Bulletin of Aichi Univ. of Education, 63（Natural Sciences）, pp. 59 − 66, March, 2014

Growth of the Medaka (III) – Formation of Scales

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ABSTRACT

We provide information on the development of scales of cycloid type and of the squamation pattern in the medaka. During the growth period from larva to juvenile, scales begin to form concurrently with the pronounced degeneration or resorption of the median fin fold in the region of the caudal peduncle. The position of scales to be formed first may be predestined on the body surface along the horizontal septum. In medaka, the body size at which the scales first appear is 8–10 mm total length (TL). The squamation begins on the horizontal septum of the posterior region of the trunk and the anterior region of the tail and subsequently extends to the cranial, the caudal and dorso-ventral sides. Scales on the skin of the prefrontal and dorsal surface of the pectoral fin appear last. During squamation formation, the diameter and circular number of a scale gradually increases, but the number of scale rows and circuli (ridges) varies greatly among individuals. The distribution of scales closely corresponds with the somites and vertebrae.

Keywords: scale, squamation, metamorphosis, larva, medaka, horizontal septum

INTRODUCTION

Metamorphosis in vertebrates such as fishes and anurans is a distinct transformation of larval to adult morphology (Blaxter, 1988). The transformation is controlled by pituitary hormones (Pickford and Atz, 1957). The morphological transition of the larval to the juvenile of teleosts is observed in internal and external organs and requires the action of thyroid hormone (TH) that is released from the thyroid stimulated by pituitary thyroid stimulating hormone (Brown, 1997). TH also stimulates the formation of larval scales in the medaka (Tomita, 1961). In the transitional stages between the larva and juvenile of medaka, various morphologies such as rib, vertebral columns, blood vesicles (Iwamatsu, 2012), fins (Iwamatsu, 2010, 2013), scales, otolith (asteriscus) and so on are newly formed, while the fin fold and ventral vein degenerate. The transformation of morphology seems to be an essential prerequisite for a rapid adaptation to new, strict environments of the growth period. The juvenile stage is characterized by specimens having the appearance of small adults: all fin rays and scales are formed, and the skeleton is completely ossified. It is generally assumed that scales protect the body surface from various changes and stimuli in environmental conditions (Fukuhara and Fushimi, 1984). As in other fishes, medaka in the early stage of growth has no scales in the skin, although the body surface of all adults is covered with scales. In the medaka (*Oryzias latipes*), appearance of scales is recognized as a transition from the larval to the juvenile, and scale development is closely correlated with age, or size of the body (Kobayashi, 1936; Inaba and Nomura, 1950). The size and circular number of scales may be used as measures to study meristically growth processes in teleosts.

Recently it has been reported that a *rs-3* locus which maps to medaka linkage group (LG) 21 encodes an ectodysplasin-A receptor (EDAR), which is involved in the proliferation or differentiation of scales (Kondo *et al.*, 2001). A mutation at the *rs-3* locus of medaka leads to an almost complete loss of scales in adult. A screen for mutants with scale defects is expected to identify key molecules of scale development. Little basic study, however, has been conducted on meticulous description on scale development and squamation of this fish.

The present study was undertaken to obtain basic information on scale formation and pattern of squamation in the period of metamorphosis during growth.

MATERIALS and METHODS

The medaka fish, *Oryzias latipes* (d-rR strain), used in the present study were reared in a rectangular glass aquarium (60×35×30 cm, 3 females and 2 males per about 60 liters of water) under reproductive conditions (L14: D10, 26–28°C). Under these conditions, mature females mate with males and spawn early every morning. After females were netted, fingers were used to pluck off clusters of chorion-hardened eggs that hung from their urogenital pores. During the rearing period, fish were fed a balanced diet containing one part each of shrimp powder, parched barley flour, Tetramin (West Germany) and Otohime no.1 (Nisshin-seifun) four times a day. After hatching, larvae were reared by being fed the balanced diet in rainwater containing green algae in a rat PC (polycarbonate) cage.

