

CHROMATOPHORE BEHAVIOR IN THE ISOPOD *LIGIA OCCIDENTALIS*
DANA, 1853

BY

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INTRODUCTION

The alteration of body color in *Ligia* was observed by Tait (1910). He demonstrated that the melanophores of *Ligia oceanica* contained dispersed pigment when the animals were on a dark background. Similar responses for this species have been observed by Smith (1938); for *Ligia exotica* by Enami (1941a), Nagano (1949), and Fingerman (1956) and for *Ligia baudiniana* by Kleinholz (1937). Diurnal rhythms of pigment dispersed by day and pigment concentrated at night were described by Enami (1941a), Fingerman (1956), and Kleinholz (1937).

These studies were primarily concerned with determining the physiological mechanisms of control of melanin dispersion or concentration. Little consideration has been given to the adaptive significance of the melanophore responses. The present study was undertaken to examine the pigment responses in the chromatophores of *Ligia occidentalis* Dana, 1853 under varied conditions of light and dark and to correlate the responses with the habits of the animals in their natural environment.

This study developed from a class project in ecological physiology taught by Professor A. C. Giese at Hopkins Marine Station of Stanford University. I wish to express my appreciation to Dr. L. R. Blinks, Director of Hopkins Marine Station, for making space and facilities available for this study. This investigation was conducted while the author was studying marine biology by means of a National Science Foundation science faculty fellowship.

MATERIALS AND METHODS

Ligia occidentalis was collected along the rocky shore in the vicinity of Pacific Grove, California, and at Yankee Point, 10 miles south of Pacific Grove. The stock supply of isopods was kept in battery jars. Each jar was supplied with a small dish of water into which the animals could dip their uropods. Algae were supplied for food. All experiments were carried out at 18-21°C.

The room in which the animals were kept and in which the experiments were conducted was completely darkened. As an added precaution, those experiments in which the animals were kept in complete darkness were carried out in a dark

ground. Smith's results show a slow initial response while that of *L. occidentalis* was rapid.

Two interesting differences in the behavior of the chromatophores of animals that have been acclimated to a yellow or black background and then shifted to the opposite background can be seen in figures 5 and 6. Firstly, the pigment in the xanthophores of animals acclimated to a yellow background and shifted to a black background did not become as concentrated as the pigment in the xanthophores of animals acclimated to a black background. This is in contrast to the pigment in the melanophores which had about the same concentration in the acclimated and the tested conditions. Secondly, the pigment in the melanophores tended to disperse after first concentrating in animals acclimated to a black background and shifted to a yellow background. This dispersion of the black pigment is associated with the longer period of time required by the pigment in the xanthophores to disperse.

Changes in the concentration and dispersion of pigment in the chromatophores of isopods are controlled by hormones (Carlisle and Knowles, 1959 : 55). However, the studies of the mechanisms of hormonal control have presented diverse results. These experiments are summarized in Table I. Examination of the table shows a considerable disparity in the results of making injections into intact recipients even within the same species. Inspection of the table also indicates that the acclimation condition of both donor and recipient is very important in determining the effects of injection experiments.

The importance of the factor of acclimation is further emphasized by studies on *Orconectes clypeatus* (Fingerman and Aoto, 1958) that demonstrated that under the conditions of long term acclimation, dispersing hormone increased in the circumesophageal connectives of crayfish on a white background whose red pigment was concentrated and that concentrating hormone increased in crayfish on a black background with dispersed red pigment. Since most of the experiments with *Ligia* were concerned only with the melanophores, some of the individual experiments could be interpreted to support the hypothesis that a single hormone is present. However, the entire body of data can be better understood if the presence of both a concentrating and a dispersing hormone is postulated. Smith (1938) postulated such a condition in *L. oceanica* based on the rates at which lightening and darkening took place under various experimental conditions.

When the reactions of both xanthophores and melanophores are considered, two hormones seem to be the minimum number that will account for the observed behavior of the chromatophores. The rates of change of the pigment in the chromatophores occurred too rapidly to be accounted for by the presence or absence of a single hormone. All of the pigment reactions which are illustrated in figures 1-6 can be rationalized on the hypothesis that the target organs react differently to the concentrations of the hormones in the blood. Such a schema is presented in Table II. The amount of hormone necessary to produce a complete reaction is given in the table in relative terms. An examination of figures 1-6 indicates that a complete reaction was not the typical response. Thus, the responses that occurred were

TABLE II

A hypothetical schema of hormone activity that controls the dispersion and concentration of pigment in the chromatophores of Ligia occidentalis. D = dispersing hormone, C = concentrating hormone.

