

COMPARATIVE STUDY OF THE DEVELOPMENT OF LIMBS IN LARVAE OF THE COMMON FROG *Rana temporaria* (AMPHIBIA: ANURA) AND IN *Salamandrella* (CAUDATA)

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The development of limbs in larvae of the common frog (*Rana temporaria*) was studied using a standard histological technique. In the anuran limb bud like in the urodele one the major central arteries extend along the medial and lateral (opposite) sides of the mesenchymal core. These arteries are periodically connected with each other by means of anastomoses that penetrate the mesenchymal core and divide it into the pre- and postaxial parts. The ulna (fibula) and radius (tibia), ulnare (fibulare) and radiale (tibiale), and digits III and IV are laid down on both sides of these medio-lateral anastomoses. This fact and some others support the idea that the stem blood vessels determine, at least in part, the arrangement of cartilaginous anlagen in the limb bud. Digits of urodeles and anurans that have the same numbers in the traditional numeration system occupy a different position relatively to the central arteries. A new numeration system is proposed based on the notion of a zero digit, a digit that is the first to be laid down in ontogeny.

Key words: Amphibia, limb skeleton, limb blood vessels, morphogenesis.

In a recent description of the limb development in Siberian salamander (*Salamandrella*) a special attention was paid on the blood vessels that form a regular network in the limb bud in early ontogeny (Borkhvardt, 1994a). In this case arteries and veins were only used as the topographical markers, but in other papers their morphogenetic role was discussed and opinion voiced that vessels literally break up the skeletogenous mesenchymal core of the limb bud into some portions and thereby determine (in part) the position of the future skeletal elements (Borkhvardt, 1992, 1994b). The main aim of the present work was to investigate the relations between the blood vessels, skeletogenous tissue, and cartilages in the limb buds of the other tetrapod. The new data allow us to compare the limb skeletons of urodeles and anurans within the same “coordinate system.”

MATERIAL AND METHODS

Spawn of the common frog (*Rana temporaria* L.) was collected in the environs of St. Petersburg, larvae were reared in aquaria. Tadpoles were fixed in Bouin's fluid. Staging was done according to Daba-

gian and Sleptsova (1975). The limbs were embedded in paraffin, sections of 7 and 10 μm were stained with hematoxylin and eosin. The limbs were cut in three mutually perpendicular planes: 1) transversely to the proximo-distal axis of the limb bud (transverse sections); 2) in the preaxial – postaxial planes (PP-planes) which connect the preaxial and postaxial edges of the limb bud (preaxial-postaxial or PP-sections); 3) in the medio-lateral planes (ML-planes) which connect the medial and lateral surfaces of the limb bud (medio-lateral or ML-sections).

RESULTS

The hind limb buds are more suitable for preparing regular sections, therefore, they were used for the detailed examination and description; information about the front limbs is cited in discussion. The larval limb is conventionally divided into two parts by a transverse plane which cuts the limb in that place where its postaxial edge adjoins the tadpole body; the part proximal to this plane is designated as “the base,” and that distal to it is called “free” (about terms see also Borkhvardt, 1994a).

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Development of the Hind Limbs

Stage 39. A free part of the limb buds is slightly protruded. In the proximal limb region the dorsal (future lateral) and ventral (future medial) peripheral mesenchyme is organized into the subectodermal layers consisting of 1–2 rows of cells. The central mesenchyme, the mesenchymal core, is loose, especially in its preaxial half. There are many small blood vessels within the core. The only stem vessel, the postaxial vein, begins at some distance from the limb apex.

