PHYSIOLOGY OF THE MELANOPHORES IN THE CRAB SESARMA RETICULATUM [1]

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In 1948 Brown noted that the black pigment in all crabs, which had been observed, concentrated maximally after eyestalk removal if the animals were not exposed to excessively high intensities of illumination. Later, however, Enami (1951) reported that the pigment in melanophores of specimens of *Sesarma intermedia, S. haematocheir,* and *S. dehaani* dispersed maximally after eyestalk ablation.

Regulation of color change has been studied in a detailed manner in very few species of crabs. In the fiddler crab, *Uca pugilator,* the species whose chromatophores have been studied most intensively, the melanophores are controlled by pigment-dispersing and pigment-concentrating substances (Carlson, 1936; Sandeen, 1950; Brown and Fingerman, 1951; Fingerman, 1956a). A black-pigment-dispersing principle has been found also in the crabs *Hemigrapsus oregonensis* by Bowman (1949), *Callinectes sapidus,* the blue crab, by Fingerman (1956b), *Eriocheir japonicus* by Matsumoto (1954), *Uca rapax* by Burgers (1958), and *Macropipus vernalis* by Burgers (1959). No evidence, however, has been adduced for a melanin-concentrating principle in these crabs. Enami (1951) reported that extracts of central nervous organs from the three species of *Sesarma* evoked concentration of melanin when injected into eyestalkless individuals.

The responses of melanophores to changes in light intensity and temperature have been observed in *Uca pugilator* (Brown and Sandeen, 1948) and *Callinectes sapidus* (Fingerman, 1956b). An albedo response operated to disperse the black pigment when specimens of both species were placed on a black background and to concentrate this pigment in crabs on a white background. Enami (1951) stated that background responses were not exhibited by the three species of *Sesarma* which he observed. With respect to temperature, the melanin in *Uca* tended to concentrate as the temperature increased above or decreased below 15° C. In contrast, the black pigment in *Callinectes* became progressively more concentrated as the temperature increased from 10° to 28° C.

The present investigation was undertaken primarily to expand the small amount of information available concerning chromatophores of brachyurans other than *Uca,* and secondarily to compare the behavior of the melanophores in specimens of *Sesarma reticulatum* from the Woods Hole area with the findings reported by Enami (1951) for three species of *Sesarma* in Japan. Problems such as the chromatics of eyestalkless specimens, background responses, and endocrine regulation of the melanophores were considered.

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Materials and Methods

Adult specimens of the grapsoid crab *Sesarma reticulatum* were collected at West Falmouth, Massachusetts, for use in this investigation. In the laboratory crabs were maintained under constant illumination in aquaria containing sea water approximately 2 cm. deep. The intensity of illumination incident on these crabs was 40 ft. c.

Melanophores on the walking legs were observed with the aid of a stereoscopic dissecting microscope and transmitted light and were staged according to the system of Hogben and Slome (1931). Stage 1 represented maximal concentration of the melanin, stage 5 maximal dispersion, and stages 2, 3 and 4 the intermediate conditions.

Tissue extracts were prepared in sea water as described in detail by Sandeen (1950). The dose of each extract injected into assay animals was 0.05 ml. The concentration of each extract was one-third of a complement per 0.05 ml. Eyestalkless specimens, some of which served as assay animals, had had both eyestalks removed at least 12 hours prior to use. The wounds were cauterized to minimize loss of blood.

Student's t test was used for determination of the level of significance between the means. The 5% level was considered the maximum for a significant difference. Standard deviations and standard errors for the differences between means were also calculated.

Experiments and Results

Rhythm of pigment migration

Twenty intact specimens were placed into a darkroom during the afternoon of July 19, 1960. At midnight the average stage of the melanophores of 10 specimens was determined and the crabs were returned to the darkroom. The same procedure was followed at 8:00–8:30 AM, noon–1:00 PM, 5:00 PM, and midnight Eastern Daylight Saving Time for the next seven days.

The means are presented in Figure 1. Inspection of this figure reveals that the melanin was more dispersed by day than at night. The chromatophore indices determined at midnight and at 8:00–8:30 AM for the entire series of observations were compared to determine the statistical significance of the difference between the means. This difference was highly significant.