Environmental condition	Xanthophore		Melanophore	
	Pigment Stage	Hormone and amount necessary to produce the pigment stage.	Pigment Stage	Hormone and amount necessary to produce the pigment stage.
Diurnal rhythm in constant dark:				
Day	Dispersed	D small	Dispersed	D large
Night	Concentrated	C large	Concentrated	C small
Two weeks constant dark	Concentrated	C large	Dispersed	D large
Constant illumination:				
Yellow background	Dispersed	D small	Concentrated	C small
Black background	Concentrated	C large	Dispersed	D large

a result of the relative concentrations of the two hormones in the blood. One example, from figure 6, should illustrate this point. Animals acclimated to a black background showed dispersed pigment in the melanophores (chromatophore stage 5.0) and concentrated pigment in the xanthophores (chromatophore stage 2.3). This could be the result of large amounts of dispersing hormone and medium amounts of concentrating hormone. When the animals were shifted to the yellow background, the amounts of both hormones decreased. The xanthophores reacted more quickly and the pigment dispersed. The pigment in the melanophore concentrated slowly as the level of the dispersing hormone fell. The two hormones came into a balance so that the xanthophores had a stage of 4.2 and the melanophores, 3.0. A subsequent increase in the dispersing hormone resulted in the dispersion of pigment in both chromatophores. Fingerman (1958) reported red pigment concentrating and dispersing hormones and white pigment concentrating hormone in the crayfish *Orconectes clypeatus*. However, the hormonal systems so far elucidated in the Crustacea have been so varied, that the composition of one system can not be used to predict the composition of another system. It is quite clear that further work is necessary before the endocrine system of *Ligia* can be known.

Reactions to Different Intensities of Light

The previously described experiments demonstrated that *L. occidentalis* can rapidly acclimate to background. Not only must this acclimation be a response to the shade of the background; it must also be a response to the light intensity of the incident light. Animals have been shown to respond to the albedo, the ratio of incident light striking the eye from above to reflected light striking the eye from below (Brown, 1950 : 685). The two components of the albedo have been separated in the experiments reported in this paper. The experiments in which the light

was held constant and the background varied have been described. The following experiments were conducted on a yellow background at different light intensities. Six animals were used in each experiment. The experiments were conducted after 9 : 00 P.M. in order to test the ability of the animals to respond to light stimulation when the pigment in the chromatophores was concentrating. The results of these experiments are presented in Figure 7.

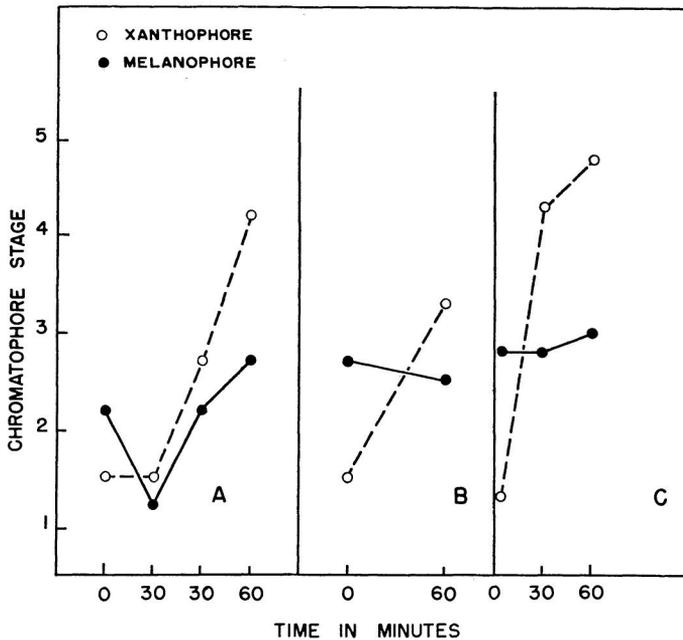


Fig. 7. Pigment concentration and dispersion in the chromatophores of *Ligia occidentalis* on a yellow background. A. 0-30 minutes, the animals were under 5 ft. c. of light. At the end of 30 minutes, the light intensity was raised to 32 ft. c. Experiment ran from 9 : 45 P.M. to 11 : 15 P.M. — B. The animals were under 17 ft. c. of light. Experiment ran from 9 : 15 P.M. to 10 : 15 P.M. — C. The animals were under 150 ft. c. of light. Experiment ran from 11 : 00 P.M. to midnight.