Stage 40. Limb buds are rounded in their transverse section. Their PP-plane extends from the larval body slightly downwards. At the base of the limb bud and at the beginning of its free part the mesenchymal core is a dense homogeneous cylinder. It is surrounded by the looser mesenchyme. The subectodermal layer is thin as before. The powerful sciatic nerve (n. ischiadicus) enters the postaxial half of the limb base and soon divides into the n. peroneus and n. tibialis. These nerve branches enclose the mesenchymal core like a horse-shoe, are displaced to the middle ML-plane, and extend in the distal direction along the lateral and medial boundaries of the core. The central artery enters the limb near the n. ischiadicus and is also divided into the lateral and medial trunks, the *lateral* and *medial central arteries*, which run alongside the nerves but penetrate into the limb somewhat further. At the place of bifurcation of the sciatic nerve the mesenchymal core contains scanty blood vessels. Distally a group of small anastomoses appears; they connect the medial and lateral central arteries and cut the core into the looser preaxial and denser postaxial portions. Distal to this region the mesenchymal core “runs,” the arterial network loses its well-defined organization. The postaxial vein extends from the very apex of the limb bud, while the preaxial vein originates more proximal. Each stem artery or vein consists of several trunks which periodically divide and fuse again.

Stage 41 (Fig. 1). At the place of branching of the sciatic nerve, the central cells of the mesenchymal core condense forming a short rudiment of the femur. Further distally, the core maintains homogeneity for a considerable distance, and then *shin anastomoses* arise within it connecting the medial and lateral central arteries with each other. Among these anastomoses a large dominant trunk begins to form that in the adult frog penetrates the shin bone (os cruris) and

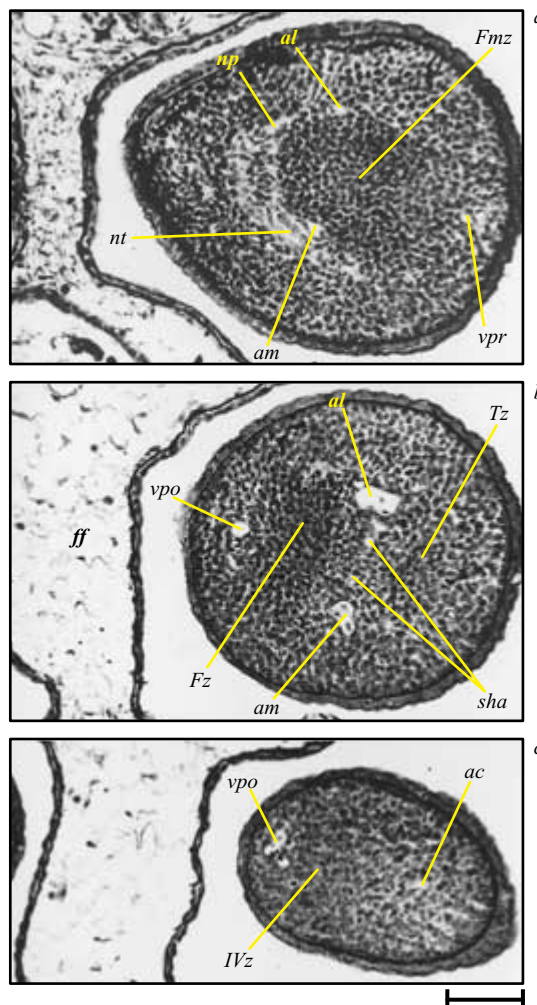


Fig. 1. Larva of *Rana temporaria*, stage 41. Hind left limb. Transverse sections in stylopodial (a), zeugopodial (b), and digital (c) regions. b) 160 μm distal to a; c) 230 μm distal to b. ac — Central artery; al — lateral central artery; am — medial central artery; ff — preanal fin-fold; Fz — zone of fibula; Fmz — zone of femur; np — nervus peroneus; nt — nervus tibialis; sha — shin anastomoses; Tz — zone of tibia; vpo — postaxial vein; vpr — preaxial vein; IVz — zone of the fourth digit. Scale bar 85 μm .

transports blood from a. poplitea to a. tibialis anterior (a. interossea anterior). Behind this region the core becomes slightly denser and then loosens again; small vessels within it increase in number but do not show regular organization. The central arteries extend almost to the very apex of the limb bud where they join with the initial portion of the postaxial vein that begins at the preaxial side and goes round the distal part of the mesenchymal core. The dense apical