For observation of scales, larvae were anesthetized in a saline solution containing a mixture of 7 parts of phenylurethane and 3 parts of ethanol. The scale was examined in the anesthetized fish using a stereoscopic microscope (×20, Olympus SZX12) equipped with a calibrated ocular micrometer. Size and number of circuli (ridges) of scales were measured in a given region of the body, i.e. in the horizontal septum and the upper and lower lateral regions along the haemal and neural spines of the 14th–15th vertebrae, because the size and shape of the scales are different in the body regions, and the scales on this mid-lateral region with the highest body depth seem to be the most suitable for realizing their differentiation and development. Body size was represented by total length (TL, from the snout to the extremity of the caudal fin) by which the stage of fish development was determined.

For examination of scales in obscure regions of the head and abdomen, deeply anesthetized fish were fixed in 0.1 % glutaraldehyde-saline for 24 hr. The specimens were treated with 0.5% KOH for several seconds and stained with 0.05 % alizarin red S (Nakarai Chemicals Ltd.) for 5–6 hrs after they were rinsed in distilled water. The stained specimens shrunk to about 93.3% in TL and were completely rinsed in tap water and finally cleared in 50% glycerol for observation. Growth stages of fish were assigned following the author's developmental criteria (Iwamatsu, 1994, 2004, Iwamatsu *et al.*, 2003). Names used for skeletal structures follow Owen (1984) and Yabumoto and Uyeno (1984).

OBSERVATIONAL RESULTS and DISCUSSION

The round or oval shaped scales of medaka are smooth-rimmed and termed cycloid without radii (Kobayashi, 1936). According to the previous studies (Inaba and Nomura, 1950; Tomita, 1961) in the orange-red medaka and the see-through medaka (Iwamatsu *et al.*, 2003), the round scales appear first on the mid-bilateral lines (lateral stripe, or horizontal septum) of the body in larvae about 9 mm TL (7.0–8.5 mm body length in Inaba and Nomura, 1950). First appearance and formation of scales varies among individuals under different breeding conditions (light, temperature, water volume and quality, diet, population of individuals and so on). The differentiation and development of scales are affected by thyroxine (Tomita, 1961).

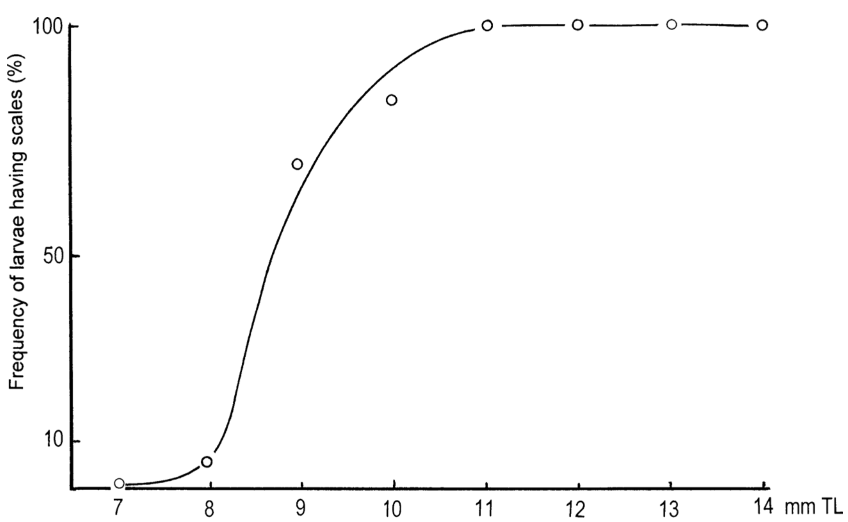

Fig. 1 Frequency of appearance of larvae with scales in growth period.