Relationship between light intensity, background, and chromatophore stage

The objectives of this set of experiments were to determine during the day phase and the night phase of the cycle of pigment migration (1) if specimens of *Sesarma reticulatum* exhibit a background response and (2) the response of the melanophores to changes in total intensity of illumination. For the first experiment 10 intact, recently collected specimens were placed into each of six black and six white pans at 8:00 AM. The animals in one black and one white container then were exposed to one of these intensities of illumination, 2, 17, 40, 70, 250, and 1110 ft. c., for two hours at which time the melanophores of each crab in the 12 containers were staged. This experiment was repeated on another day with one change in the procedure; the crabs were placed under the various intensities at noon instead of at 8:00 AM.

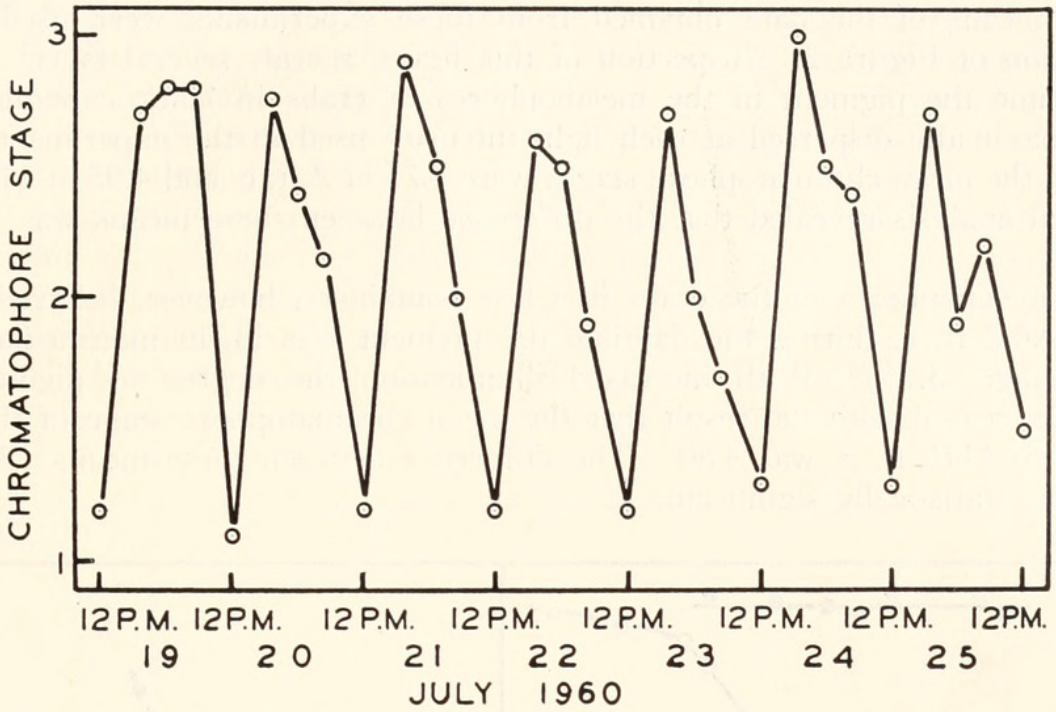

FIGURE 1. Relationship between melanophore stage and time of day in crabs maintained in darkness.

The next two experiments were performed at night between 10:45 PM and 3:00 AM. Ten intact crabs were placed into each of four black and four white pans. The crabs in one black and one white container were then exposed to one of these light intensities, 2, 32, 280, and 1110 ft. c., for two hours. The chromatophore stage of each crab was then determined. This experiment was also performed one more time.

