NOTES ON SOME PROBLEMS OF ADAPTATION: 6. RELATION OF LIGHT TO THE PIGMENTATION OF ASCIDIANS.[1]

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1. Experiments concerned with the re-formation of the test of the blue-black *Ascidia atra*, subsequent to its injury or partial removal, have demonstrated (Hecht, '18a, p. 236) that "the newly formed test material is pigmented in the usual way," even in the case of animals operated upon at night and maintained during regeneration in complete darkness. Since, "moreover, a new, pigmented test will form on the right face of the animal under the intact, opaque, old one, when the latter has been accidentally separated from the ectodermal surface which secretes it," Hecht concluded that the characteristic pigmentation is not due to the presence of light—in the sense, at least, that it cannot result from "a photic stimulus only." This conclusion is of interest, since it at first sight conflicts with some facts afforded by related species, and also with facts which may be made out from the distribution of *A. atra* itself in nature. Hecht's experimental result I can fully confirm, but inasmuch as pale, relatively unpigmented individuals are to be encountered in dark situations, the relation of the illumination to pigmentation seemed far from clear.

It might be assumed that if, in the normal course of development, pigmentation were simply a qualitative result of metabolic events, the quantitative course of transformations leading to pigment deposition in the test might be determined, once for all, by illumination early in the ascidian's life; if this were correct, experiments upon adult animals might be quite incompetent to decide whether or not light has anything to do with pigmentation—and effects substantially of this category are not unknown (Goldfarb, '10).

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2. Owing to the nature of its normal mode of occurrence, which is most frequently in bright, sunlit situations in shallow water, *A. atra* is rendered quite conspicuous by its dense blue-black coloration—so much so, in fact, that some observers (cf. Mortensen, '17) have not hesitated to suggest the operation of "warning" coloration in this connection. In view of this fact, and of the direct relation, in *Ascidia*, between pigmentation and blood metabolism (Hecht, '18a), the finding of pale individuals in dark situations required further analysis.

Simple ascidians having transparent or translucent bodies are commonly found to frequent dimly illuminated spots. Examples are given by *Ascidia curvata*, quite transparent and at Bermuda found almost always under fair-sized stone slabs, although taken by me also among masses of the alga *Valonia*, the dead cells of which are closely imitated by it (!); by *A. californica* (Ritter and Forsyth, '17), from the undersides of rocks; and by *Ciona intestinalis*, "especially given to clinging to the undersides of floats, buoys, and the like." The original assumption of a darkened habitat by these forms is probably due to the fact thar the ascidian larva, at first photopositive, becomes sooner or latet negatively heliotropic, before attaching; in different species this reversal of behavior with respect to light may be much delayed, or may even not appear at all. (In another place I shall discuss experiments concerned with the behavior of the tunicate tadpole.) It is of interest to note that the adult *Ciona* is quite reactive to light, in a sensory way, and also that in the laboratory these animals die rather quickly in diffuse light, although in dim light or darkness they are able to live for some days (Hecht, '18a); whereas *A. atra*, adult, is insensitive to photic excitation (Hecht, '18b; Day, '19).

Very pale examples of *A. atra*, however, were, as stated, found under stones in various places at Bermuda. Some of these were carefully gathered, and used in the following experiment:

"Aug. 1 to 7, 1918. Under stones on the shore of Trunk Island, Harrington Sound, 4 very pale *A. atra* secured; blue-black pigmentation was apparent only at the very tips of the siphons. These were kept in laboratory dishes lighted by a northwest window, some little direct sunlight falling upon the animals late each afternoon. The tunicates were perceptibly blacker on the second day, and rather dense black by the seventh day."

The experiment was several times repeated. The individuals concerned were 1.2 to 2 cms. long.

