Researches on the Coloration of the Skins of Flat-fishes.

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In the year 1890 I tried an experiment upon young flounders, with the object of discovering what would be the effect upon the lower sides of the fish if these sides were continually exposed to daylight. Under ordinary conditions the upper side of the fish is dark-coloured, the lower side white; and the upper side is exposed to light, while the lower side being usually in contact with the ground, and always turned away from the sky, is protected from the light. A connection between the difference of the two sides in relation to light and in coloration naturally suggests itself. If the difference in coloration is due to the difference in the exposure of the sides to light, when the lower side of the fish is kept exposed to the light it ought to become coloured.

When the flounder or other flat-fish is first hatched it has chromatophores on both sides equally, and these chromatophores disappear from the lower side during the metamorphosis. It seemed more likely that illumination of the lower side would prevent to some extent this disappearance, than that it would cause the reappearance of chromatophores on the lower sides of older specimens. My first experiment (described in the Zool. Anzeiger, 1891) consisted, therefore, in taking a few young flounders which had not completed their metamorphosis and rearing them in a glass bottle supported on a plate of glass, underneath which was placed an inclined mirror reflecting the light from a window vertically upwards. I covered the sides and top of the bottle with an opaque cover made first of brown paper, afterwards of cloth, so that the light was to a great extent prevented from entering the bottle in any direction except from the mirror. The rearing of flounders from this stage to maturity, although requiring minute and constant attention, presents no great difficulty. The methods of feeding them and maintaining a circulation of the water containing them were described in this Journal, vol. i, No. 4, 1890. A circulation was kept up in the bottle over the mirror in the experiment by connecting it with another bottle by means of a siphon outflow tube, the aperture of the tube being protected by silk bolting cloth, so that the little fish could not escape.

I was absent from Plymouth in July and the early part of August in 1890. When I returned I noticed that the little flounders continually clung with their lower sides to the darkened sides of the bottle, so that the object for which the apparatus was arranged was to a great extent defeated. I tried to prevent this by confining the fish beneath a horizontal partition of coarse cloth fitted into a cylindrical glass vessel substituted for the bottle, but the cloth did not allow of sufficient renewal of the water beneath it, and the fish were found all dead one morning, having been killed by suffocation. There were thirteen of these fish, and all except one had some pigment on the lower side. The greatest extent of the pigmentation was over the region along the edges of the lower side, from the base of the dorsal and ventral fins inwards, the region corresponding to the muscles of the fins. As far as could be observed in the course of the experiment (it was not possible to make a minute examination without risking the life of the fish), the pigmentation present at the end of the experiment was not due to a retention of the pigment present on the lower side before the transformation of the larval fish was complete, but the original chromatophores had disappeared from the lower side as usual, and had been redeveloped under the action of light.

In my next experiment I took four flounders belonging to the same brood as those of the first experiment. These were some of a number which had been reared under ordinary conditions, which had long passed their transformation and had no pigment on their lower sides. They were about five or six months old, and between 2 and 3 inches long. I removed the covering from the sides of the vessel, and left off using any partition inside it, keeping only an opaque cover on the top. In consequence of this the fish could not protect their lower sides from the light by clinging to the sides of the vessel, and their upper sides were illuminated by light passing through the sides, as well as their lower sides by the light from the mirror. At the beginning of 1891 I had made a wooden tank with a plate-glass bottom, which is still in use, and is shown in the figure illustrating this article. It is 3½ feet long, 2 feet 3 inches broad, and 11 inches deep, and I procured large mirrors to place beneath it. In this tank the four flounders lived and grew. A recurrence of the old difficulty of the fish clinging to the opaque sides took place, and I met this as far as possible by keeping the water in the tank very shallow. After this experiment had lasted six months I observed a commencement of pigmentation on one of the four fish. At the end of June, 1891, one of the four died. I had placed bricks in the tank to keep the fish in the centre of the glass bottom, and this specimen had got fixed between a brick and the side of the tank.

In September one of the three survivors had developed pigment all over the external regions of the lower side; in the other two pigment could not be detected with certainty. In this month another specimen, fortunately not the pigmented one, died. The remaining two lived on till July, 1892, when another died. This one was 23 cm. (about 9 inches) long, and had a large number of separate spots of pigment on the lower side. These spots were of considerable size and dark. Under the microscope they were found to consist of chromatophores exactly similar to those which constitute the pigmentation of the upper side. The fourth specimen is still alive at the present time. It is now three years old, and has lived in the apparatus since September, 1891. It is now deeply pigmented all over the lower side with the exception of a very small area.