At 9 : 45 P.M. six animals were subjected to 5 ft. c. of light stimulation. After thirty minutes, there was no change in the xanthophores and there was some concentration of pigment in the melanophores. At that point, the light intensity was increased to 32 ft. c. (Fig. 7A). The pigment of both chromatophores dispersed, the yellow pigment dispersing almost twice as much as the black. In two further experiments the animals were treated with 17 ft. c. light at 9 : 15 P.M. (Fig. 7B) and with 150 ft. c. light at 11 : 00 P.M. (Fig. 7C). All three experiments demonstrated that *L. occidentalis* reacted to light with an expansion of pigment at the time in its diurnal rhythm when the pigment is concentrating. Enami (1941a) obtained similar results with *L. exotica*.

The activity of the pigment in the chromatophores showed a relationship to light intensity. Under dim light the black pigment concentrated; as the light

intensity increased, the amount of concentration decreased; a further increase in light intensity resulted in a pigment dispersion, tending to level off at a chromatophore stage of 3.0, the value associated with 150 ft. c. light intensity on a yellow background in the experiments described earlier. From no response under dim light, the pigment in the xanthophores dispersed at an increasing rate with increasing light intensity. These results show that *L. occidentalis* acclimated to change in albedo resulting from changes in incident light.

DISCUSSION

Since the primary response of the chromatophore system of *L. occidentalis* was acclimation to the shade of the background, one can infer that the functional significance of the chromatophore system is protective coloration.

Observations of the animals' activities in their natural environment lends support to such a hypothesis. *Ligia* is found along rocky shores just above the spray line. During low tide it ventures down into the intertidal area (Ricketts and Calvin, 1952 : 13). During low tides *Ligia* was active, being found on the rocks or among the algae. When disturbed, the isopods made short, rapid runs, dashing under a projecting knob on the rock or around the edge of the rock, or under a ledge where the animals flattened against the rock. The escape pattern was one of concealment for the animals did not attempt to run long distances nor to seek out narrow crevices in which they could hide. This is not to say that some animals did not run for a comparatively long distance nor that some did not get into crevices, but 90 % of the animals reacted as described above. Since the animals were difficult to see against the rocks, they could be easily collected by walking among the rocks where they were active and causing them to make a short dash. Once an animal made the short dash and flattened against the rock, it could carefully be approached and caught.

Ligia was not active at high tide. The animals aggregated under rubble in moist areas above the high tide line. When disturbed, the animals demonstrated essentially the same escape reaction as above except that the isopods dashed under nearby rubble.

The behavior pattern of escape is correlated with background coloration. But of what significance is the diurnal rhythm? The diurnal rhythm appears to be a mechanism that maintains the chromatophores of the animal in the proper state so that when *Ligia* becomes active, it is already at least partially protectively colored. When *Ligia* collect under rubble during the day, the reduction of light reaching the eye could result in a concentration of pigment (Fig. 7A, B). When the animals emerged during a daytime low tide, they would be pale against the darker rocks. At least thirty to sixty minutes would elapse before the animals become acclimated to the background (Fig. 6). During this time they would be more susceptible to predation. Those animals who reacted more rapidly to the change in light and background when they emerged from under the rocks would have a light selective advantage over the others. The genetic mechanism whereby

the high individual response to environmental stimulation could become genetically assimilated, in this case as a diurnal rhythm of chromatophore dispersion and concentration, has been discussed by Stern (1959, p. 188). Thus such a mechanism as the diurnal rhythm would have greater selective advantage and would persist and become typical of the species. However, the diurnal rhythm is flexible enough that it does not override the more basic background acclimation. Hence persistent illumination suppresses the rhythm. The rhythm is also suppressed in constant dark and at first sight the adaptive significance of this is not apparent. The dispersion of the pigment in the melanophores and the concentration of the pigment in the xanthophores and the morphological color changes parallel those conditions in animals which have been kept on a black background under constant illumination. Smith (1938) demonstrated by means of blinding techniques that a lack of stimulation of ventral elements of the eyes resulted in a melanophore stage of 4.7. In total darkness the melanophore stage was 2.7. The eyes of animals on a black background receive little ventral stimulation and in total darkness the eyes receive none. That part of the mechanism of response to light mediated by the ventral elements of the eye eventually dominates the response and the animals become so dark that they can hardly be seen against the black background in dim light. Thus the entire pattern of pigmentary responses can be interpreted as part of a system of background acclimation.