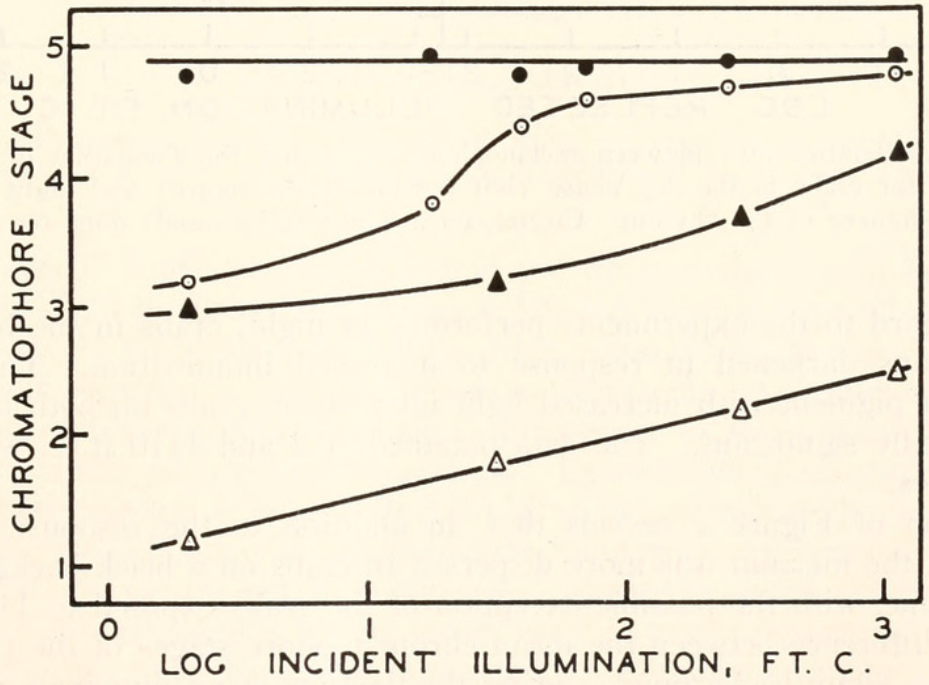

FIGURE 2. Relationships between melanophore stage and the logarithm of the incident light intensity for crabs in the day phase of the rhythm and on a black background (dots), day phase and on a white background (circles), night phase amd on a black background (solid triangles), night phase and on a white background (empty triangles).

The means of the data obtained from these experiments were used in the preparation of Figure 2. Inspection of this figure reveals several facts. During the daytime the pigment in the melanophores of crabs in black containers was nearly maximally dispersed at each light intensity used in the experiments. For example, the mean chromatophore stages were 4.75 at 2 ft. c. and 4.95 at 1110 ft. c. Statistical analysis revealed that the difference between these means was not significant.

The melanophores of the crabs in white containers, however, behaved differently. At 2 ft. c. during the daytime the pigment was in an intermediate state (mean stage, 3.15). With increased illumination the degree of pigment dispersion increased with the result that the mean chromatophore stage of the crabs exposed to 1110 ft. c. was 4.80. The difference between these means (3.15 and 4.80) was statistically significant.

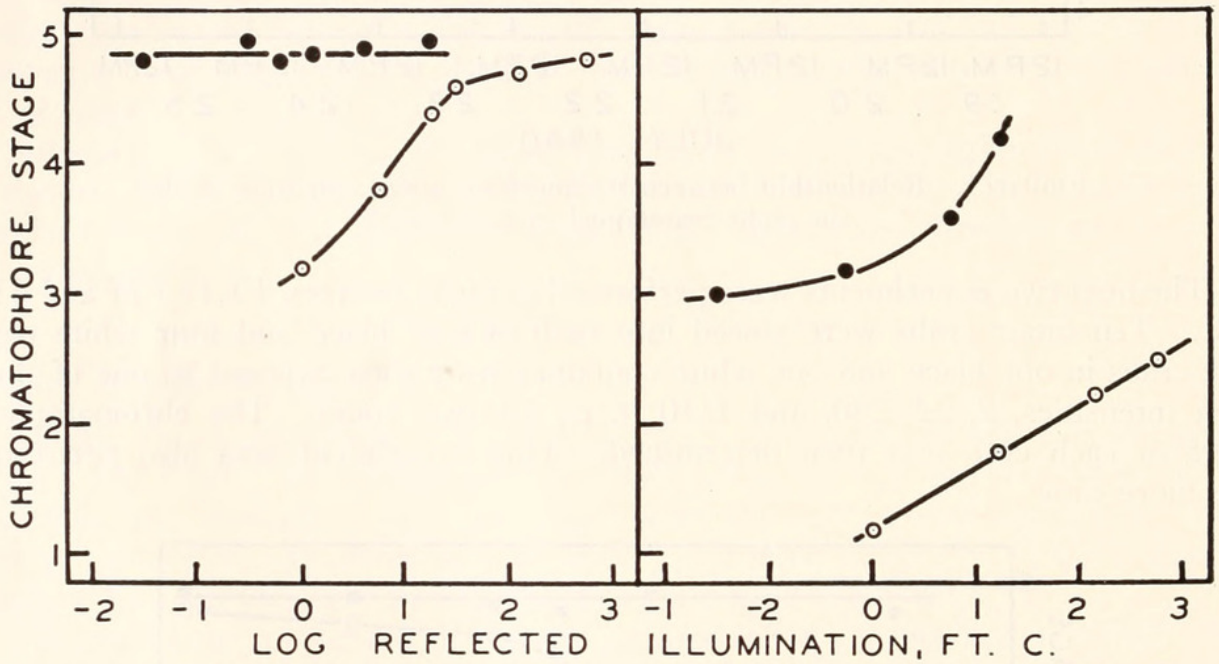

FIGURE 3. Relationships between melanophore stage and the logarithm of the reflected light intensity for crabs in the day phase (left portion of the figure) and night phase (right portion of the figure) of the rhythm. Circles, on a white background; dots, on a black background.