1) Scale development

Larvae less than 8.0 mm TL have not yet formed scales. The scales are recognized as mere circular plates (112–126 μm in diameter) in the body surface of the horizontal septum (lateral stripe) in larvae just before the circulum (ridge) is detectable. In medaka larvae 8.1–8.9 mm TL, one out of 19 larvae has first scales with a single circulum, concurrent with the pronounced degeneration of the median fin fold in the region of the caudal peduncle. First scales on a longitudinal mid-line are somewhat

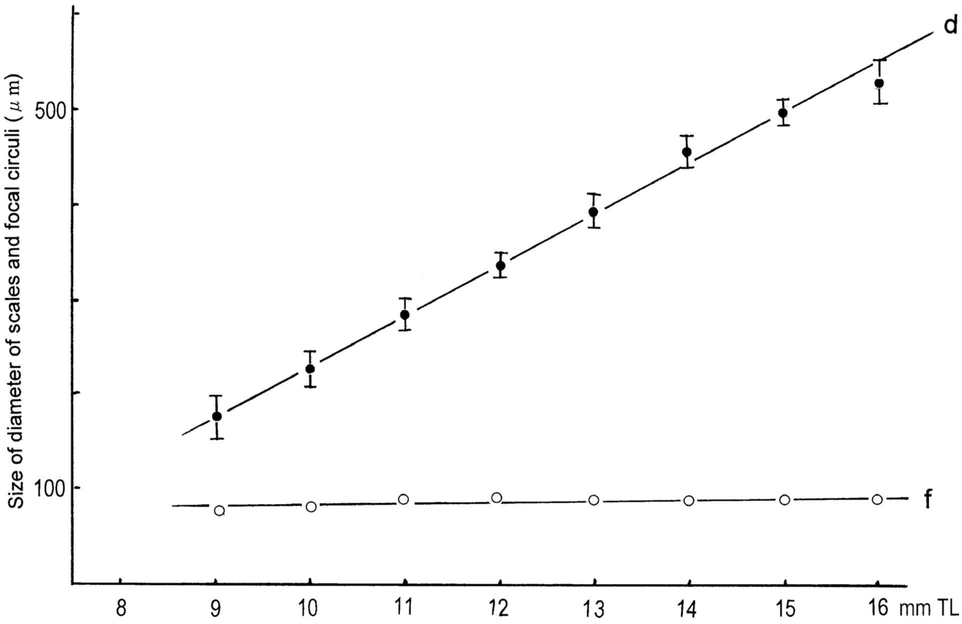

Fig. 2 Changes in a diameter of scales and focal circuli in growth period.
d: the antero-posterior diameter of scales, f: diameter of focal circuli.

dorso-ventrally elongate and elliptical and exhibits a comparatively regular shape. Scales with 1–6 circuli are detectable in specimens about 70.6 % in 9.0 mm TL, 83.3 % in 10 mm TL and 100 % in 11 mm TL (Fig. 1). In zebrafish, when the fish increases in length rapidly, the skin does not organize as rapidly (and scale development can obviously be initiated) even in large fish (Sire *et al.*, 1997). Several first scales appear simultaneously and always along the horizontal septum, in which guanophores exist at intervals.

According to the previous studies, the diameter of first detectable scales with a single circulum is 50–80 μm (Tomita, 1961) or 90–132 μm (Inaba and Nomura, 1950). In the present observation on the 14th–15th vertebrae, the size of first-appeared scales with a single circulum is 88.0 ± 2.7 μm (n = 11) in antero-posterior diameter and the distance between scales is mean 97.0 ± 7.4 μm (n = 6). Size (diameter) of scales increases linearly, whereas diameters of focal circuli show almost no change (Fig. 2). In larvae 9.0–9.7 mm TL, the scales with two circuli (82.7 ± 3.8 μm in diameter of a focal circulum, n = 11) are 143.5 ± 5.4 μm in diameter (n = 8) and have at a distance of mean 39.6 ± 3.6 μm (n = 6) each other along the horizontal septum. About 20 % of larvae of this body size also have scales with three circuli (Fig. 3). The number of scales with two circuli which lie along the vertebrae (horizontal septum) is 10–15 on the region from the 9th to the 26th vertebrae in larvae about 10 mm TL (Fig. 4). In case of a larva 10.4 mm TL, the scales with a single circulum are observed in two regions from the 7th to the 10th vertebrae and the 28th to the 29th vertebrae, and the scales with two circuli are distributed linearly in the restricted region from the 11th to the 27th vertebrae. Larvae 11 mm TL exhibit scales with 4 (about 45 %) and 5 (20 %) circuli. The number of circuli of a scale increases in proportion to body growth. Fig. 3 indicates a developmental process of scales with more than 3 circuli in most larvae (about 85 %) 11 mm TL, more than 4 circuli in larvae (about 95 %) 12 mm TL, more than 5 circuli in larvae (about 85 %) 13 mm TL, and more than 6 circuli in larvae (about 85