ZUSAMMENFASSUNG

Die Chromatophoren von *L. occidentalis* paßten sich schnell an dunklen und hellen Hintergrund an. Die Isopoden waren heller gefärbt auf hellem Untergrund und dunkler auf dunklem Untergrund. Bei Tieren, die Lichtintensitäten von 150 foot candles ausgesetzt waren, zeigte der Grad der Pigmentverschiebung in den Chromatophoren eine Beziehung zu der vom Untergrund reflektierten Lichtmenge. Der 24 Stunden Rhythmus (Dispersion des Pigmentes bei Tage, Konzentration bei Nacht) wurde sofort unterdrückt durch konstante Beleuchtung, durch totale Dunkelheit nach 12 Tagen. Die Ergebnisse der bisherigen Untersuchungen über die hormonale Kontrolle der Pigmentaktivität werden erörtert und die Existenz von mindestens zwei kontrollierenden Hormonen postuliert. Die Veränderungen in den Chromatophoren stehen im Zusammenhang mit den Gewohnheiten und dem Verhalten der Art im Sinne einer positiven Schutzfärbung.

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box built for that purpose. However, the stock animals were not so treated and were subjected to light whenever the light in the room was turned on.

Background responses were carried out in plastic dishes which were painted yellow, black, red, white, and green. Illumination, unless otherwise indicated, was provided by a fluorescent lamp so placed that the animals received 150 ft. c. of light.

Chromatophores were staged according to the method of Hogben and Slome (1931). Stage 1 represents maximum concentration of the pigment, stage 5 represents maximum dispersion of the pigment, and stages 2, 3, and 4 represent intermediate conditions. Chromatophores on the dorsal surface along the medial side of the left uropod were staged with the aid of a stereoscopic dissecting microscope and reflected light.

EXPERIMENTS AND RESULTS

Diurnal Rhythm of Chromatophore Behavior

Since diurnal rhythms of melanophore activity had been described for *L. baundiniana* by Kleinholz (1937), and for *L. exotica* by Enami (1941a) and Fingerman (1956), *L. occidentalis* was examined to determine the nature of the rhythm in this species. Six animals collected during the morning were staged and placed in complete darkness at 2 : 00 P.M. Both xanthophores and melanophores were staged at intervals for four days (Fig. 1). A typical rhythm of pigment dispersion by day and pigment concentration by night was evident in both types of chromatophores. Maximum concentration occurred by 10 : 00 P.M. and maximum dispersion occurred by 8 : 00 A.M. and was maintained through the early afternoon. This experiment was repeated four times using different animals with the same results. Ruck (reported by Bullock, 1955) found the diurnal rhythm of *L. occidentalis* to be temperature independent over a wide range of temperature.

Two of the above experiments were continued for nearly two weeks by which time most of the animals were dead. In both there was an indication that the diurnal rhythm of pigment dispersion and concentration was suppressed. In order to test this further, a fresh stock of animals was gathered and the entire stock placed in the dark box. Twelve were selected at random and placed in a separate dish in the dark box. The chromatophores of these twelve were staged twice daily, between 8 : 00 A.M. and 12 : 00 A.M. and between 10 : 00 P.M. and 12 : 00 P.M. These periods for staging were chosen because the previous experiments indicated that maximum conditions of expansion and contraction of pigment occurred during these intervals. Whenever an animal died, or showed signs of molting, it was replaced by an animal from the stock jar. Thus the chromatophore stage at any time was based on the average of twelve individuals, although more than twelve individuals were used in the course of the experiment. The results of these observations are presented in Fig. 2. After about one week the amplitude of the rhythm of pigment dispersion and concentration decreased; after twelve days the rhythm was suppressed and changes in the pigment condition of the