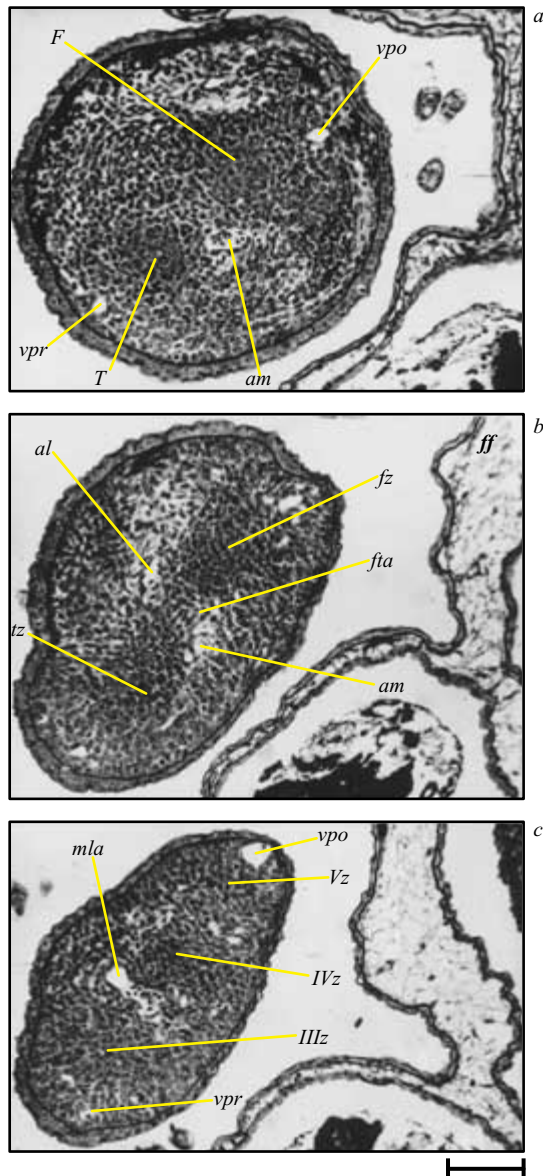


Fig. 2. Larva of *Rana temporaria*, stage 42. Hind right limb. Transverse sections in zeugopodial (a), tarsal (b), and digital (c) regions. a — Just in front of the dominant shin anastomosis; b — 190 μm distal to a; c — 140 μm distal to b. F — Fibula; fta — ft-anastomoses; fz — zone of fibulare; mla — anastomosis between medial and lateral central arteries; T — tibia; tz — zone of tibiale; IIIz, IVz, Vz — zones of the third, fourth, fifth digits. Other designations as in Fig. 1. Scale bar 85 μm .

mesenchyme lying postaxial to the central arteries is the material of the future fourth digit.

Stage 42 (Figs. 2, 3). Proximally, the limb buds are rounded in the transverse section, more distal they

