight in excess of 10,000 daltons.

Introduction

Various mechanisms of communication involving a variety of sensory modalities are instrumental in the interactions between species associated in symbiotic relationships in marine and freshwater ecosystems (Davenport, 1966; Ache, 1974; Fricke, 1975). Chemical communication, often highly specific, consistently achieves prominence in the initiation and maintenance of such interspecific associations (Davenport, 1955; McCauley, 1969; Mackie and Grant, 1974; Barbier, 1981).

Unionid bivalve molluscs frequently serve as hosts for symbiotic freshwater mites of the genus *Unionicola* (Acarina: Unionicolidae) (Mitchell, 1955; Davids, 1973; Hevers, 1980; Vidrine, 1980). Presumably, the host's mantle cavity could be a refuge from predators or perhaps environmental stresses, while the water circulated through the mantle cavity may convey food to the symbionts. The host itself can be exploited as a source of nutrition (Baker, 1977) or as a site for oviposition and metamorphosis (Mitchell, 1955).

The specificity of these molluscan-acarine symbioses may be influenced by different aspects of the biology of the organisms involved in them, such as behavior, population dynamics and distributional patterns. For example *Unionicola formosa* is widely distributed in eastern North America (Vidrine, 1980) and throughout its range exhibits varying degrees of host specificity as revealed by field data (Dobson, 1966; Roberts, 1977; Gordon *et al.* 1979; Vidrine, 1980). Specificity has also been demonstrated in the host recognition behavior of *U. formosa* (LaRochelle and Dimock, 1981). In addition Welsh (1930) reported that *U. ypsilophora* (probably

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[2] Present address: Department of Zoology, University of Georgia, Athens, GA 30602.

[3] To whom reprint requests and correspondence should be directed.

Abbreviations: APW, artificial pond water; MH, mantle homogenate.

U. formosa, see Roberts *et al.*, 1978) reversed completely its normally positive phototaxis when tested both in water from the mantle cavity and in gill extract of its host, the freshwater mussel *Anodonta cataracta*. Subsequently Welsh (1931) indicated that this behavior could only be induced in the presence of the mite's own molluscan host, *i.e.*, the host-induced negative phototaxis was species-specific with respect to its induction.

With the exception of the observations of Welsh (1930, 1931) there has been no further study of the factors affecting the host-induced negative phototaxis of *U. formosa* except for an analysis of the spectral sensitivity of the response (Roberts *et al.*, 1978). The present paper quantifies the dose-response relationships of the negative phototaxis of *Unionicola formosa* induced by a tissue homogenate of its molluscan host in the southeastern U. S., *Anodonta imbecilis*, and examines the species-specificity of the induction of this behavior. Results of initial chemical characterization of the substance of host origin that elicits the negative phototaxis of *U. formosa* are also presented.

Materials and Methods

Organisms

With the exception of *Elliptio complanata* which was purchased from Carolina Biological Supply Co., the animals used in this study were collected as needed from the farm pond of Mr. James Honeycut, Mt. Pleasant, Cabarrus County, North Carolina, where *Anodonta imbecilis* is sympatric with *Anodonta cataracta*. Because the density of female *U. formosa* in host bivalves increases in a linear fashion with increasing bivalve length (Dimock, 1979; Gordon *et al.*, 1979), only mussels > 60 mm in total length were used in the experiments. Since female *U. formosa* are far more numerous than males (Dimock, 1979), only females were used.

All *U. formosa* employed in this study were collected from *A. imbecilis*. Mites removed from host mussels were first held for at least 24 h in artificial pond water (APW) (Dietz and Alvarado, 1970). They were then transferred twice to fresh APW for 1 h, after which they were rinsed in 2 changes of APW immediately before use in the phototaxis assay. All mites were used within 36 h of isolation from their host.

Mantle homogenate (MH)

The precise standardization of a chemical stimulus for behavioral studies, although highly desirable, is impossible if one is working with a crude homogenate of a tissue. All tissue homogenates employed in the present study were, therefore, quantified on the basis of protein concentration as determined by the Bradford technique (Bradford, 1976) standardized with bovine serum albumin (Sigma Chemical Co.). In order to avoid potential contamination with *U. formosa's* eggs which are deposited in the host's gills (Vidrine, 1980), only mantle tissue of the mussels was used to prepare experimental homogenates [Mantle Homogenate (MH)].