There can be no doubt that the pigmentation in this experiment was due to the exposure of the lower sides of the fish to light. There were only four fish used, and two of them which lived long enough developed pigment which continually increased in extent. This is 50 per cent., and although pigment occurs on the lower sides of flounders living under natural conditions as an occasional abnormality, the percentage of such specimens is nothing like 50 per cent. It is important to point out that these four specimens were taken from a number reared in the aquarium in tanks with sand at the bottom, and subjected to no artificial conditions except captivity. In this Journal, vol. ii, No. 3, I have given the result of the examination of all such specimens reared from the brood of 1890. There were ninety specimens altogether, and one of these had a few small patches of pigment on the lower side. These were two years old when examined, and a more rigid control experiment could scarcely be required.

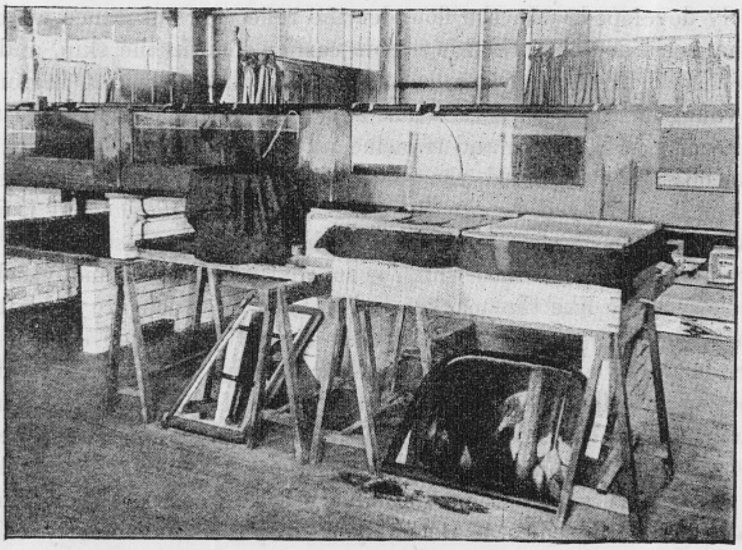
In another experiment I took one of this same brood (not one of the ninety just mentioned, but one taken before the examination referred to) which had one small spot of pigment close to the pectoral fin, and placed it in the apparatus, where its lower side was exposed to the light. In a few months the pigmentation of the lower side had extended over the greater part of that side.

Other similar experiments are described in greater detail in the full memoir by Dr. MacMunn and myself recently communicated to the Royal Society. Other experiments are now in progress, and a figure of the apparatus in use, prepared from a photograph, is here given. Besides the wooden tank already described and seen in the figure, there is also a large bell-jar. Both vessels are supported on trestles, and the large mirrors are placed beneath, upon the floor of the Laboratory. The vessels are placed in front of the tanks on the south side of the Laboratory and opposite the south windows, the supply of water being conveyed into the vessels by siphons from the Laboratory tanks. The fish are seen by reflection in the mirrors. At present the entrance of light is absolutely prevented by coverings of black cloth, or wooden covers lined with black cloth, except through the bottoms of the two vessels. The smaller fish are some reared from the brood of 1892, and the results exhibited by these are not yet published anywhere; the two larger fish are sole survivors from two separate experiments, and each of these is almost completely pigmented on the lower side.

During the period of time over which these experiments have extended, I have been studying, in collaboration with Dr. MacMunn, the anatomy and the physical and chemical properties of the elements to which the coloration is due. The results of these studies are fully described and illustrated in the memoir communicated to the Royal Society. A general account is all that can be given here. In the skins of flat-fishes the chromatophores have been described by Pouchet and other zoologists. They are of two kinds. Those of one kind are black or dark brown, have very definite outlines, and are contractile. They are stellate in form, having, when expanded, branching processes stretching out from the centre in all directions; but these processes can be partially or entirely retracted, and when completely contracted the chromatophore has a circular outline, being really nearly spherical in shape. The chromatophores of the other kind are yellow in colour. In the flounder the yellow deepens to orange at the centre. Usually the outlines of the coloured chromatophores are much less distinct and definite than those of the black, and it generally looks as though the pigment had diffused to some distance into the surrounding tissue. Nevertheless, contraction and dilatation of these yellow chromatophores takes place.