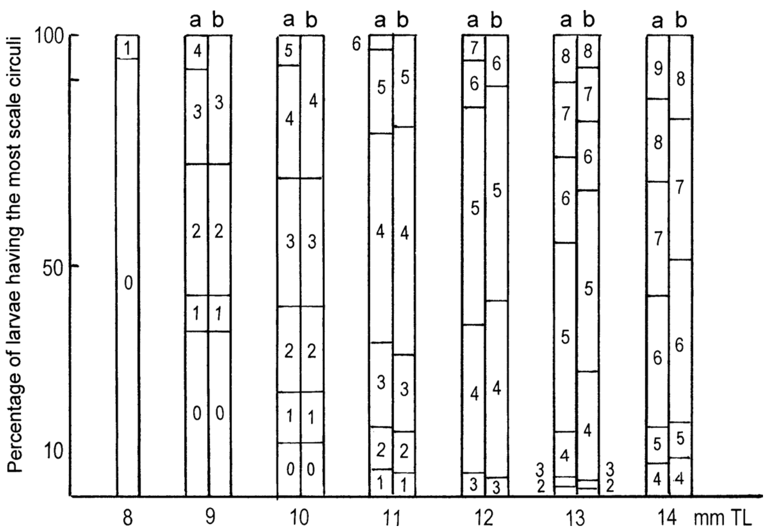

Fig. 3 Changes in number of the anterior and posterior circuli of the scale in growth period.
a: anterior circuli, b: posterior circuli.
Number in each column indicates the number of circuli.

%) 14 mm TL. Scales with less than 3 circuli do not overlap.

The anterior region of imbricate scales with more than 4 circuli is embedded deeply in the dermis (Iwamatsu, 2006) under two exposed posterior circuli of the front scale in which melanophores exist in the wild medaka. In individuals more than about 10 mm TL, scales with 4 or more circuli are imbricate with one another at the antero-posterior (Fig. 4) and at dorso-ventral regions of scales in other scale rows. In imbricate scales with circuli more than 4, the number of circuli of a scale is greater in the anterior (basal) than that of the posterior (apical) circuli (Fig. 3). The intervals between circuli (inter-circuli) are also different at the anterior and posterior areas of the scale during body growth. Namely, the circular intervals at the anterior area of a scale are narrower than that of the posterior area before the body size reaches 12 mm TL, while the former becomes in reverse wider than the latter when larvae grow into more than 12 mm TL (Fig. 5). According to Sire *et al.* (1997), during scale development, the epidermal basal layer cells produce substances which are deposited at the epidermal-dermal boundary. These substances can attract fibroblast-like cells, which interact with the epidermal-basal cells to build scales at the appropriate place. They considered that epidermal-dermal interactions continue in the posterior region of the scale and are probably involved in morphologically slight differences in the anterior and posterior regions of the scale.