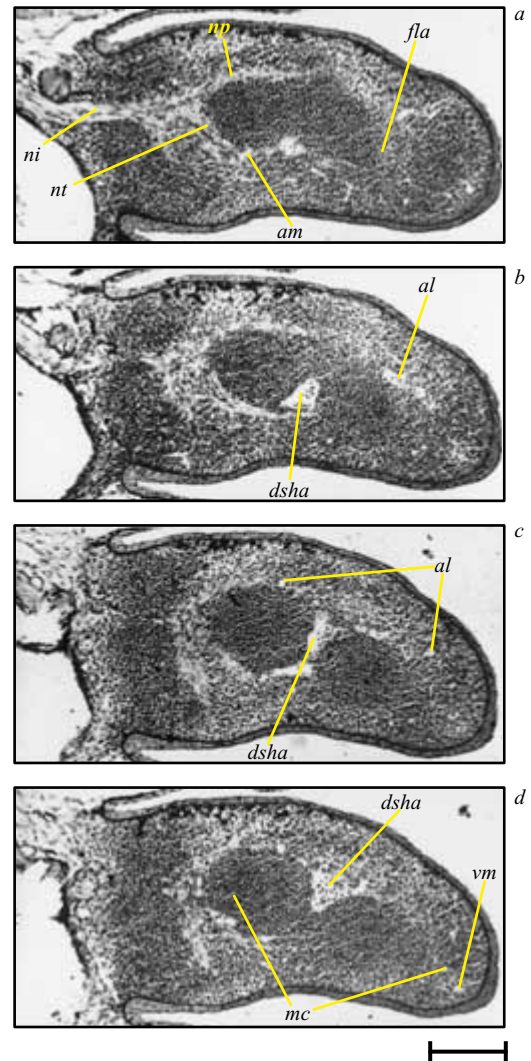


Fig. 3. Larva of *Rana temporaria*, stage 42. Hind right limb. Medio-lateral sections disposed in postaxial to preaxial direction. b — 20 μm preaxial to a; c — 10 μm preaxial to b; d — next to c. dsha — Dominant shin anastomosis; mc — mesenchymal core; ni — nervus ishiadicus; vm — marginal vein. Other designations as in Figs. 1, 2. Scale bar 165 μm .

flatten in the medio-lateral direction. The PP-plane extends obliquely downwards, in the distal half of the limb bud the anlage between it and the vertical plane is about 45°. The regular subectodermal layers are absent in the limb base and in the proximal region of its free part; the peripheral muscles begin to form there. Nerves reach the tarsal region. The cartilaginous femur wholly lies within the base of the limb. Behind the sciatic nerve, the mesenchymal core bro-

adens in the PP-plane. The dominant shin anastomosis is very powerful, small anastomoses are also preserved in front and behind it. Short fibula and tibia become cartilaginous on both sides of shin anastomoses. Distal to them, the core becomes homogeneous again, still further one more anastomotic network (*ft*-anastomoses) appears which connects the medial (a. interossea posterior) and lateral (a. interossea anterior) central arteries and divides quite a loose tissue of the future fibulare and tibiale. Distal to this region, the mesenchymal core consolidates again. There, the central arteries ramify, and branches extend to the postaxial vein between the dense mesenchymal column of the fourth digit and the yet unorganized tissue of the future fifth digit. The pre- and postaxial veins unite into a single marginal vein.

Stage 43. The cartilaginous femur slightly projects within the free part of the limb, with muscular anlagen above it. The femur and shin cartilages (fibula and tibia) are separated by a thick layer of dense undifferentiated mesenchyme. Fibulare is a compact mesenchymal rudiment, tibiale is hardly distinguishable within the preaxial tissue. A large vessel, the future a. perforans tarsi inferior, begins to form among *ft*-anastomoses. A solid mesenchymal rod of the fourth digit is highly prominent, the third and the fifth digits are poorly formed. All the three digits are separated from each other by arteries extending to the marginal vein, and the medial and lateral trunks are connected by a series of anastomoses. Similar arteries stretch preaxially to the third digit and limit the yet unorganized material of the second digit.

Stage 44. At the beginning of the free limb portion the angle between the PP-plane and vertical plane is about 45°, in the region of the digital plate both planes coincide. The lateral central artery reduces proximal to the powerful shin anastomosis so that the medial trunk (a. poplitea) becomes a main stem vessel of that region. Short cartilaginous fibulare and tibiale are wholly divided by *ft*-anastomoses. The digital plate is wide. Metatarsal IV becomes cartilaginous, the third and the fourth digits are solid mesenchymal rods, and the second digit only begins to form. Muscles and nerves reach the base of the digits.

Stage 46 (Fig. 4*a*). The proximal ends of the fibula and tibia are united by young cartilage. Massive fibulare in its distal part extensively grows towards a more slender tibiale. The base of the fourth digit is disposed along the fibular line, the base of the third one along the tibial line. Metatarsals III and V and the