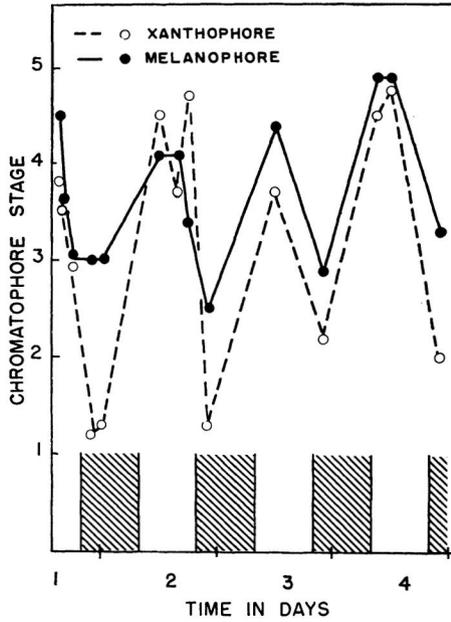


Fig. 1. Diurnal rhythm of pigment dispersion and concentration in the chromatophores of *Ligia occidentalis*. The shaded bars indicate the hours 8 P.M. to 8 A.M.

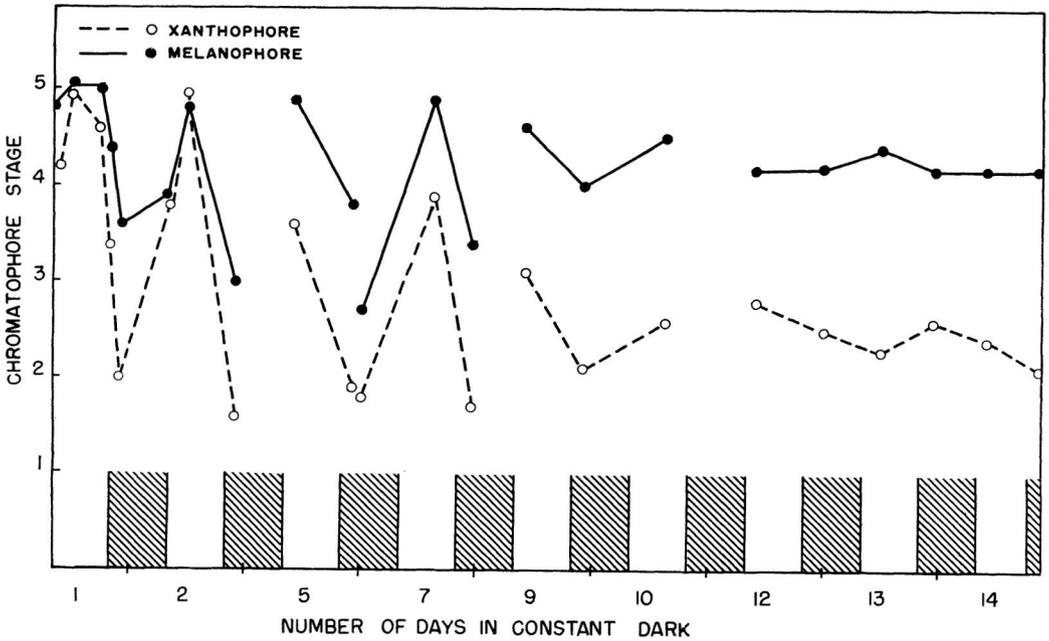


Fig. 2. Diurnal rhythm of pigment dispersion and concentration in the chromatophores of *Ligia occidentalis* kept in constant dark. The shaded bars indicate the hours 8 P.M. to 8 A.M.

chromatophores were no longer associated with environmental day-night changes.

Morphological color changes also occurred. After five days dark pigment began to form in the center of many of the xanthophores. This was particularly evident in the middle portion of the uropod where xanthophores were concentrated and melanophores were few or absent. In subsequent days the pigment became darker and spread over the xanthophore. At the end of the two weeks the xanthophores were almost completely obscured by the development of melanin over them. These changes were accompanied by changes in the melanophores. The number of processes greatly increased and tended to intermingle. This intermingling produced a network of black pigment which had the effect of greatly darkening the animals.