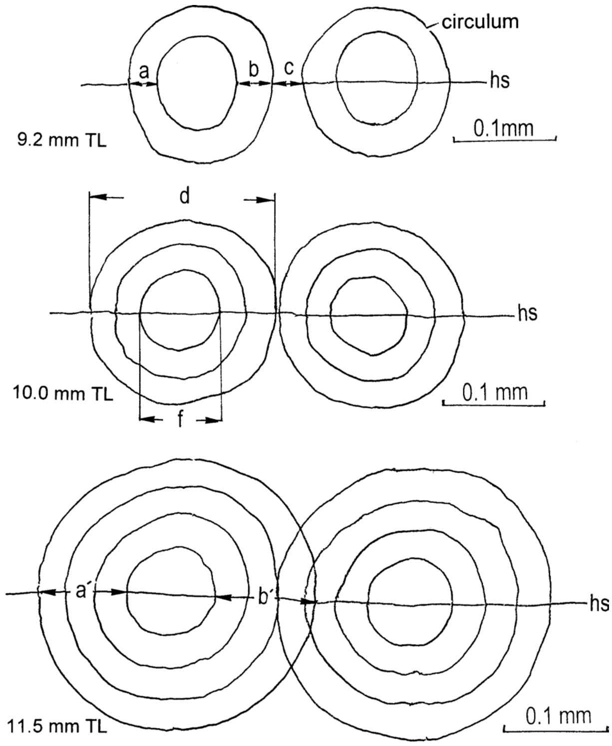

Fig. 4 Development of scales and number of circuli on the horizontal septum during metamorphosis.
a: width between the anterior circuli, a': width of the anterior portion of the scale, b: width between the posterior circuli, b': width of the posterior portion of the scale, c: distance between scales, d: the antero-posterior diameter of the scale, f: the antero-posterior diameter of the focal circulum, hs: horizontal septum.

In a scale on the centra of the 14th–15th vertebrae, the number of circuli increases with growth, but there is a surprising difference among individuals of the same TL (Fig. 3). Why large variations in circular number among individuals take place is unknown: it is probably due in part to individual genetic differences because the present breeding conditions are the same except for overpopulation of breeding individuals. Therefore, the circular number seems to be unreliable to determine the age of medaka fish, although scale growth has been extensively used to determine the age and rate of body growth in fishes. The diameters of scale foci (central circuli) are of a constant mean of 87.8 ± 1.1μm (range 56–112 μm, n = 97) despite increase of the scale size, whereas diameters of the scale (outermost circulum) increase in proportion to the total length (Fig. 2). The diameter of the scales at the region of the 14th–15th vertebrae seems to be a convenient meristic character for judging growth stage of the medaka.

In the cranial and the proximal region of caudal fin rays, the number of circuli in some scales does not always differ between the anterior and posterior sides of a scale.

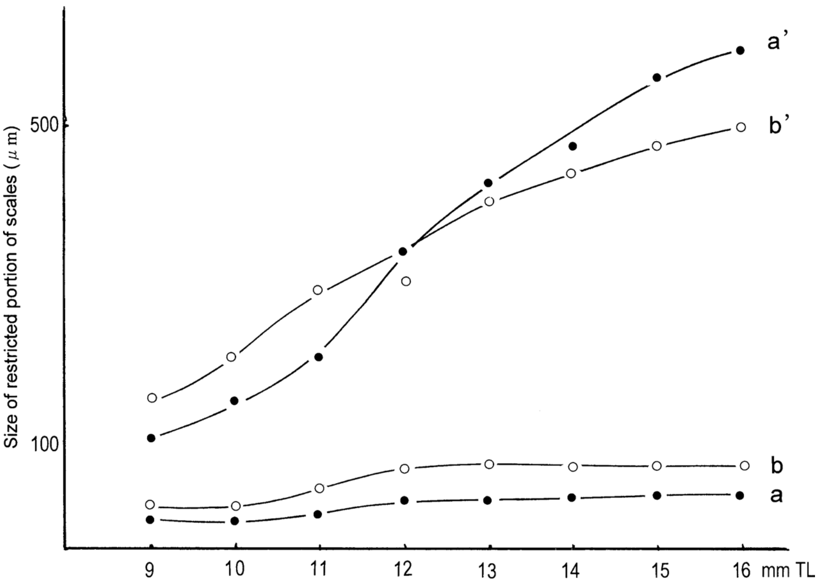

Fig. 5 Meristic changes of scales in growth period.
a: width between the anterior circuli, a': width of the anterior portion of the scale,
b: width between the posterior circuli, b': width of the posterior portion of the scale.

