MORPHOLOGICAL ANALYSIS OF CHROMATOPHORES IN THE SKIN OF TROUT

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Abstract

The aim of the study was the morphological analysis of chromatophore cells in the skin of trout. Based on the material representing three species (brown trout, rainbow trout, and char), the histological examination of 60 specimens of the skin from dorsal, lateral, and ventral sides of the body was carried out. Histological analysis of the trout’s dermis revealed the variability of chromatophores regarding their morphology, distribution in the skin, and physiological state. There were distinguished melanophores in the pigment aggregation state or pigment dispersion state. There were found two types of iridophores, containing light-reflecting platelets in the cytoplasm. Histological study demonstrated the changes in the trout’s dermis connected with appearance of nuptial coat during the spawning season.

Key words: trout, skin, melanophores, iridophores, melanosomes.

Chromatophore is a pigment-containing or light-reflecting cell found in the skin of various vertebrates like fishes, amphibians, and reptiles (2, 9, 11). The main function of fish chromatophores is protection of the body against ultraviolet radiation. Skin colours of fish are generated as a result of absorption of light rays by pigmentary substances contained in dendritic melanophores, xanthophores, and by scattering and reflection of light by the iridophores (5). In contrast with birds and mammals, fish chromatophores are not component of epidermis, but are situated in the dermis. These cells derive from the neural crest cells (6).

According to recent studies, the colour forming pattern of fish skin results from the cooperation of many types of neighbouring chromatophores and their positioning in the skin (6). The aim of this study was morphological analysis of chromatophores in trout skin including distribution of these cells in the dermis, interactions between chromatophores in forming of colour pattern, and monitoring of changes in chromatophores population cells during reproductive cycle of trout.

Material and Methods

Histological examinations of the skin were performed on three species of trout: char (Salvelinus fontinalis), brown trout (Salmo trutta m. fario), and rainbow trout (Oncorhynchus mykiss). Research was carried out on 60 specimens (females and males), which were caught in the fish breeding ponds. The animals were anaesthetised with tricaine methanesulphonate MS-222 and killed by decapitation. Samples of skin were taken every second month during the whole year, in order to check any changes in chromatophores connected with the appearance of nuptial coat during the spawning season. The skin fragments were sampled (ca 8 mm) from three body parts: dorsal, lateral, and ventral. For the observations in light microscope, tissues were preserved for 48 h in 4.0% formalin solution, then dehydrated and embedded in paraffin. Serial sections, (5-7 µm thick), were stained with haematoxylin and eosin according to Delafield, with alcian blue (pH=2.5) staining method. The microscopic observation was made under light microscope Nicon Eclipse 80I, equipped with Nomarski differential interference contrast system.

For the transmission electron microscopy (TEM), the skin fragments were prefixed in 3.5% glutaraldehyde in a 0.1M phosphate buffer, pH 7.2. The postfixation was performed in 1.0% osmium tetroxide in the same buffer. After dehydration in a graded alcohol series, the samples were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Tesla BS-500 transmission electron microscope.

Results

The trout skin is composed of two main layers: epidermis, which is non-keratinised, stratified squamous epithelium, and dermis. The dermis is diversified into two layers: stratum laxum (built of loose connective
tissue with numerous capillary blood vessels, nerves, and scales) and *stratum compactum* (composed of dense connective tissue comprising parallel bundles of collagen fibres). The dermis is connected with the muscles by the layer of subcutis containing fat cells.

In the trout skin, the most abundant chromatophores were melanophores. Melanophores had cytoplasmic projections, which stick out from the centre of the cell and intertwined with collagen fibres of connective tissue (Fig. 1). The diameter of melanophores ranged from 10 µm to 100 µm. These cells were detected in various physiological stages: in the state of pigment granules dispersion and in the phase of pigment aggregation. The translocation of the pigment within the melanophore influenced morphological view of the cell. In the aggregation state, all the pigment granules were concentrated in the centre of the cell and the melanophore was oval in shape (Fig. 2). In the phase of dispersion, the pigment granules were spread within the cytoplasmic processes and such a melanophore had dendritic shape (Fig. 1). In the central part of the melanophore, the bilobate nucleus was observed. Melanophores exhibited the colour from brown to black. TEM analysis revealed the structure of pigment granules. Melanin was stored in the cytoplasm in the form of membrane-bound vesicles - melanosomes with uniform electron density (Fig. 3). Some of the melanosomes were surrounded by membranes of the endoplasmic reticulum. Between melanosomes, free ribosomes were observed. In the vicinity of the nucleus, there were detected elements of the cytoskeleton: microtubules and microfilaments.

The examination of the skin under the light microscope revealed the iridophore cells containing light-reflecting platelets in the cytoplasm and forming glowing areas in the skin (Fig. 6). The colour of reflection changed from red/orange to blue and depended on a variable orientation of the platelets in the iridophore cytoplasm. In contrast with the light reflecting iridophores, melanophores were visible in polarised light as the black cells. The iridophores were concentrated in two regions: between *stratum compactum* and subcutis (Fig. 4) as well as in the vicinity of the basement membrane of the epidermis (Fig. 7). The iridophores from the borderline between the dermis and subcutis were elongated, flattened cells, lying with their long axes parallel to the skin surface. These cells were intertwined with each other (Figs 4, 5). In the cytoplasm of the elongated iridophores, there were visible flattened nuclei and light-reflecting platelets, oriented at various angle to the cell surface. In the layer formed by the elongated iridophores, there were found melanophores in aggregation or dispersion state (Fig. 6).