MH was prepared by macerating fresh mantle in an iced tissue homogenizer with APW and then centrifuging at 48,000 × *g* at 4°C for 2 hours. The supernatant was frozen at −80°C in microcentrifuge tubes (0.5 ml, Brinkmann) until it was used, since preliminary experiments had revealed that the effect of MH on phototaxis is not altered by freezing and storage for 30 days.

Phototaxis

Phototaxis was monitored in a 120 × 20 × 20 mm chamber constructed of black lucite (except for transparent ends) which was provided with a removable partition that subdivided the chamber into five 22 mm compartments. The chamber was illuminated horizontally from one end with a 100 W tungsten filament spot photographic light that was filtered first through an infrared filter (Corning #1-75) and a "hot-mirror" (Baird Atomic) to reduce the radiation above 700 nm. The light intensity (measured at the chamber) and wavelength were then filtered (Ditric Optics Co.) to 10^{-1} $\mu E/m^2/sec$ and 500 nm, the optimum light stimulus for negative phototaxis (Roberts *et al.*, 1978). Light intensity was measured with a Lambda Instrument Corp. Quantum Sensor (Model LI-185).

A typical test involved dark-adapting 30 mites in 1 ml of APW in a 12 × 75 mm plastic tube (Falcon 2003) for 1.5 h at 20°C. The mites were then poured in the dark into the center section of the chamber which had previously been filled with 29 ml of the desired test medium also at 20°C. After 30 seconds the stimulus light was turned on and the partition was removed for 90 seconds; it was then reinserted and the number of mites in each section was recorded.

Where appropriate the data were analyzed by the Kolmogorov-Smirnov goodness of fit test (Zar, 1974), one-way ANOVA, and Student Newman Kuels (SNK) test. The data are presented as percent response based on the number of mites in the section of the chamber closest to the light source (positive phototaxis) or the section farthest from the light source (negative phototaxis). Every experiment was repeated three times with different mites in each replicate for a total of 90 animals per experiment.

Dose-Response

Concentrations of MH (0.2 to 30 μg of protein/ml) prepared from *A. imbecilis* were tested in the chamber for their effects on the phototaxis of *U. formosa.*

Host specificity

The specificity of the induction of negative phototaxis was tested by comparing the responses of *U. formosa* to the mussels *Anodonta imbecilis*, *Anodonta cataracta*, and *Elliptio complanata.* Two different experimental approaches were employed. In the first, 12 live mussels of each species were scrubbed in APW to remove any debris from the shells and the water from the mantle cavity was gently removed. Each species was then placed in a clean aquarium with 2000 ml of APW for 38 h, after which 30 ml of water was obtained from the mantle cavities and used as the test medium. In the second approach, MH of each of the three species was prepared as described above and tested at a concentration of 6.67 μg protein/ml.

Ultrafiltration and heat sensitivity

Fifteen ml (30 mg protein) of MH of *A. imbecilis* was ultrafiltered to 1 ml with an Immersible Cx-10 ultrafilter (nominal moleclular weight exclusion of 10,000 daltons, Millepore Corp.). The low ($<10^4$ daltons) molecular weight fraction, which contained no detectable protein, was subdivided into three aliquots which were each made up to 30 ml with APW and tested for their effect on phototaxis. The high molecular weight fraction was tested at 6.67 μg protein/ml.

Unfractionated MH was heated at 60, 80 and 100°C for 30 min in a water bath and each solution was then bioassayed.

Trypsinization

The effect of digestion by trypsin on the potential of MH from *A. imbecilis* to induce negative phototaxis was examined by treating MH (7 mg protein) with 10 μg of trypsin (Difco) in a total volume of 1.0 ml for 2 h at 25°C. From this solution 30 μl aliquots were bioassayed in a final volume of 30 ml (MH = 6.67 μg protein/ml; trypsin = 0.01 μg/ml).

Control experiments tested the effects of exposure to trypsin on the positive and negative phototaxis of *U. formosa*. Mites were tested for the sign and magnitude of their photobehavior in APW with 0.01 μg trypsin/ml. Also, mites that had been incubated for 1 h in 10 μg trypsin/ml were tested in MH (6.67 μg protein/ml) to determine if trypsin affected the mite's detection of the chemical signal.