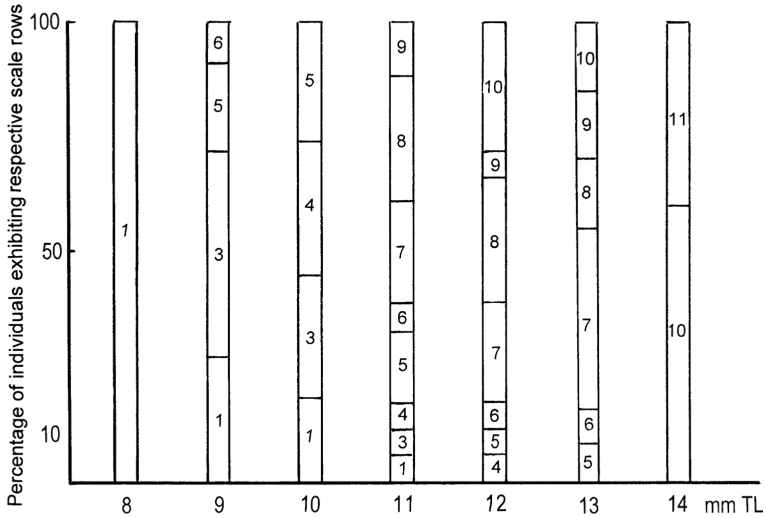

Fig. 6 Development of the scale rows during metamorphosis.
Number in each column indicates the number of scale rows.

2) Development of squamation

The ossified scales are first recognizable as a single circulum on the horizontal septum of bilateral sides prior to the initiation of fin formation of the pelvic fins. The first row of scales that are located on the horizontal septum constitutes scales with a single or two circuli. In the medaka, the location where the squamation initiates by a first scale row is a single locus, as seen in most teleosts (Conley and Witt, 1966; Priegel, 1966; Andrews, 1970; Fijita, 1971; Fukuhara, 1976; White, 1977; Fukuhara and Fushimi, 1981; Sire *et al.*, 1997). As the body grows proceeds, the scaled area expands from the horizontal septum towards the dorso-ventral portions, followed by extention in the cranio-caudal direction. This developmental pattern of squamation is the same even in different environmental conditions during growth, as observed in zebrafish (Sire *et al.*, 1997). In other teleosts the squamation process is simultaneously initiated in two (Fukuhara and Fushimi, 1984; Sire and Arnulf, 1990), three (Park and Lee, 1988), and four (Cooper, 1971) loci. However, the factors to determine the locus of initiation of first scale formation still remain unexplored.