With regard to the experiments performed at night, crabs in the black and in the white pans darkened in response to increased illumination. The increased dispersion of pigment with increased light intensity in crabs on both backgrounds was statistically significant. The data obtained at 2 and 1110 ft. c. were used in these analyses.

Inspection of Figure 2 reveals that, in addition to the response to incident illumination, the melanin was more dispersed in crabs on a black background than on a white one, with the possible exception of the crabs exposed to 1110 ft. c. by day. The difference between the mean chromatophore stages of the crabs on the black and the white backgrounds during the daytime at an illumination of 2 ft. c. was statistically significant but not at 1110 ft. c. However, the differences between the crabs on the two backgrounds during the nighttime were significant at both 2 and 1110 ft. c.

The findings presented in Figure 2 bear out the cycle of pigment migration shown in Figure 1. At each light intensity used the pigment of crabs on both backgrounds was more dispersed by day than at night. Statistical analysis revealed that the differences between chromatophore stages obtained at 2 and 1110 ft. c. during the nighttime and daytime were highly significant.

To demonstrate clearly that specimens of *Sesarma reticulatum* show a true background (*i.e.*, albedo) response and not merely a response to the amount of light reflected from the background, the data of Figure 2 were replotted in terms of the intensity of light reflected from the black and the white backgrounds (Fig. 3). The white background reflected one-half the incident illumination and the black background 1/60. The existence of an albedo response was evident be-

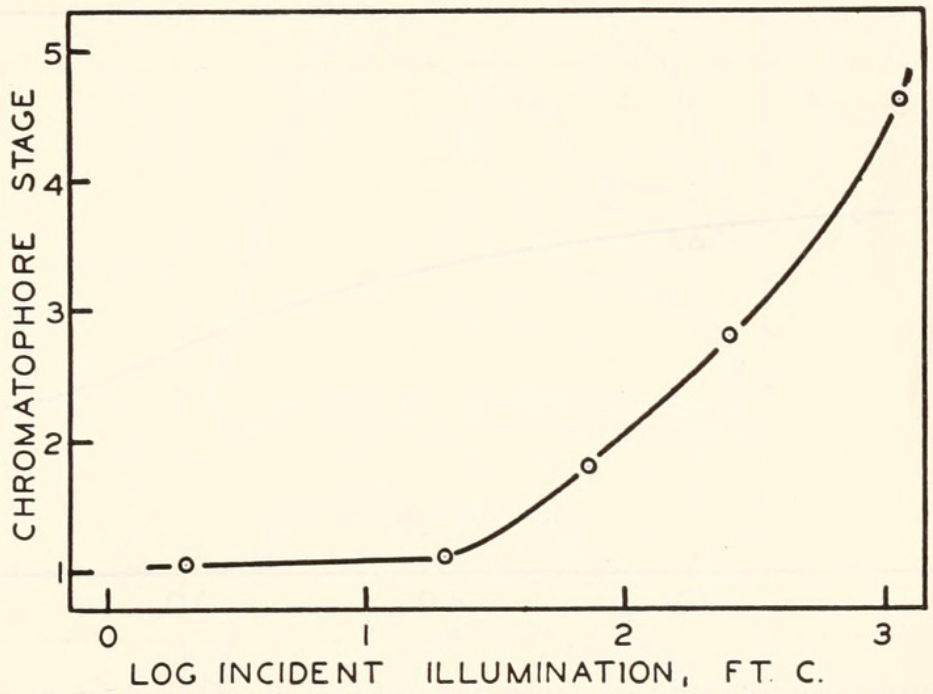

FIGURE 4. Relationship between melanophore index of eyestalkless specimens of *Sesarma reticulatum* on a white background and the logarithm of the incident light intensity.

cause the data obtained on the black and the white backgrounds did not overlap although the light intensity had increased 505-fold whereas the white background reflected only 30 times more light than did the black background.