Responses to Constant Illumination

Animals collected in the morning were staged and placed on yellow and black backgrounds at 2 : 00 P.M. Six animals were placed on each background. The

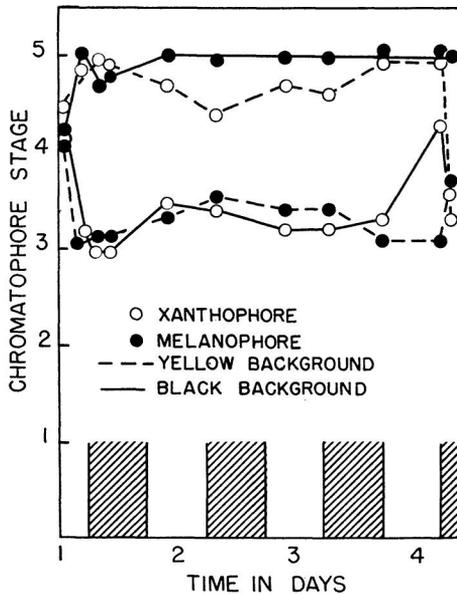


Fig. 3. Pigment concentration and dispersion in the chromatophores of *Ligia occidentalis* on yellow and black backgrounds under constant illumination of 150 ft. c. The shaded bars indicate the hours 8 P.M. to 8 A.M.

chromatophores were staged at intervals for four days. During this period the rhythm of pigment dispersion and concentration was suppressed (Fig. 3). The animals were dark on the black background and light on the yellow background. Enami (1941a) reported similar results for *L. exotica* which were illuminated for 1½ days on white and black backgrounds. Fingerman (1956) illuminated *L. exotica* at 40 ft. c. light intensity on black and white backgrounds. A third group was kept in the dark. All three groups showed a rhythm for one day, although the

amplitude of change in dispersion and concentration of pigment was much less in the illuminated animals. Kleinholz (1937) reported an absence of a rhythm at night in *L. baudiniana* upon an illuminated black background. The different results obtained by Fingerman and the others may be a result of the intensity of illumination as the light intensity used in this study was more than three times greater than that used by Fingerman. Since Enami and Kleinholz did not report the intensity of illumination, further analysis of the differences reported under constant illumination is not possible.

Background Responses of *L. occidentalis*

The preceding experiment demonstrated that the xanthophores and melanophores of *L. occidentalis* responded differently to background. These responses were further tested by placing six animals in each of five plastic dishes painted green, black, red, white, and yellow. The chromatophores were staged after 24

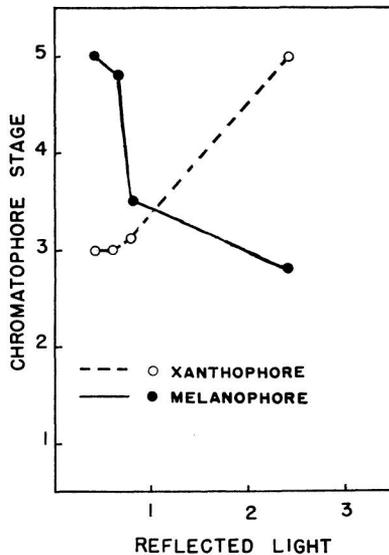


Fig. 4. The degree of pigment dispersion in the chromatophores of *Ligia occidentalis* after 24 hours on yellow, white, red, green and black backgrounds. The color of the background is reported as reflected light. Reflected light is recorded as units on the Weston MII exposure meter.

hours. The reflected light was determined with a Weston MII exposure meter. Since the white and yellow dishes gave the same light values, the animals in these two dishes were treated together. The chromatophores were staged and these values plotted against reflected light (Fig. 4). Since the incident light was constant, the chromatophore condition can be considered a result of the amount of reflected light from the background. Nagano (1948) found a similar reaction in the melanophores of *L. exotica* on colored backgrounds. However, on light backgrounds the pigment concentration was much greater than that reported here.

Nagano illuminated his animals with a 60 watt lamp placed 50 centimeters distant from the animals. A rough approximation indicates that about 25 ft. c. of light were reaching the animals. The increase in pigment concentration is consistent with the results obtained for *L. occidentalis* under different intensities of light reported below. Smith (1938) also reported that the pigment was more concentrated in the melanophores of *L. oceanica* on both black and white backgrounds under a dim light than under a bright light.

Animals were kept on yellow and black backgrounds under constant illumination for one week. After four days, the melanophores of the animals in the black dishes began spreading over the xanthophores. This development of intermingling processes continued throughout the remainder of the experiment. The xanthophores of the animals in the light background became much paler and tended to develop processes which were spreading over the melanophores. However, the amount of morphological change was much less in the animals on the light background at the end of the week. Fingerman and Lowe (1957) reported that the red chromatophores of *Cambarellus shufeldti* underwent morphological change on a black background similar to that described above for the melanophores of *L. occidentalis*.