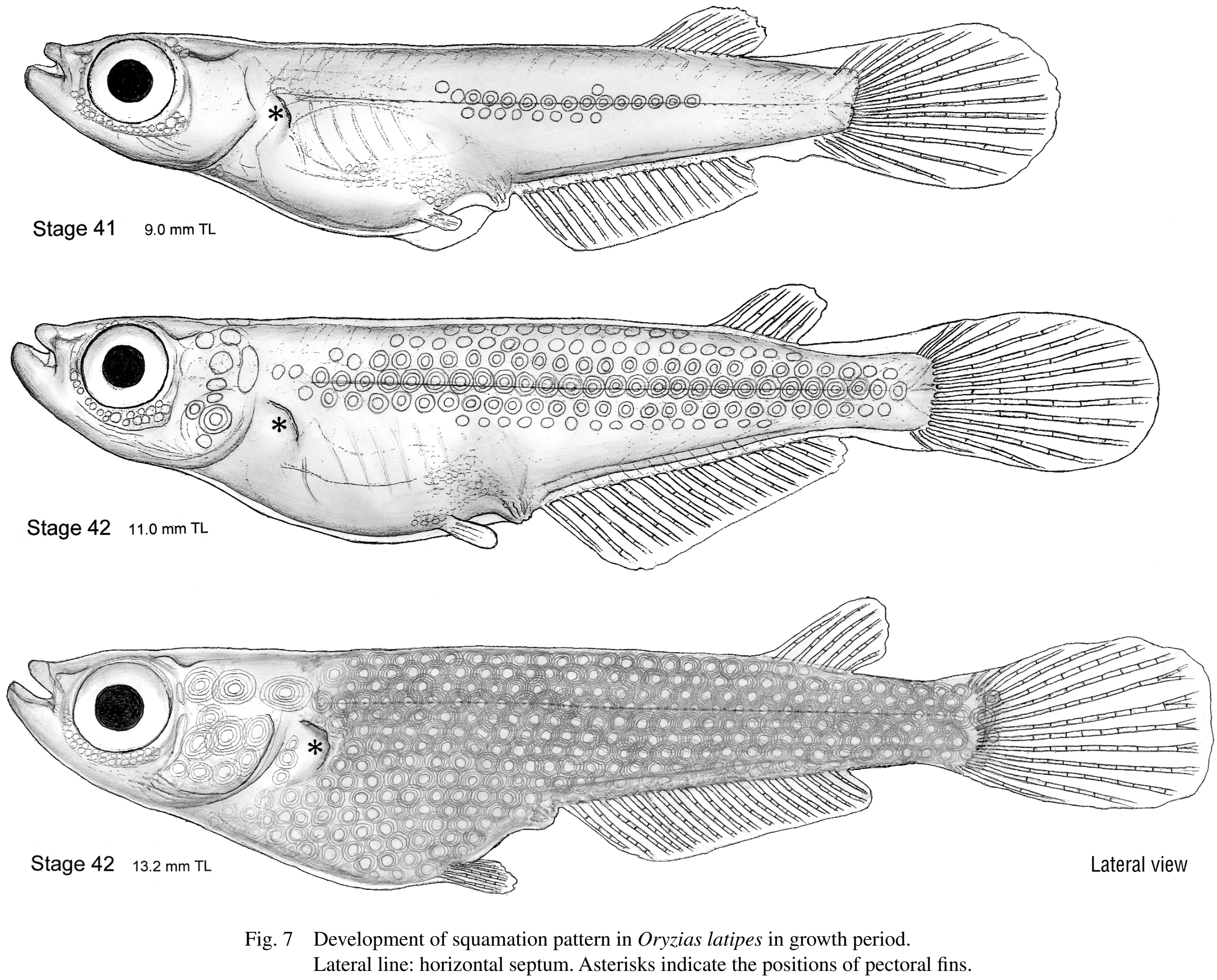

Fig. 7 Development of squamation pattern in *Oryzias latipes* in growth period.
Lateral line: horizontal septum. Asterisks indicate the positions of pectoral fins.