The chromatophores are of considerable size, easily seen with a low power of the microscope when a slice of the fresh skin is examined. But there are present much smaller elements which affect the coloration. These are angular plates of opaque substance of fixed form, having no colour, but reflecting light strongly. They are called iridocytes.

In the flounder, in the skin of the upper side a layer of chromatophores and iridocytes occurs close beneath the epidermis outside the scales, which are small and rudimentary. In the deeper part of the skin there are scarcely any chromatophores and no iridocytes, but on the inner surface of the skin there occurs another layer of chromatophores, black and yellow as in the superficial layer. Associated with the chromatophores here there are no iridocytes, but the place of the latter is taken by a continuous layer of opaque reflecting substance similar to that of which the iridocytes are composed. On the lower side of the fish chromatophores are entirely absent, but the iridocytes of the superficial layer are well developed. It is not these, however, which cause the opaque whiteness of the lower side of the flounder, for the layer containing these can be removed with a razor, and the whiteness of the skin remains. This characteristic of the lower skin is due to a thick, dense, continuous layer of reflecting substance on the inner surface of the skin, corresponding to the layer mentioned above in similar position on the upper side. This layer is much thicker on the lower side than on the upper.



The character and location of the elements of coloration are quite similar in other flat-fishes, but they are not always developed to the same degree. The chief variation is in the subcutaneous reflecting layer, which is in some species, *e. g.* the Megrim (*Arnoglossus megastoma*), almost entirely absent, represented only, whether on the upper or lower side, by separate small plates quite similar to iridocytes, but not so regularly arranged. In fact, comparative observations of different species, and the history of the development in the flounder, prove that the internal reflecting layer is actually derived from a layer of separate iridocytes which enlarge until they become continuous. This explains why the lower skin in the young flounder

absorption bands in the spectroscope, and easily bleached under the action of light when removed from the living body.

To return finally to the effect of the action of light on the lower side of the flounder. Analysis shows that the result is a development of black and yellow chromatophores exactly similar to those of the upper side. At the same time there is a gradual diminution in the amount of the reflecting substance in the argenteum, while a change in the superficial iridocytes has not been observed. Whence do is not opaque white, but bluish and translucent. The chromatophores, both black and coloured, are the first elements of coloration to develop, appearing in the skin of the embryo even before it is hatched. The external iridocytes appear next, and are found in the flounder during its transformation when it is $\frac{1}{5}$ to $\frac{1}{4}$ of an inch in length. The internal reflecting layer of the lower side develops late and very gradually. It first appears in streaks along the lines of the intermuscular septa, when the flounder is about $1\frac{1}{2}$ inches long and extends gradually. When the flounder is 3 to 4 inches long, the white opacity is usually fully developed. The peritoneum contains the same elements of coloration as the skin, namely, chromatophores and reflecting substance, and it is an important and significant fact that in the normal flat-fish the chromatophores are present only in the peritoneum of the upper side, while in that of the lower side they are absent or very scarce, and on this side the reflecting tissue is more largely developed. In the flounder the reflecting substance appears in the peritoneum of the lower side earlier than in the skin, and as it is visible through the walls of the body the abdominal region in the young flounder is marked out as a white area, while the rest of the lower side is bluish and translucent.

As might be expected, the elements of coloration in other fishes are not essentially different from those of flat-fishes. But it is a striking peculiarity in the flat-fishes that they are destitute of the silveriness and iridescence which is so characteristic of many fishes, especially those like the mackerel and herring which are migratory or pelagic. Investigation shows that the silveriness of such fishes depends almost entirely on a thick subcutaneous or internal layer of reflecting substance corresponding to that of the flat-fish. These layers, in fact, are homologous, the difference in appearance being due to a difference in the structure of the reflecting layer, which in the flat-fish is granular, in the silvery fish is composed of minute parallel rods or needles. Both layers may be conveniently called the argenteum. In other fishes as in flat-fishes, chromatophores, black and coloured, occur in an external layer and an internal, and where the chromatophores are most developed the argenteum is evanescent, and *vice versâ*. In other fishes there are also bodies corresponding to iridocytes, but they vary in form and arrangement. In fishes whose skins are iridescent, as the herring, this quality is due to a layer of parallel rods or prisms of reflecting substance, which in the herring line the inner surface of each scale, or more accurately are present between those parts of the scales which overlap one another. The scales themselves are never iridescent. This iridescent substance is obtained from the scales of certain fishes, especially the fresh-water bleak (*Albunus lucidus*), and placed in the interior of thin glass beads to make artificial pearls. The coloured chromatophores of fishes are always of some shade of yellow or orange, deepening to red; in some the colour is a distinct red, as in the gurnards and red mullet. Green fishes occur, *e. g.* the green pipe-fish (*Siphonostoma typhle*) and the mackerel, but in such cases the colour is not due to green chromatophores. The coloured chromatophores in such fish are of a lemon-yellow colour by transmitted light, though it approximates to green when viewed at certain angles by reflected light. The green colour exhibited by the fish is due to the mixture of this yellow colour with the black of the black chromatophores, just as a mixture of gamboge and black among artists' pigments produces a green. No blue pigment either occurs in any fishes that I have examined, blue colours being due to the reflections of iridocytes, modified by black chromatophores.