The next experiment was designed to determine the relationship between illumination and the degree of pigment dispersion in the melanophores of eyestalkless specimens of *Sesarma reticulatum*. Eyestalkless specimens in white pans were exposed between 8:20 and 10:20 AM to the following illuminations: 2, 20, 70, 250, and 1110 ft. c. The melanin was maximally concentrated in crabs exposed to 2 ft. c. and was nearly maximally dispersed in crabs at 1110 ft. c. This experiment was repeated. Means were used in the preparation of Figure 4. The degree of pigment dispersion in eyestalkless specimens can be readily altered by changing the intensity of incident illumination. This pigment migration is presumably a direct response of the melanophores to light. The difference between the means at 2 and 1110 ft. c. was statistically significant.

Relationship between temperature and melanophore stage

The aim of this set of experiments was to determine whether the melanophores of *Sesarma reticulatum* are sensitive to temperature changes. During the daytime 10 intact crabs were placed into each of five white pans which contained sufficient sea water to cover the crabs. These crabs were then exposed to an illumination of 2 ft. c. The white background, daytime, and light intensity of 2 ft. c. were chosen because under these conditions the melanophores of the crabs were at approximately stage 3 (Fig. 2). Through use of water baths each pan was exposed for two hours to one of the following temperatures: 3.5°, 10°, 22.5°, 30°, and 36° C. The average stage of the melanophores of each crab was determined at the end of the exposure period. This experiment was performed twice.

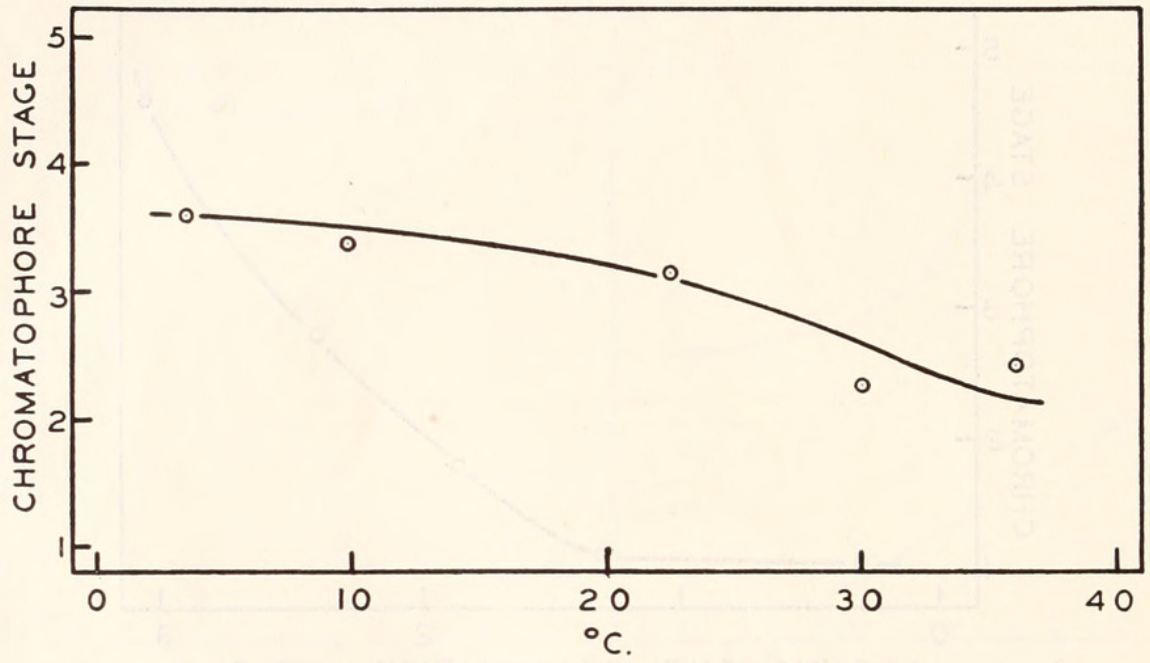

FIGURE 5. Relationship between temperature and melanophore index of intact specimens on a white background under an incident illumination of 2 ft. c. during the daytime.

An inverse relationship between temperature and chromatophore stage was apparent (Fig. 5). For purposes of statistical analysis the data obtained at (1) 3.5° and 10° C., and (2) 30° and 36° C. were grouped. The difference between the means of these two groups of data was statistically significant.