Beneath the basement membrane of the epidermis, the iridophores of globular shape were detected (Fig. 7). They had oval large nuclei, located in the centre of the cell. Transmission electron microscope analysis of globular iridophores showed that the platelets were present in the peripheral part of the cytoplasm (Fig. 8). The singular globular iridophores were surrounded by cytoplasmic processes of melanophores (Figs 7, 8). These two types of chromatophores formed under the epidermis the structures, which looked like chain link. In samples of light coloured skin, the globular iridophores formed complexes containing from five to seven cells, which were visible as clusters (Fig. 9).

The xanthophores were not distinguishable from the other chromatophores in a light microscope. The xanthophore pigment, consisting of fat and water soluble carotenoids and pteridines, was lost during alcohol dehydration and tissue preparation. There were identified singular cells containing pale yellow intracytoplasmic vacuoles, which could be presumably xanthophores.
Fig. 3. Melanosomes in the cytoplasm of melanophore. TEM, 6,000x

Fig. 4. The layer of elongated iridophores in hypodermis. HE, 250x

Fig. 5. The iridophores in the hypodermis. HE, 1,000x

Fig. 6. Iridophores layer associated with melanophore. Polarised light, 1,000x

Fig. 7. Globular iridophores surrounded by melanophore processes. HE, 400x

Fig. 8. Globular iridophore (the arrow indicates a reflecting platelet) encompassed with melanophore. TEM, 8,000x
The histological analysis revealed that the distribution and concentration of chromatophores in the trout skin depend on the location on the body area and physiological state of an individual. The highest concentration of the chromatophores was observed in the dorsal skin and the lowest one in the ventral skin.

The fish, which manifested nuptial coat during spawning season, had more chromatophores in the dermis. In skin samples collected during spawning season, there were observed increases in melanophore concentration in all analysed body areas. The melanophores were seen mainly in a dispersion state and formed large clusters. These cells made up a continuous layer adjacent to basement membrane of the epidermis and were abundantly scattered in the stratum laxum of the dermis, where surrounded the blood vessels and the scale pockets (Fig. 10). Numerous dispersed melanophores were also seen in the complexes with globular iridophores in the upper dermis and with the elongated iridophores in the subcutis.

In the skin samples collected after spawning season, there were relatively fewer melanophores and proportionally greater number of iridophores. The globular iridophores were present beneath the epidermis in four-seven cell complexes and were associated with singular melanophores in the aggregation state (Fig. 9). The elongated iridophores were numerous in the hypodermis.

**Discussion**

Dermal chromatophore units discovered in the skin of amphibians and reptiles consist of xanthophores, iridophores, and melanophores (2, 9). According to the dermal chromatophore unit concept (2), the dispersion of melanin granules in melanophores can modify bright colours imparted by light reflecting iridophores through obscuring iridophore cells. In dispersion state of melanophores, the animal skin is dark. The aggregation of melanin granules in melanophores uncovers the iridophore cells, which scatter and reflect light to the xanthophore layer above. Xanthophores filter the light and create tones of the skin.

The examination of trout skin indicated the cooperation between melanophores and iridophores in forming skin colouration, which seems to be similar to chromatophores of amphibians and reptiles. Melanophores in trout skin form complexes with globular and elongated iridophores. The dendritic melanophore processes, which encompass the globular iridophores, absorb the incoming light before it reaches the reflecting platelets of iridophores and, in consequence, the skin takes on a dark colouration. In the bright skin, clusters of globular iridophores are associated with aggregated melanophores, which results in the exposition of iridophores to light and producing of bright colours. Similarly, to the melanophore-iridophores complexes present in the stratum laxum, the elongated iridophores in the hypodermis were also observed with close association with melanophores in various physiological stages.

Trout, similar to other salmonids, assume nuptial coat manifested as change of skin colour, just before and during the spawning season. The colouration of the skin is in this time darker and more intensive. Histological examination of skin samples collected during spawning season revealed increases in the concentration of chromatophores in the dermis.

The pigment translocation could be under hormonal or neuronal control (3). Aspengren et al. (1), indicated that noradrenaline is a neurochemical, which influences the pigment translocation. According to Logan et al. (7), pigment translocation is regulated by melanocortins, melatonin, and MCH (melanin concentrating hormone). Relation between chromatophores physiological state and reproductive cycle of trout proves that hormonal regulation has a significant influence on the pigment cells.
Rodionov et al. (8) and Tuma et al. (10) described the mechanism of pigment intracellular transport in fish melanophores and proved that melanosomes are transported along radial microtubules by motor proteins: kinesins and dyneins. The analysis of trout melanophores in a TEM revealed the presence of microtubules in the vicinity of the nucleus. Hawkes (5) studied chromatophores in the skin of another salmonid fish - Coho salmon (Oncorhynchus kisutch). He found also two morphological types of iridophores: elongated and globular, and revealed that light reflecting platelets of iridophores are composed of guanine and hypoxanthine crystalline material. Harris and Hunt (4) indicated the variability in iridophore structure and described four kinds of these cells in the skin of Atlantic salmon. Morphological research of pigment cells and histological examination of trout skin could be helpful in the veterinary ichthyopathology, especially that trout are often bred by fish farmers.

References