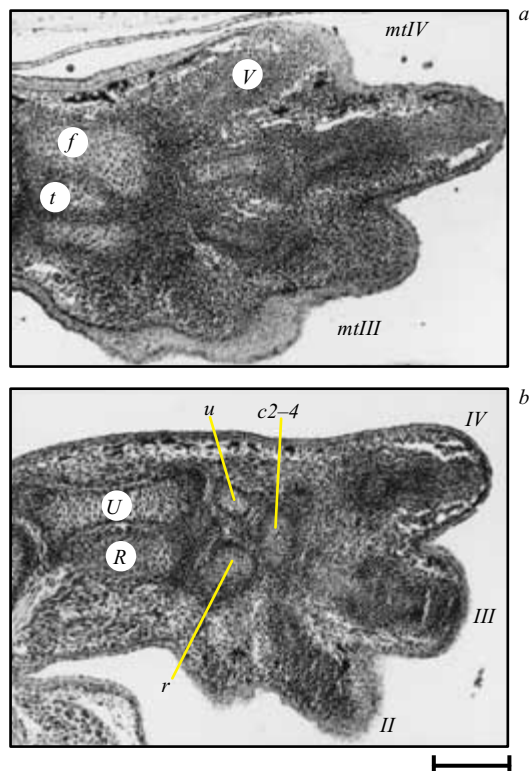


Fig. 4. Larvae of *Rana temporaria*, stages 46 (*a*) and 47 (*b*). Hind (*a*) and front (*b*) limbs. Preaxial-postaxial sections. *c2-4* — Carpale 2-4; *f* — fibulare; *mtIII*, *mtIV* — metatarsalia III, IV; *R* — radius; *r* — radiale; *t* — tibiale; *U* — ulna; *u* — ulnare; *II-V* — digits II-V. Scale bar 165 μ m.

first phalanx of the fourth digit become cartilaginous. The second digit is a solid mesenchymal rod, branches of the central arteries separate it from tissue of the future first digit.

Stage 48. Both fibula and tibia and fibulare and tibiale are fused at their ends. There are all five digits, and also the cartilaginous rudiments of the tarsalia distalia and praeallux.

DISCUSSION

A change in the limb position relative to the tadpole body was studied only for the hind limbs. Like in Siberian salamander, the right and the left hind limb buds of frog grow backwards parallel to each other, but the PP-plane in their free part at first lies almost horizontally. Only in tadpoles of stage 44 the PP-plane in the proximal region of the free part of the limb extends approximately at 45° to the vertical

plane, and the digital plate coincides with the latter; the limb is as if being twisted around its proximo-distal axis (for intermediate stages see Figs. 1, 2).

Mesoderm filling the frog limb bud "behaves" according to a familiar plan (Borkhvardt, 1994a, 1994b). Very soon, a thin subectodermal layer of regularly organized cells forms that in future will take part in muscle development. A subectodermal layer is better represented at the medial and lateral sides of the limb bud and is underdeveloped in the pre- and postaxial regions where the stem veins are disposed. A solid mesenchymal core forms in the central region of the limb bud, and relatively loose tissue separates it from the subectodermal layer (Figs. 1a, b; 3). During the comparable stages the limb buds in frog are noticeably larger than in Siberian salamander and contain much more mesenchyme, especially in the core region.

Blood Vessels, Skeletogenous Mesenchyme, and Skeleton

At stage 39, the frog hind limb bud is a slightly backwardly stretched knob. There is a dense vascular network within it so that cells of the loose mesenchyme as if fill in the holes of this network. As the central mesenchyme condenses, blood vessels become displaced to a peripheral zone. The stem central arteries, branches of the common central artery, stand out among these vessels and extend in a distal direction along the medial and lateral surfaces of the mesenchymal core. This process of regulation and concentration is largely completed at stage 41 when the central arteries can be traced up to their junction with the initial portion of the postaxial vein that begins at the preaxial side of the limb bud and bends round the mesenchymal core apex (Figs. 1, 3).

The medial and lateral central arteries are periodically connected with each other by means of anastomoses which penetrate the mesenchymal core and, like a fence, divide it into the pre- and postaxial parts. Such anastomotic networks form in the shin region (shin anastomoses, Fig. 1b), in the region of future fibulare and tibiale (*ft*-anastomoses, Fig. 2b) and in the digital region where they originally divide the tissue of the future third and fourth digits. As the digital plate broadens, the central arteries give off branches to the marginal vein so that these branches limit in turn the zones of the fifth, second, and first digits (Fig. 2c). The transverse arterial flows concentrate in

the shin and tarsal regions where the one dominant trunk forms within each anastomotic network; this process runs more quickly in the shin region where the dominant vessel may be found already at stage 41. As this vessel increases (Fig. 3b-d), the proximal portion of the lateral central artery reduces (Fig. 2a) and finally fully disappears. Distal to the large shin anastomosis both central arteries, the medial one (a. interossea posterior) and the lateral one (a. interossea anterior = a. tibialis anterior) remain for life; in the adult frog they communicate, as before, with each other in the shin and tarsal regions (Gaupp, 1899).