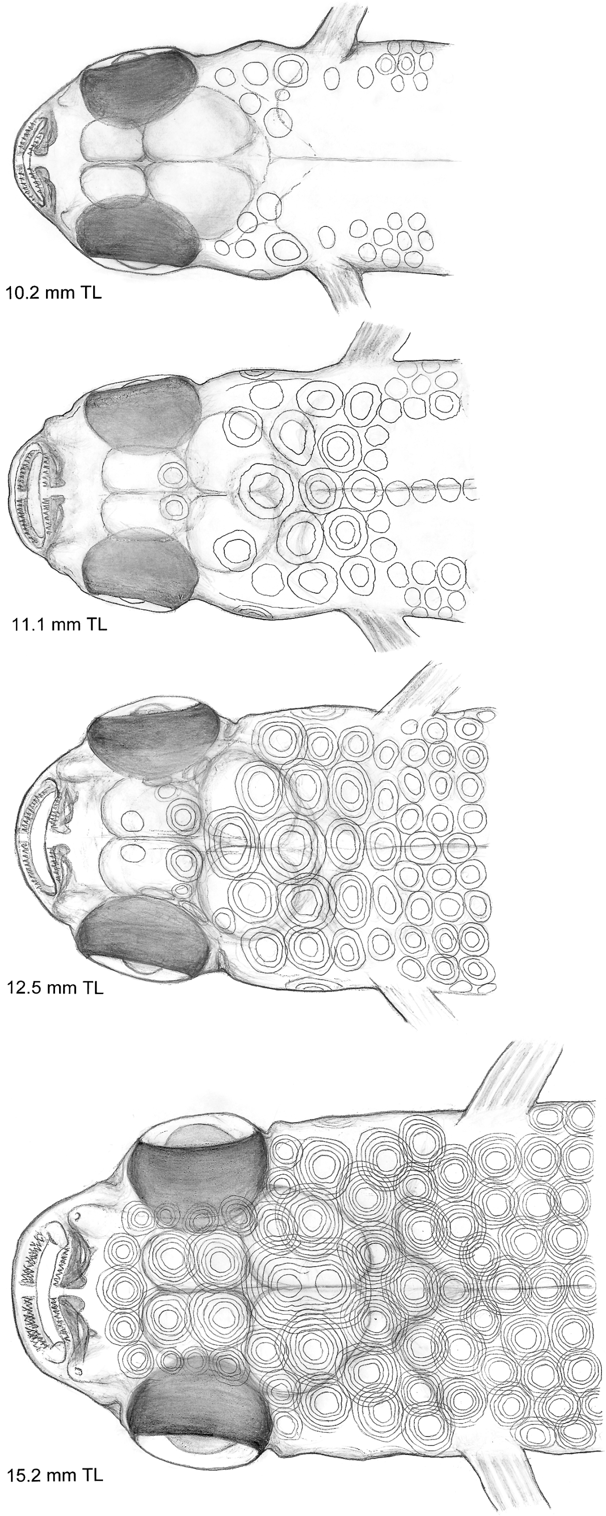

Fig. 8 Change in distribution of developing scales in the head area in growth period.

When larvae grow to about 9 mm TL, the squamation initiates linearly by appearance of scales on the horizontal septum from the posterior region of the trunk to the anterior region of the tail. In this body size, about 27 % of larvae exhibit a first scale row on horizontal septum, and three (45 %), five (about 20 %) and six (9 %) rows of scales in the dorsal (upper lateral) and ventral (lower lateral) sides of the horizontal septum (Fig. 6). Larvae more than 10 mm TL already possess 29–30 scales to be the same number on the lateral line as that in the adult. Larvae 9–10 mm TL have 1–6 scale rows in the dorsal and ventral sides of the horizontal septum. Most larvae (about 83 %) about 11 mm TL show more than 5 rows of scales (Fig. 7), but row numbers vary even in individuals of the same body size. When the body size reaches 13 mm TL, most larvae (about 85 %) exhibit 7–10 longitudinal rows of scales. In this body size, the surface of the cranial area and a proximal region of the caudal fin become covered with scales (Fig. 7). When larvae attain the body size of more than 14 mm TL, they exhibit 11 rows of scales on lateral sides, as seen in adult (Fig. 6). The scales subsequently spread over the body surface by the additional formation of new rows along the horizontal direction. By 16 mm TL, scales are distributed on the whole body surface, as described previously (Iwamatsu *et al.*, 2003).

On the supraoccipital of larvae 10 mm TL, scales are not seen but scales are seen at the upper area of the opercula. In larvae about 11 mm TL, the squamous area reaches the surface of the parietal and the prefrontal bones (Fig. 8). Scales are recognized on the vault (supraoccipital, parietal, prefrontal) and opercula (Fig. 7), but not on the surface of the lower jaw between both opercula. In larvae about 12 mm TL, irregular shaped scales frequently form on the posterior valut of the occipital. Small scales on the prefrontal form latest in squamation process (Fig. 8).

On the operculum, or gill cover, 3–7 scales with 1–4 circuli appear first in larvae about 11 mm TL. A large scale with 5 circuli is observed in at a central region of the operculum in larvae 13 mm TL. In larvae 14 mm TL, 8 scales with 6–8 circuli are observed on the operculum. In larvae about 15.5 mm TL, the scale number on the operculum increases up to 12–13 that are equal to the number in the adult.

In some larvae about 11 mm TL, a few scales appear on the hypurals. Scales with a single or a few circuli on the hypurals appear prior to those on the proximal area of the caudal fin rays. When TL reaches more than 12 mm, scales are subsequently found on the proximal region of the caudal fin rays that are attached to the hypurals (Fig. 7).

Thus, the squamation pattern in the medaka begins in the central regions of the horizontal septum, and ends in the cranial and the middle dorsal area in the anterior region of the trunk. In the transformation stage between larva and juvenile, the larvae gradually attain adult characters of meristic features by 14–15 mm TL although the formation and development of scales progress variably by individuals. At present, in addition to the problem on the cause of these considerable variations, it remains to be clarified how the position of first scales is determined.

ACKNOWLEDGEMENTS

The author is grateful to Dr. Lynne R. Parenti of National Museum of Natural History, Smithsonian Institute and Dr. Hirokuni Kobayashi for critical reading of manuscript, and Dr. Satoshi Hagino of Sumika Technoservice (Takarazuka, City, Hyogo Prefec.) for providing valuable literatures.

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(Received September 30, 2013)