Results

Phototaxis

The distribution of mites among the five compartments of the test chamber when held for 90 seconds in total darkness in APW with or without mantle homogenate was not significantly different from an expected distribution of 20% of the animals in each compartment (Kolmogorov-Smirnov; $P > 0.05$). However, their distribution in response to the stimulus light in APW was highly significantly different (Kolmogorov-Smirnov; $P < 0.01$; $\bar{X}$% positive phototaxis = 87%) from that in the dark and clearly substantiated their positive phototaxis when tested in APW free of any chemical influence of *A. imbecilis*. The phototactic response of *U. formosa*, however, was significantly negative (Kolmogorov-Smirnov; $P < 0.01$; $\bar{X}$% negative phototaxis = 50%) when the animals were exposed to the stimulus light both in water from the mantle cavity of *A. imbecilis* and in supra-threshold concentrations of MH of *A. imbecilis*.

Dose-response

The results of the dose-response experiments (Fig. 1) revealed that MH at a concentration of 0.83 μg protein/ml was an adequate stimulus to induce negative phototaxis. Furthermore, while all concentrations of MH > 0.83 μg protein/ml induced responses that were significantly different from the responses to concentrations lower than that dose (ANOVA; SNK; $P < 0.05$), the responses of *U. formosa* to all concentrations > 0.83 μg protein/ml were not significantly different from each other (ANOVA; $P > 0.05$).

Host specificity

The sign and magnitude of the phototaxis of *U. formosa* in the presence of MH from various species of mussels are depicted in Figure 2. It is obvious that only MH from *A. imbecilis* induced negative phototaxis by the mites. This marked specificity also occurred when *U. formosa* was tested for its sign of phototaxis in water from the mantle cavities of these three species of mussels. In those experiments water from *A. imbecilis* induced 40% negative and 4% positive phototaxis; that from *A. cataracta*, 4% negative and 83% positive phototaxis; and that from *E. complanata*, 8% negative and 82% positive phototaxis.

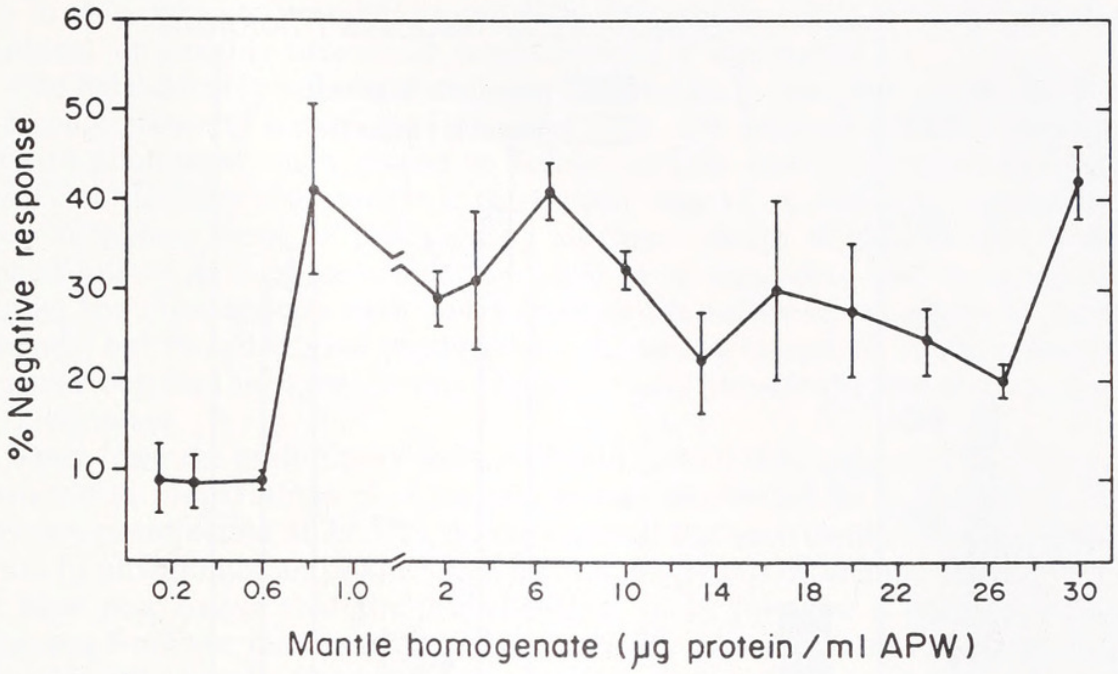

FIGURE 1. Dose-response relationships of the negative phototaxis of *Unionicola formosa* induced by mantle homogenate of *Anodonta imbecilis*. Data points are the $\bar{X} \pm SE$ (N = 90). All concentrations of mantle homogenate ≥ 0.83 µg protein/ml elicited negative phototactic responses that were not different from each other (ANOVA, $P > 0.05$) but that all were significantly greater than the responses to lower concentrations (ANOVA; SNK; $P < 0.05$).