Endocrine regulation of the melanophores

The object of the final set of experiments was to determine the role of endocrines in mediating pigment migration in the melanophores of *Sesarma reticulatum*. For use in the first set of experiments of this group extracts of the optic ganglia, supraesophageal ganglia, circumesophageal connectives, and thoracic ganglia were prepared in the manner described earlier under Materials and Methods. Each extract was injected into five eyestalkless specimens in a white pan under an illumination of 2 ft. c. Under these conditions the melanin was maximally concentrated. The control consisted of eyestalkless crabs which received sea water injections. Each extract dispersed the black pigment.

This experiment was repeated twice. The data of the three experiments were averaged and the means were used in the preparation of the upper portion of Figure 6. The amount of pigment dispersion produced by each extract was highly significant.

The next experiment was similar to the previous one with the single change that the crabs were exposed to an illumination of 560 ft. c. Under this light intensity the pigment was in an intermediate degree of dispersion (see Fig. 4). Substances that concentrate as well as disperse pigment can sometimes be detected

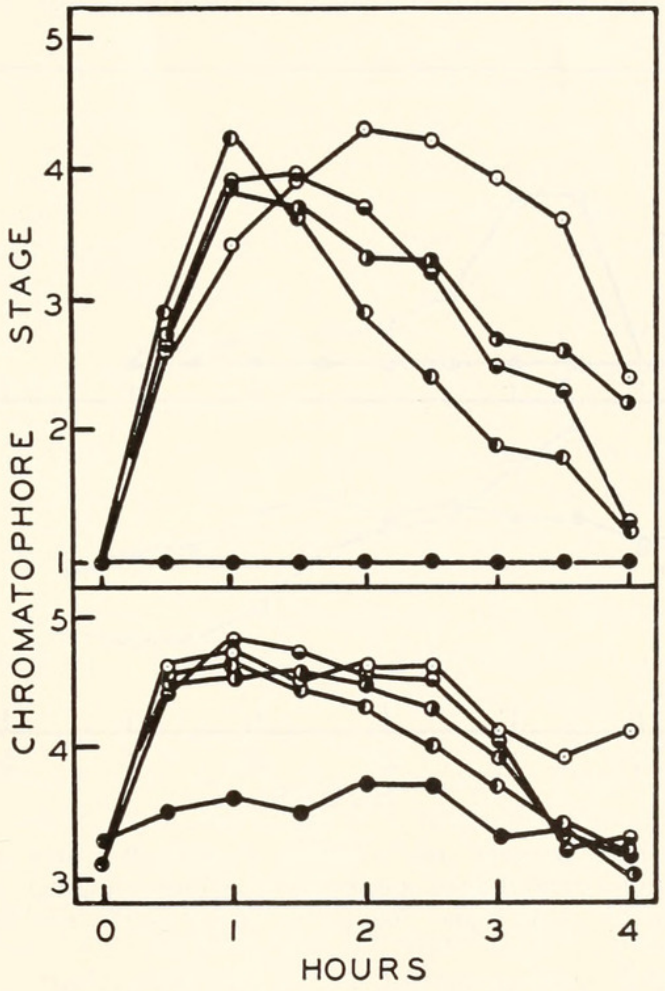

FIGURE 6. Relationships between melanophore stages and time following injection of extracts of central nervous organs into eyestalkless specimens in white containers under an illumination of 2 ft. c. (upper portion of figure) and 560 ft. c. (lower portion of figure). Optic ganglia, circles; supraesophageal ganglia, circles half-filled on bottom; circumesophageal connectives, circles half-filled on left; thoracic ganglia, circles half-filled on right; control, dots.

when the pigment of assay animals is in an intermediate state (Fingerman and Lowe, 1958). A pigment-dispersing substance was apparent but not a pigment-concentrating one. The experiment was repeated twice. The averaged data of the three experiments are shown in the lower portion of Figure 6. Each extract produced a statistically significant amount of pigment dispersion as was the case when extracts of these organs were injected into specimens with maximally concentrated melanin.

The remaining experiments involved assay of extracts prepared from sinus glands. Each extract was injected first into five eyestalkless crabs with maximally

concentrated black pigment and pigment dispersion was noted. This experiment was repeated twice with the same results. The means were used in the preparation of the upper portion of Figure 7 where each point represents 15 crabs. The amount of pigment dispersion was statistically significant. Controls consisted of eyestalkless specimens which received sea water injections.