In the tadpole front limb the arterial blood is distributed, as a whole, according to the same plan (Fig. 5) but the dominant trunk is not formed among anastomoses which divide the zeugopodial elements, the ulna and the radius. Accordingly, in adult frog the arterial blood flows from the medial (posterior) to the lateral (anterior) side only in the carpal region where a. interossea communicates with the arcus dorsalis manus (Gaupp, 1899). Such a distinction between the definitive blood circulation in the front and hind limbs suggests an idea that the principal disposition of stem arteries is not dependent on the arrangement of skeletal elements. Since it is known that this distinction and some other important features of the limb blood system appear in early morphogenesis it can be concluded that the general plan of blood circulation in the limbs is being established without any influence of skeletal or muscular structures.

In Siberian salamander larvae the medial and lateral central arteries lie much closer to each other, than those in tadpoles, undoubtedly because of the small size of urodele limb buds, including their mesenchymal core. Because of looseness and small volume of mesenchyme that divide the central arteries of *Salamandrella* it is difficult to recognize anastomoses connecting the medial and lateral vessels. Only today, during new studies, they were discovered at the previously used (Borkhvardt, 1994a) slides, although the precise arrangement of transverse anastomoses along the proximo-distal limb axis remains unknown. Now, it is clear that connections between the medial and lateral central arteries and also between their branches, e.g., interdigital ones, is a striking feature of the blood system in amphibian limb buds. There are similar anastomoses in adult frog as well (Gaupp, 1899), urodeles also have at any rate one such anastomosis, namely, a. perforans of a mesopodial region (Francis, 1934).