Characterization of mantle homogenate

Only the high molecular weight fraction ($>10^4$ daltons) of ultrafiltered MH elicited negative phototaxis (Table I). The magnitude of the negative response induced by this fraction was significantly higher ($P < 0.05$, t test) than that elicited by unmodified homogenate.

Heating MH at 80°C and at 100°C for 30 minutes destroyed its capacity to induce negative phototaxis (Table I). Although heating MH at 60°C for 30 min resulted in a significant reduction in the negative response (and a concomitant increase in positive phototaxis), the potential of MH to induce negative phototaxis was not completely eliminated by this milder heat treatment (Table I).

Trypsinization of MH resulted in a significant reduction of the magnitude of negative phototaxis and restored the level of positive phototaxis to about 60% of that which occurs in plain APW (Table I). Exposure of mites to trypsin in the absence of MH had no significant effect on their positive phototaxis. In addition, the incubation of *U. formosa* for 1 h in a 1000-fold higher concentration of trypsin than that to which they were exposed in the trypsinization assay did not significantly affect their subsequent photonegative behavior in the presence of unaltered MH (Table I).

DISCUSSION

Chemical influences on the sign of an organism's phototaxis are not unknown. Thorson (1964) showed that a reduced salinity could reverse the normally positive phototaxis of various pelagic marine larvae. Lucas (1936) demonstrated that the copepod *Eurytemora hirundoides* reversed its positive phototaxis in the presence of abundant diatoms. In the present study adult female *Unionicola formosa* changed

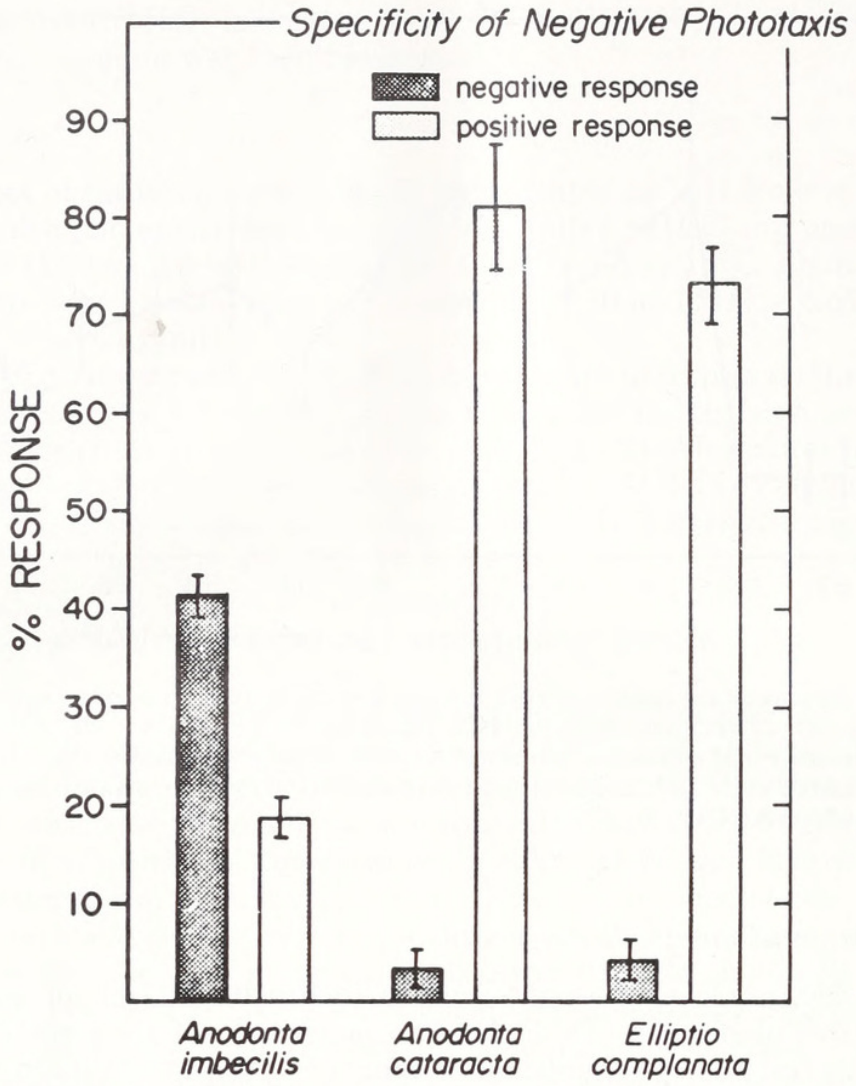

FIGURE 2. Specificity of the negative phototaxis of *Unionicola formosa* induced by mantle homogenate (6.67 μg protein/ml) from various unionid bivalves. Histograms depict $\bar{X} \pm SE$ (N = 90). The total for positive and negative response is less than 100% because of the design of the bioassay (see text).