When an extract of sinus glands was injected into eyestalkless crabs whose black pigment was in an intermediate stage of dispersion as a result of exposure to an illumination of 560 ft. c., a transitory increase in the degree of pigment dispersion occurred. This was followed by migration of the pigment to a more

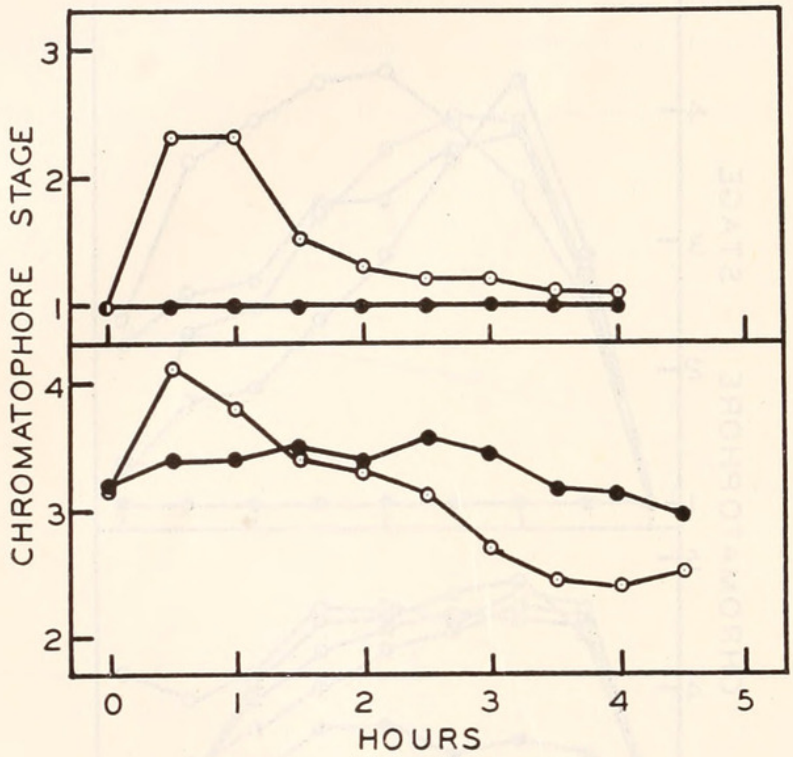

FIGURE 7. Relationships between melanophore stages and time following injection of extracts of sinus glands into eyestalkless specimens in white containers under an illuminatoin of 2 ft. c. (upper portion of figure) and 560 ft. c. (lower portion of figure). Sinus glands, circles; control, dots.

concentrated level than observed in the control group. This experiment was repeated three times. Means are presented in the lower portion of Figure 7 where each point represents 20 crabs. Statistical analysis of these data revealed that the amounts of both dispersion and concentration of melanin were statistically significant.

DISCUSSION

Specimens of *Sesarma reticulatum* showed a rhythm of color change which was manifested by a pale phase at night and a dark phase during the daytime (Fig. 1). Similar cycles have been observed in the fiddler crab, *Uca pugilator,* by Abramowitz (1937), in the blue crab, *Callinectes sapidus,* by Fingerman (1955), and in the swimming crab, *Macropipus vernalis* by Burgers (1959). Enami (1951) did not report on the possibility of a rhythm of color change occurring in the three species of *Sesarma* he used.

The responses to increased illumination of the melanophores in *Sesarma reticulatum, Uca pugilator* (Brown and Sandeen, 1948), and *Callinectes sapidus* (Fingerman, 1956b) are qualitatively alike. In intact specimens of these three crabs increased total illumination resulted in greater dispersion of the melanophore pigment. Enami (1951) stated that the three species of *Sesarma* he used exhibited a direct response to illumination, but he did not indicate the direction of the response. He also reported that these three species of *Sesarma* did not show a background response. However, specimens of *Uca pugilator, Callinectes sapidus,* and *Sesarma reticulatum* showed, in addition to the response to total illumination, an albedo response which called for more dispersion of melanin in crabs on a black background than on a white one. An interesting point concerning *Uca pugilator* is that in spite of the albedo response, at least during the daytime, the melanin of specimens on a white background was more dispersed than in crabs on a black background because the response to total illumination was stronger than the albedo response (Brown and Sandeen, 1948). In blue crabs, however, the albedo response was stronger than the response to total illumination (Fingerman, 1956b).