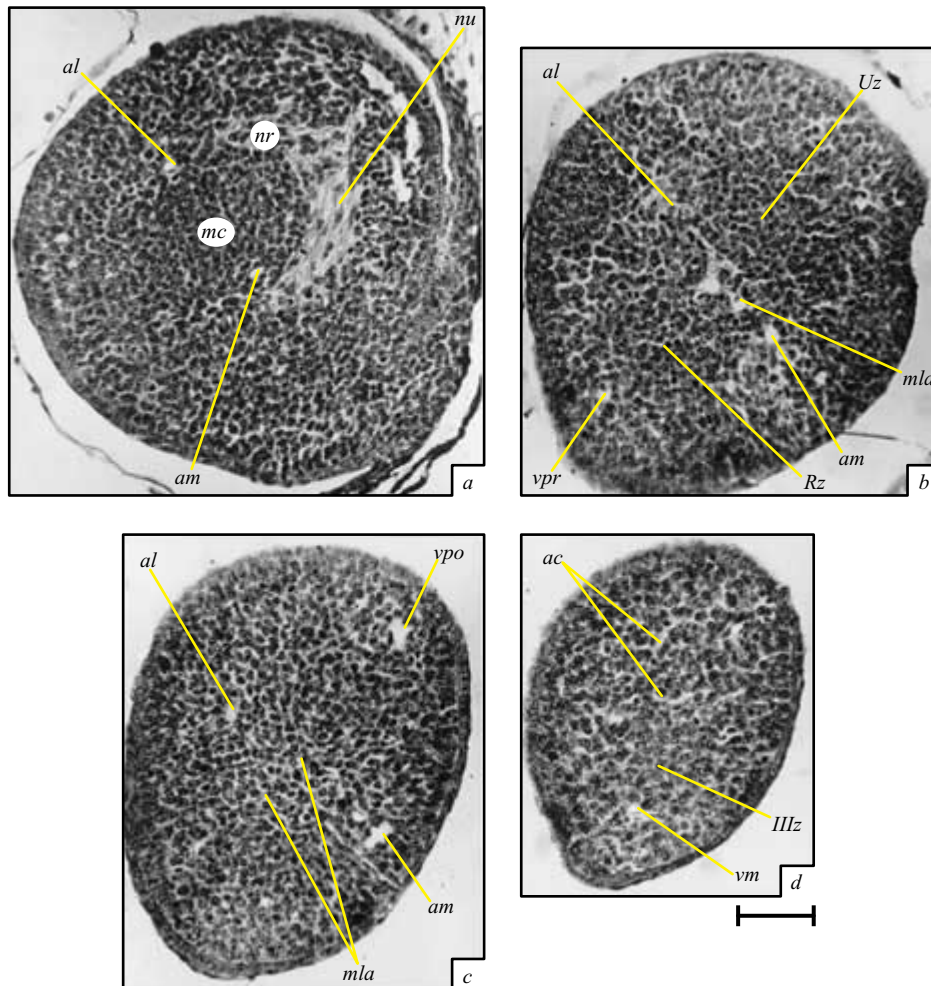


Fig. 5. Larva of *Rana temporaria*, stage 42. Front right limb. Transverse sections in stylopodial (*a*), zeugopodial (*b*), ?carpal (*c*), and digital (*d*) regions. *b* — 220 μm distal to *a*; *c* — 130 μm distal to *b*; *d* — 100 μm distal to *c*. Sections are not strictly oriented relatively to the larva body. *nr* — Nervus radialis; *nu* — nervus ulnaris; *Rz* — zone of radius; *Uz* — zone of ulna. Other designations as in Figs. 1 – 3. Scale bar 65 μm .

There is an important difference in the arrangement of arteries in the limb buds of frog and Siberian salamander. In tadpoles, central arteries are the only stem trunks, all other vessels, including the interdigital ones, branch off from them. On the contrary, in larvae of *Salamandrella* each central artery, medial and lateral, gives a large postaxial branch in a zeugopodial region (Borkhvardt, 1994b: Fig. 1*b*). Later, the medial postaxial branch (a. interossea) surpasses the central arteries in power and, partly transporting blood to the lateral side with the help of a. perforans, becomes the main artery of the paw which itself sends the interdigital vessels.

The anastomotic networks connecting the medial and lateral arteries arise in the tadpole limb buds at the time when there are no even mesenchymal rudiments of the skeleton. These networks, and especially dominant anastomoses, look like the true partitions (Fig. 3) which at first divide the mesenchymal core into the zones of the future ulna (fibula) and radius (tibia) (Figs. 1*b*, 5*b*), ulnare (fibulare), and radiale (tibiale) (Figs. 2*b*, 5*c*), digits III and IV. Later, as primary mesenchymal core broadens in its distal part and forms there a digital plate, branches of the central arteries divide the latter into the zones of the next digits (Fig. 2*c*).

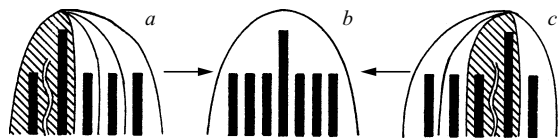


Fig. 6. Schematic representation of the right digital complexes viewed from above. *a* — Caudata; *b* — hypothetical construction; *c* — Anura. Vertical black column — digit; high column — zero digit. Shaded zone — Primary mesenchymal core; double wavy line — central artery. Curved lines indicate growth of the digital plate, i.e., broadening of the primary mesenchymal core. Horizontal arrows indicate that figure (*b*) is created by the mutual imposition of figures (*a*) and (*c*); this composition is done so that high vertical columns (zero digits) are combined.