the sign of their phototactic response from positive to negative in the presence of a chemical influence of their host, the freshwater mussel *Anodonta imbecilis*.

The negative phototactic behavior of *U. formosa* followed a more or less classical dose-response relationship with the threshold for negative phototaxis occurring in mantle homogenate with approximately 0.7 μg protein/ml. The response quickly saturated at supra-threshold concentrations (Fig. 1), suggesting that the chemoreceptors involved in the response become saturated at concentrations of the stimulus that are only slightly above threshold. However, there may be a more concentration-dependent aspect of the response, such as some photokinetic phenomenon, that has not been identified.

The host specificity of this induced negative phototaxis was very pronounced. *U. formosa* did not become negatively phototactic either in the presence of water from the mantle cavity or in mantle homogenate (8 times the minimum effective concentration from *A. imbecilis*) of *Elliptio complanata* or of *Anodonta cataracta*, a sympatric congener of the mite's host. These results are only partially in agreement with those of Welsh (1930, 1931) which indicated that *U. formosa* responded

only to *A. cataracta*. Whether or not these differences reflect methodological, geographical, or possibly taxonomic considerations is unresolved.

The behavioral specificity that we have observed in this study could potentially be a consequence of one of the following. First, the substance(s) that induces the negative phototaxis could indeed be species-specific and *U. formosa* has evolved sensory capabilities that enable it to respond only to *A. imbecilis*. Secondly, the active substance could be produced by all three species of mussels that were examined but at an ineffective concentration in *A. cataracta* and *E. complanata*. Finally, the three species each could produce the substance to which *U. formosa* responds, but the substance produced by *A. cataracta* and *E. complanata* might be masked by another agent or some inhibitor may prevent the phototactic response by *U. formosa*.

Aside from the preliminary work of Welsh (1930) that suggested that the active substance in preparations of *A. cataracta* was unaffected by boiling and resisted extensive putrefaction at 37.5°C, no information has been available on the chemical nature of any molluscan product that affects phototaxis of unionicolid water mites. We have now shown that the photobehavior of *U. formosa* is modified by some substance from the mantle of *A. imbecilis* that is heat labile and sensitive to trypsinization. The results presented here indicate that the active substance has a molecular weight of >10,000 daltons; additional data suggest that its molecular weight is probably well in excess of 10^5 daltons (del Portillo and Dimock, unpublished observations). These characteristics are consistent with the active substance being proteinaceous. Further analysis of the quantitative and qualitative characteristics of this substance may elucidate how the specificity of this phototactic behavior is mediated.

TABLE I

Effects of exposure to various chemical stimuli on the sign and magnitude of phototaxis by Unionicola formosa. *Entries are the mean ± SE for N = 90. All MH prepared from* Anodonta imbecilis.

Stimulus	Response of *U. formosa*	
	% Positive phototaxis	% Negative phototaxis
APW	88.8 ± 1.1	0.0
Unaltered MH	20.6 ± 1.8	42.6 ± 3.0
Ultrafiltered MH		
$>10^4$ daltons	10.0 ± 1.9	64.4 ± 2.2
$<10^4$ daltons	66.7 ± 1.7	2.2 ± 1.1
Heat-treated MH		
100°C, 30 min	74.7 ± 2.9	9.0 ± 2.9
80°C, 30 min	80.0 ± 3.8	6.7 ± 0.0
60°C, 30 min	30.0 ± 1.9	31.1 ± 1.1
Trypsinized MH		
0.01 μg trypsin/ml, 25°C, 2 h	55.4 ± 4.9	4.6 ± 3.1
Trypsin Controls		
APW + 0.01 μg trypsin/ml	90.7 ± 2.3	0.0
[a]Pre-treated mites + MH	9.0 ± 3.9	39.0 ± 7.4

[a] Mites were incubated for 1 h in 10 μg trypsin/ml then were tested in the presence of MH.

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