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DISPERSION OF THE DARK RED CHROMATOPHORIC PIGMENT IN THE DWARF CRAYFISH, *CAMBARELLUS SHUFELDTI:* A QUANTITATIVE ANALYSIS OF THE HOGBEN AND SLOME STAGES[1]

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ABSTRACT

The areas of the pigment in the dark red chromatophores of adult dwarf crayfish, *Cambarellus shufeldti,* were determined at each of the five stages of the Hogben and Slome scale. The increase in area as the pigment proceeded from the maximally concentrated position (stage 1) through the intermediate stages to the final, maximally dispersed condition (stage 5) was statistically significant at each interval. The area of the pigment in a stage 5 chromatophore was 5.0 times that of a stage 1 chromatophore. No such simple relationship existed with the other stages. The chromatophores of adults compared with juveniles were larger but fewer in number per unit area in the portion of the epidermis where the chromatophores were observed. The increase in area offset the decrease in density, resulting in a constant contribution by the dark red chromatophores to the total coloration of the dwarf crayfish regardless of the size of the crayfish.

Several methods of staging the degree of dispersion or concentration of the pigment in chromatophores have been utilized. These have been reviewed by Fingerman (1963). The most commonly employed method is that of Hogben and Slome (1931) who divided the range of pigment migration into five stages. Stage 1 represents maximal concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. Although this system is subjective, trained investigators have no difficulty in duplicating each other's readings with precision. In this laboratory the mean chromatophore stage of the same 10 animals determined by two investigators will not differ by more than 0.2 of a stage. However, the Hogben and Slome system is open to criticism because it is subjective and more importantly because the numbers obtained are not additive, *a priori.* For example, stage 4 was not intended to represent twice as much dispersion as seen in stage 2. On the other hand, the advantages of the Hogben and Slome system are its inherent simplicity and the opportunity to examine individual chromatophores, especially in an animal that has more than one type of chromatophore and where one pigment may be dispersing while another is concentrating as the result of changing the animal from a light to a dark background.

The present investigation was undertaken to obtain an objective basis for the Hogben and Slome stages with the dark red chromatophores of the dwarf crayfish, *Cambarellus shufeldti.* The actual area of the pigment in the chromatophores at each of the five stages was determined in order to calculate the relative extent of spreading of the pigment. In this manner the true mathematical relationships of the five stages could be calculated for this particular chromatophore. In addition, the areas of the pigment in chromatophores of two different sizes of crayfish were determined with the objective of learning whether or not the chromatophores increase in size when the crayfish does. Finally, the chromatophores were counted in

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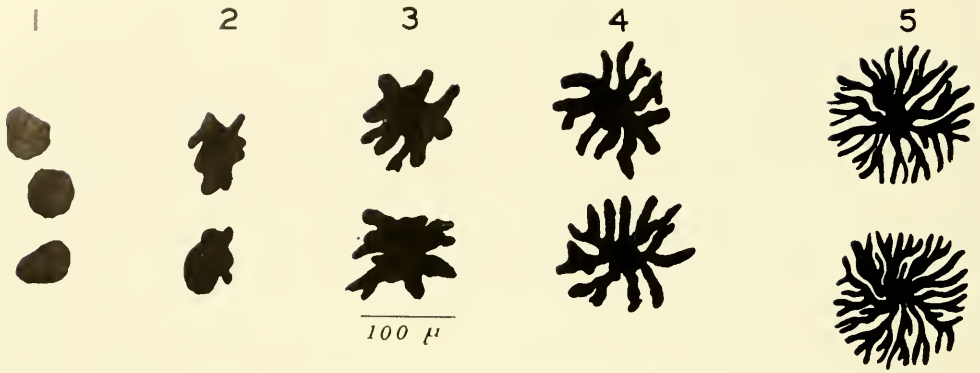

Figure 1. The five stages of the dark red chromatophores of *Cambarellus shufeldti* according to the Hogben and Slome method.

a unit area of the epidermis from juveniles and adults in order to determine whether or not the density changes as the crayfish grows.

Materials and Methods

Specimens of the dwarf crayfish, *Cambarellus shufeldti*, were collected periodically from a pond near the town of Pearl River, Louisiana. The crayfish were kept in the laboratory in aquaria containing aerated tap water about 2.5 cm deep and maintained at a temperature of 22-24°C under a constant illumination of 3.25 meter-candles.

For all of the observations only the dark red chromatophores dorsal to the heart were used. This crayfish also has smaller light red chromatophores interspersed among the dark red ones. The pigment in the dark red chromatophores will become maximally concentrated in specimens on a white background and maximally dispersed in individuals on a black one (Fingerman, 1957). After the pigment reached the desired stage the portion of the carapace dorsal to the heart was removed with scissors and forceps and placed inner side uppermost on a depression slide with enough Van Harreveld's solution (Van Harreveld, 1936) to cover the piece of carapace completely. The isolated piece was then viewed with a microscope having one ocular that contained a square grid which was calibrated. At the magnification used (125×) the square grid delimited an actual area of 0.56 mm^2. The number of dark red chromatophores within the area was determined. Then with the aid of a camera lucida the outlines of the pigment in one or more chromatophores were traced. These chromatophores were staged according to the system of Hogben and Slome. The camera lucida further magnified the chromatophores to 218×. These outlines were then enlarged an additional 6× by means of a photographic enlarger. The areas of the final tracings were then determined by means of a calibrated planimeter. The planimeter readings were finally reduced to the actual areas of the pigment in the chromatophores.