As to the histological nature of these elements, it has generally been held that they are modified connective-tissue cells. This may be true of the chromatophores, but probably is not true of the iridocytes and reflecting tissue.

From a chemical point of view the reflecting substance is composed of a definite organic compound in an almost pure state, and the opacity and reflecting properties of the reflecting tissues or elements are those of this compound, varying according to the state of aggregation in which it exists. This substance, whose formula is $C_5H_5N_5O$, is connected with the uric acid series, but its chemical relations are not well understood. It is found in small quantities in the excretions of the excretory organs of certain Invertebrates, but has never been found associated with the kidneys of Vertebrates. It was first recognised as an abundant constituent of guano, derived in that substance from the skins of the fish devoured by the sea-birds, whose excrement formed the guano. The pigment of the black chromatophores is known as melanin, an organic compound which is extremely insoluble and indestructible, and, with slight variations, occurring almost universally in the animal kingdom. The pigments of the coloured chromatophores all belong to a well-characterised class of pigments known as lipochromes or fat-pigments, being of an oily nature, soluble in alcohol, ether, and other fat solvents, giving absorption bands in the spectroscope, and easily bleached under the action of light when removed from the living body.

To return finally to the effect of the action of light on the lower side of the flounder. Analysis shows that the result is a development of black and yellow chromatophores exactly similar to those of the upper side. At the same time there is a gradual diminution in the amount of the reflecting substance in the argenteum, while a change in the superficial iridocytes has not been observed. Whence do these chromatophores come? Do they migrate along the skin or through the tissues of the body from the skin of the upper side? or are they formed *in situ*, and, if so, how? We are not prepared at present to answer these questions definitely. We believe, however, that their presence is not due to migration, but that the pigment is formed from the elements supplied by the blood at or immediately near the place where they appear. That they do not come round the edge of the body along the tissues of the skin is proved by the fact that spots and patches of pigment may appear in any position, and quite isolated, on the lower side.

It is important to mention that, although chromatophores are present on both sides of the fish in the intermediate stages of metamorphosis at which most of these experiments have commenced, the action of light on the lower side never results in the retention of these chromatophores. The latter disappear from the lower side completely, and after prolonged action of the light they reappear. It is certain, therefore, that the disappearance of the pigment from the lower side in the normal flat-fish is an hereditary character, and not due to the withdrawal of the action of light in the individual. If the latter were the case, of course the pigment would be retained permanently from the larval stage as soon as the light was allowed permanently to act upon the lower side of the fish. The disappearance of the pigment is, therefore, an hereditary family character in the Pleuronectidæ. On the other hand, the fact that in these experiments the pigment, after prolonged action of the light, actually reappears is strong evidence (to my own mind a proof) that originally, in the beginning of the evolution, the pigment disappeared in consequence of the withdrawal of the lower sides from the action of light. If this be granted, it follows, of course, that a character originally acquired has become hereditary.

Pigment occurs as an occasional variation on the lower sides of flounders living free under natural conditions. That this does not invalidate the significance of these experiments is shown by the fact that in a number reared under normal conditions in the aquarium, only 1 in 90 showed a spot of pigment on the lower side, while of 94 specimens obtained from the estuary of Hamoaze only one showed a coloured spot on the lower side. Moreover, in the experiments the extent of the pigmentation, and the number of specimens exhibiting it, steadily increase from month to month, while in nature pigment on the lower sides is not any more common in large specimens than in small.

The above is a mere brief summary of general results and conclusions. The full description of the investigations, with illustrations, is contained only in the memoir communicated to the Royal Society.