From such results it might appear that in the case of *A. atra* light has indeed a direct effect in producing dark pigmentation, as it undoubtedly has in certain molluscs (cf. List's observations, 1902, on the superficial pigmentation of mussels; these observations I can confirm from experience with the Bermudan littoral *Modiolus*). But it is known that a number of tunicates change color under laboratory conditions (Caullery, '95; cf. also Holt, '14). Accordingly, I repeated the above experiment, with the difference that the animals taken from under stones were immediately placed in darkened collecting buckets, and in the laboratory maintained in aquaria in a dark room. By the sixth day, these individuals were as dark as the similar ones exposed in the laboratory to illumination. Hence it cannot be decided from such experiments whether or not light has any direct significance for pigment-deposition in the test of *A. atra*.

3. A possible explanation was sought in another direction. Every *A. atra* found on the under side of a stone slab was relatively small, none being more than 4 cms. long. Individuals of this length collected from reef rocks, wharves, buoys and floats, moreover, were comparatively much more robust. As an index of better growth, the thickness of the test on the right side of the body was measured in several of these. This thickness, in the normally situated ascidians, amounted to 2.0 ± mm.; whereas in those living under stones the thickness of the test was but 0.8 mm. Since it is fairly certain that the blue-black granular pigment of the *A. atra* test derives from metamorphosed vanadium-containing blood cells (Hecht, '18c), and is essentially an excretory product (Crozier, '16), as seems probable for some other ascidian pigments also (Pizon, '01), it is not unreasonable to suspect that malnutrition may be in these instances responsible for faulty pigmentation. Fuchs ('14) observed, in laboratory cultures, that the siphons of young Cionæ lengthen remarkably in

proportion to the available food material, and was able to correlate this finding with the forms of *Ciona* in different natural environments. *A. atra* from among piled stones was noticed (before Fuch's paper was known to me) to have siphons very short in comparison with those of the more robust specimens from open situations. The view is, therefore, permissible that relatively deficient metabolism is in this instance responsible for subnormal pigment deposition.

The experiments of Miss Johnson ('13) regarding the pigmentation of amphibian larvæ showed the kind of materials ingested, rather than the absolute amount of food, to be important for pigment formation. In *Ascidia* we are not dealing with a melanin, however, but with a quite different type of pigment. Unfortunately, one is not able to decide as to whether the larvæ ultimately producing the pale *A. atra* located under littoral stones came in the first place to assume such sites in an accidental way, or were carried there and, because of some deficiency in vigor, failed to escape, or because they endured too long (or not long enough) in the larval phase and became attached at a time of day unsuitable for the operation of photic control of their movements.

The larva of *Ecteinascidia*, as I shall elsewhere describe, is normally liberated on a falling tide, and the duration of its free-swimming phase, together with its modes of response, are so adjusted to the tidal rhythm that in most cases a new individual (in this latter species, resulting in a colony) is laid down at about low-water level; the exact location of the site of attachment depends in an interesting way upon developmental changes in the phototropism of the larva. (Parallel changes have since been described by Grave, 1920, in the larva of *Amaroucium;* but the ethologic correlations, fairly clear in *Ecteinascidia*, have been as yet incompletely set forth for *Amaroucium.*) Should similar phenomena appear in the comportment of the larva of *A. atra*, some transforming individuals might become too photonegative, or might settle down at night, in either event perhaps becoming affixed in a dark cranny among stones.

4. Definite proof that darkness as such has no control over

the pigmentation of *A. atra* seems to me indicated by the finding of well-grown and densely black specimens in small caves practically shut off from the light. A few such were discovered with the aid of an electric torch in cavelets having but a small opening to the water. And from the under surface of a submerged rock, undercut in such a manner as to be completely sheltered from the sun, I later secured a group of fair-sized individuals with a quite normally black appearance.

5. *Summary*.—Pale, translucent individuals of the blue-black *Ascidia atra*, a species normally occurring in sunlit places, are found in darkened situations under stones. They resemble, in the absence of test-pigment, ascidians of other species habitually living in shaded spots. In spite of the fact that such pale *A. atra* may quickly blacken if placed in sunlight, it is pointed out that the absence of natural pigmentation cannot be regarded as due to darkness, but is probably a consequence of faulty nutrition.

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