All of the experiments so far described indicate that *L. occidentalis* acclimated by both physiological and morphological color changes to background. The following experiments were designed to test the time factor in acclimation to background by physiological color change.

Two sets of six animals each were acclimated for 1½ hours, one set to a black background and the other set to a yellow background. At the end of the acclimation period, the chromatophores were staged and the animals were placed on the background opposite to the one to which they were acclimated. The results are presented in Fig. 5. By the end of the first hour, dispersion of the appropriate pigment (yellow on yellow background, etc.) was almost complete. However, concentration of the pigments did not parallel the acclimated condition of concentration even after 7 hours.

Since the amount of change in concentration or dispersion of pigment that occurs when an animal is placed on a background might depend on the previous acclimation, another experiment was performed in which animals were transferred from darkness to a yellow or black background. The animals were staged at 7 : 15 A.M. The animals were selected from a stock group that had been placed in the dark the previous day. The first nine animals were placed in yellow dishes; the second nine, in black dishes. The chromatophores were staged every 30 minutes for the first two hours after the animals were placed in their respective dishes, and thereafter were staged at hourly intervals. The animals were essentially acclimated to their respective backgrounds in 30 minutes, with two exceptions (Fig. 6). The dispersion of the pigment in the xanthophores of animals on the yellow background was not complete until the end of the first hour, and the concentration of pigment in the xanthophores of animals on the black background continued

slowly throughout the time of the experiment. At 1 : 15 P.M., six hours after

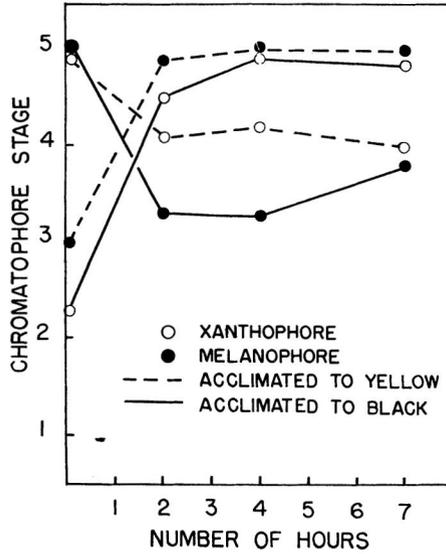


Fig. 5. Pigment concentration and dispersion in the chromatophores of *Ligia occidentalis* placed on backgrounds opposite to the ones to which they were acclimated. Six animals each were acclimated for 1½ hours to yellow and black backgrounds.

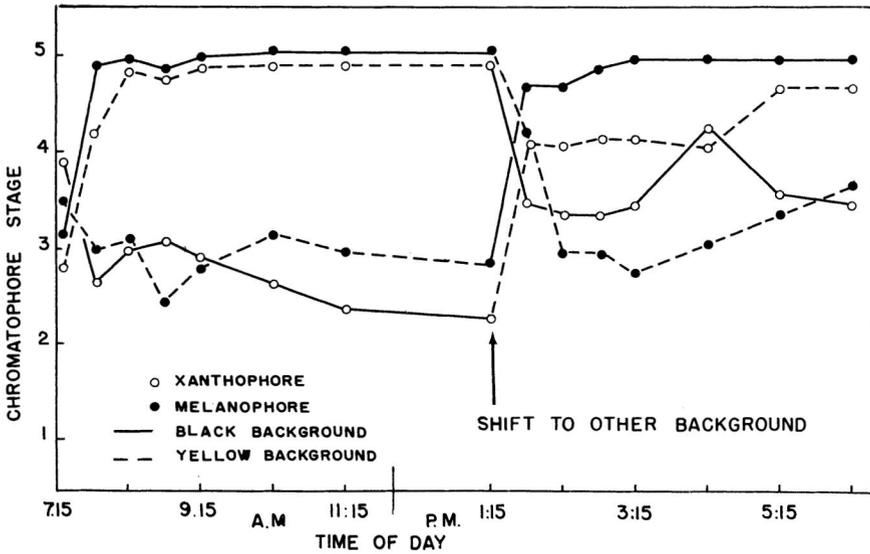


Fig. 6. Pigment concentration and dispersion in the chromatophores of *Ligia occidentalis* transferred from darkness to yellow and black backgrounds. After six hours, the animals were transferred to the opposite background.

the start of the experiment, the animals were staged and shifted to the other background. The chromatophores were staged every half hour for the first two

hours and each hour thereafter until 6 : 15 P.M. This part of figure 6 is essentially like figure 5. Most of the acclimation was completed in one hour, although some slight change occurred in the next hour in the melanophores of the animals on the black background, and in the next 2-3 hours in the xanthophores of the animals on the yellow background.