The amplitude of the response of melanophores in eyestalkless individuals of *Sesarma reticulatum* to total illumination (Fig. 4) appears to be unique among crustaceans. Maximal concentration of melanin is the typical response of brachyurans to eyestalk ablation if the crabs are not exposed to excessively high intensities of illumination (Brown, 1948). This response has been observed in a variety of crabs such as *Uca* (Carlson, 1936), *Hemigrapsus* (Bowman, 1949), and *Callinectes* (Fingerman, 1956b). As mentioned above, Enami (1951) observed three species of *Sesarma* in which the melanin dispersed maximally after eyestalk ablation instead of concentrating. Unfortunately Enami did not state the light intensity to which his crabs were exposed. At the light intensity usually recorded, about 30 ft. c., on the table tops of a laboratory, the melanin of *Sesarma reticulatum* was nearly maximally concentrated (Fig. 4), so that one would be inclined to conclude that the chromatic behavior of eyestalkless specimens of this species is more similar to that of *Uca* than to the behavior of the *Sesarma* studied by Enami. When eyestalkless *Uca* were exposed to an incident illumination as high as 3500 ft. c. (Brown and Sandeen, 1948), the black pigment dispersed only to an intermediate condition. The response of the melanophores in *Callinectes* to total illumination (Fingerman, 1956b) was not as great as was observed in *Uca* by Brown and Sandeen (1948).

In response to a rise in temperature, the black pigment of *Sesarma reticulatum* concentrated significantly (Fig. 5). This was also the response shown by the melanophores of *Callinectes* (Fingerman, 1956b). Such behavior suggested a thermoregulatory function of the black pigment. Crabs lightened at high temperatures and thereby reflected more heat from the body surface. Enami (1951) stated that the *Sesarma* he used showed a response to temperature but he did not define the nature of this response.

Extracts of central nervous organs from *Sesarma reticulatum* caused dispersion but no concentration of the pigment in melanophores (Fig. 6). However, extracts of the sinus glands caused melanin dispersion which was followed by a significant degree of melanin concentration (Fig. 7). Enami (1951) found that extracts of sinus glands and central nervous organs concentrated pigment when injected into eyestalkless specimens of *Sesarma intermedia, S. haematocheir,* and *S. dehaani.*

His experimental procedure did not allow assay for melanin-dispersing hormone, if this substance does exist in the crabs he used. The species of *Sesarma* used by Enami are not only intriguing with respect to the dispersed condition of the pigment in the melanophores of eyestalkless specimens but also with respect to the fact that the immediate effect of extracts of sinus glands from crabs investigated by Enami was concentration of pigment. Extracts of sinus glands from crabs utilized by the other investigators mentioned above always produced some dispersion of pigment.

It is interesting to note that in the assays of sinus glands of *Sesarma reticulatum* the pigment-dispersing effect preceded the pigment-concentrating one (lower portion of Figure 7). In contrast, when extracts of sinus glands from the dwarf crayfish, *Cambarellus shufeldti,* were assayed, concentration of dark red pigment occurred before dispersion (Fingerman, 1959). Red pigment in crayfishes becomes maximally dispersed after eyestalk removal (Brown, 1948). Crayfishes do not possess melanophores. Further investigation may reveal (1) the significance of this difference in behavior of the pigment-dispersing and pigment-concentrating principles in *Sesarma reticulatum* and *Cambarellus shufeldti* and (2) the reason why after eyestalk ablation the dark pigment concentrates in some crustaceans and disperses in others.

Summary and Conclusions

1. The grapsoid crab *Sesarma reticulatum* displayed a rhythm of color change which operated to disperse the pigment in the melanophores by day and concentrate it at night.

2. The crabs darkened as the intensity of illumination increased. A background response was also apparent. Melanin was more dispersed in specimens on a black background than on a white one, especially at low light intensities.

3. The degree of dispersion of the pigment in the melanophores of eyestalkless crabs was a direct function of the incident illumination. In dim light the pigment was maximally concentrated and in bright light was nearly maximally dispersed.

4. The melanin was less dispersed at high temperatures than at low. A thermoregulatory function of the melanophores was postulated.

5. Migration of melanin in *Sesarma reticulatum* is mediated by pigment-dispersing and pigment-concentrating principles.

6. The results were discussed in relation to pertinent data concerning the melanophores of crabs which have been investigated previously.

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