Student's *t* test was utilized in the statistical analysis of the data. A probability value of 0.05 was considered the maximum value for a statistically significant difference.

Results and Discussion

The areas of the pigment mass in the dark red chromatophores of adult dwarf crayfish, 2.4-2.6 cm total length, were determined for each of the five Hogben and Slome stages. These measurements were performed on 20-25 chromatophores of each stage. Figure 1 consists of drawings depicting the five stages of the dark red chromatophores. In stage 1 the pigment is maximally concentrated and no processes are visible. Stage 2 is identified by the rough edges indicating the beginnings of processes as some of the pigment is starting to flow into the branches of the chromatophores. Stage 3 is characterized by the presence of distinct processes and a large central mass of pigment. In stage 4, compared with stage 3, the processes have enlarged and the central mass of pigment has decreased. Stage 5, the most dispersed stage, is readily identified and distinguished from

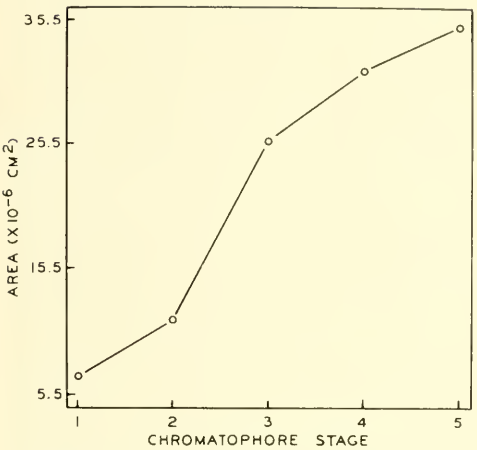

Figure 2. Relationship between the Hogben and Slome stages and the surface area of the pigment in the dark red chromatophores of adult specimens of *Cambarellus shufeldti.*

stage 4 by the branching and thinning of the processes and the minimum size of the central pigment mass as a consequence of the migration of so much pigment from the center into the processes.

The mean surface areas for each of the five stages of the dark red chromatophores from adults are shown in Figure 2. It is quite clear from Figure 2 that the area of the pigment mass undergoes its greatest increase between stages 2 and 3. In order to compare the areas of the pigment in each of the five stages ratios of the areas were calculated. The more dispersed stages increased in size over stage 1 by factors of 1.7 for stage 2, 3.7 for stage 3, 4.5 for stage 4, and 5.0 for stage 5. The surface area thus keeps increasing but only for stages 1 and 5 are the areas proportional to the numerical stages. The measurements were analyzed statistically and it was found that the differences between the areas of stages 1 and 2 and between stages 2 and 3 were highly significant ($p < 0.001$ in both cases) as were the differences between the areas of stages 3 and 4 and between stages 4 and 5 ($p < 0.01$ in the latter two instances).

Measurements were also made of 20 dark red stage 1 chromatophores in juvenile dwarf crayfish, 1.7-1.9 cm total length. The mean surface area of the maximally concentrated pigment in the juveniles was 5.8×10^{-6} cm^2 compared with an area of 7.0×10^{-6} cm^2 for stage 1 chromatophores of adults. The area of the pigment in adult stage 1 chromatophores is, therefore, 1.21 times larger than in comparable chromatophores of juveniles. The difference was statistically significant, $p < 0.05$.

The means of the numbers of dark red chromatophores within 0.56 mm^2 of the carapace dorsal to the heart were 40.7 for the adults and 48.2 for the juveniles, the ratio is 0.84:1. The difference was statistically significant, $p < 0.05$. As noted above, the ratio of the areas of the juvenile to adult stage 1 chromatophores was 1.21. The product of these two ratios is 1.02, essentially unity. In other words, the decrease in density of the dark red chromatophores in adults was compensated for by an increase in area with the result that the net coloration due to the chromatophores was unchanged as the crayfish grew.

Summary and Conclusions

1. The areas of the pigment in the dark red chromatophores of adult dwarf crayfish, *Cambarellus shufeldti,* were determined for each of the five stages of the Hogben and Slome scale.

2. The differences in area among the five stages of the Hogben and Slome system were statistically significant. The area of the pigment in a stage 5 chromatophore (maximally dispersed pigment) was 5.0 times as large as that in a stage 1 chromatophore (maximally concentrated pigment) but the areas of the pigment in chromatophores of stages 2, 3, and 4 were not simple multiples of stage 1.

3. The areas of the pigment in dark red chromatophores and their numbers in a unit area (0.56 mm^2) of the epidermis from the carapace dorsal to the heart of juveniles and adults were compared. The area of the adult chromatophores was 1.21 times larger than that of the juveniles but the density of chromatophores in the adults was 0.84 times that in the juveniles. Because the product of these two ratios (1.02) is for all practical purposes unity, it can be concluded that the decrease in chromatophore density was offset by the increase in area resulting thereby in a constant contribution by the chromatophores to the total coloration of the dwarf crayfish as it grew.

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