Enami (1941) reported that in *L. exotica* acclimated to black and white backgrounds, concentration of black pigment took one hour and the dispersion of black pigment took six hours when the animals were shifted to the opposite background. However, when Fingerman (1956) performed a similar experiment with the same species, he found that the black pigment reached almost the identical condition in 60 minutes to which its opposite number had acclimated. Smith (1938) reported that melanophores of *L. oceanica* acclimated to a black background reached equilibrium in 1½ hours when placed on a white background, but animals acclimated to a white background required six hours before pigment dispersion reached equilibrium. An examination of figures 5 and 6 shows that the melanophores of *L. occidentalis* also reached equilibrium more quickly in those animals transferred from a dark to a light background than in those animals transferred from a light to a dark background. However, figure 6 shows that the initial response is greater when animals are changed from a light to a dark background. The initial response of xanthophores of animals acclimated to a dark background and switched to a light background is as great as the initial response of melanophores of animals acclimated to a light background and transferred to a dark background. But the xanthophores require a much longer period of time before the pigment is fully dispersed.

Animals kept in the dark and then placed on light and dark backgrounds show a similar response. Dispersion of pigment in both xanthophores and melanophores was rapid, but with dispersion of pigment in the xanthophore somewhat lagging that in the melanophore. Concentration of pigment was equally rapid. The melanophore fluctuated around a chromatophore stage of 3.0. The cause of this fluctuation is unknown; however, the melanophores can be considered to have reached equilibrium. However, a steady concentration of pigment occurred in the xanthophores from the second to the sixth hour.

Smith (1938) found that the melanophores reached equilibrium more quickly when transferred from darkness to a black background than when changed from a white to a black background. The concentration of pigment required more time when animals were shifted from darkness to a white background than when animals were shifted from a black to a white background. However, when animals were taken from the dark and placed on black and white backgrounds, dispersion of the pigment in the melanophores was only slightly slower than concentration. This latter result is in close agreement with the experiment reported here. The chief difference between the results of Smith and the experiments reported here is in the initial response of the melanophores of animals changed from a black to a light back-

TABLE I

Summary of hormone experiments on the melanophores of Ligia

Animal	Worker	Source of hormone	Type of back-ground acclimation of donor	Type of back-ground acclimation of recipients	Results	
<i>Ligia exotica</i>	Enami (1941b)	Blood transfusion	Black	White	Darkening.	
			White	Black	Slight lightening followed by darkening.	
			White	White	Average chromatophore stage changed from 1.6 to 2.9 in 30 minutes.	
		Head extracts of 10 animals		Black		Considerable paling followed by darkening. Average chromatophore stage changed from 4.8 to 4.4.
				Black	White	Average chromatophore stage changed from 1.3 to 2.9 in 30 minutes.
				Black	Black	Some paling followed by darkening. Average chromatophore stage changed from 5.0 to 4.2.
Head extracts of 100 animals		average chromatophore stage of 3.9	White	Average chromatophore stage changed from 1.8 to 3.8 in 60 minutes.		
			Black	Blanching followed by darkening. Average chromatophore stage changed from 5.0 to 4.8 in 60 minutes.		
			Black	Slight pigment dispersion. Marked pigment dispersion.		
<i>Ligia baudiniana</i>	Fingerman (1956)	Extracts of sinus glands, thoracic nerve cord, and optic ganglia, cerebral ganglia-circumesophageal connectives	not given	Black White		
	Nagano (1949)	Aqueous extracts of the head	White White at night	Black Black	Slight concentration. Considerable concentration.	
<i>Ligia baudiniana</i>	Kleinholz (1937)	Head extracts in sea water	White	Black	Some concentration.	
			Black Black	Black White	Marked concentration. Marked concentration.	