New data support the idea that the arterial vessels play a part of the longitudinal “separators” that break up the skeletogenous tissue into the autonomous portions and thereby determine the arrangement of cartilaginous rudiments along the preaxial – postaxial axis. Therefore, it is expedient to compare the developmental mode and the definitive structure of the mesopodial skeleton in *Salamandrella* and *Rana*. As stated above, two pairs of stem arteries pass in the mesopodial region of the limb buds of Siberian salamander that divide the mesenchymal core into the three longitudinal columns (Borkhvardt, 1994a, 1994b). The so-called median column forms between the central arteries and its large branches, and certain specific elements of the urodele carpus (tarsus), namely, intermedium and centralia, arise within it. In tadpoles, the central arteries do not bifurcate, the median column is not formed, and the middle mesopodial elements are absent; only two cartilages, ulnare (fibulare) and radiale (tibiale), are laid down on both sides of the only pair of stem arteries (see below).

The role of arteries as the transverse “separators” (Borkhvardt, 1994b), on the contrary, now becomes less probable. Only one strong transverse anastomosis between the pre- and postaxial veins was found in the hind limb buds of tadpoles at stage 40. Possibly, this anastomosis is the penultimate generation of the apical portion of the common marginal vein (see Borkhvardt, 1994a) but it very soon disappears and it is difficult to judge about the morphogenetic role of this vessel.

Digits

The anuran limbs contain different numbers of digits. In the hind limb there are five prominent toes which are numerated as *I–V*, and a small praehallux.

There is a short preaxial ray in the front limb as well but there are only four “true” digits. Long ago all these five rays were more often numerated as *I–V* (Gegenbaur, 1864; Gaupp, 1896; Sewertzoff, 1908; Schmalhausen, 1915) but now such a set of symbols is seldom used. Usually, only prominent fingers are being numerated, as *I–IV*, whereas a small preaxial ray is named “prepollex” (Holmgren, 1933; Saint-Aubain, 1981; Shubin and Alberch, 1986).

Observation of the larval limbs of *Rana* shows that the two extreme postaxial fingers in their position relatively to the zeugopodial and mesopodial elements (Fig. 4*b*) and still earlier relatively to the central arteries (further on “central artery”) most of all correspond to the third and fourth toes (Fig. 4*a*). If the hind limb is accepted as the model one (because it evokes no controversy) one must support the modern scheme of designation of anuran fingers. At the same time, the existing general principle of counting out the digits of tetrapods does not seem to be an ideal one in itself.

In the traditional numeration system digits are counted from the edge of the paw. The edge, however, is not a stable point because it “moves” both in ontogeny and phylogenesis. There is a more reliable landmark in the paw, namely, the central artery, which corresponds as a whole to the central proximo-distal axis of the limb bud. When using the artery as the topographical criterion it is easy to see that those digits of urodeles and anurans that are traditionally numerated by the same figures occupy a different position relatively to the central artery. In *Salamandrella*, the latter divides the first (*I*) and the second (*II*) digits, in *Rana* the third (*III*) and the fourth (*IV*) ones. These “central” digits can be marked by the same symbols and a new universal system can be created that could count out the remaining digits from them. Something like this is being proposed below but attention is paid to certain other features in choosing a “bearing” digit.

Apparently, in all the tetrapods the development of the digital complex begins with laying down of only one digit that usually forestalls the others even if a little; it is like this even in two-hoofed mammals whose definitive limb is strictly symmetrical (Kurnosov, 1960). In urodeles, the second digit is the earliest to arise, in the front four-dactylose limb of anurans the third one, in pentadactylose limb of anurans and amniotes the fourth one (Shubin and Alberch, 1986). In amphibians, the “earliest” digit is always laid

down approximately at the same place, at the central proximo-distal axis of the limb or near it (Figs. 1c; 2c; 5d; 6a, c; Schmalhausen, 1915: Tables 1–4, 9–10; Saint-Aubain, 1981: Figs. 2, 9; Borkhardt, 1994a: Fig. 4); the central artery inevitably limits this anlage at one side.

I propose to name the digit that is the first to arise as a “zero digit” (0), irrespective of its position relative to the central artery whether pre- or postaxial. So, the temporal, and not spatial, criterion is selected as the basic one for marking a “bearing” digit. This criterion is more suitable in practice because it is easier to detect the first anlage (mesenchymal condensation) than to ascertain the relation between digits and vessels when observing the paw morphogenesis. But it is more important that the selected numeration system most clearly displays some regularities of limb development (see below). Moreover, the two systems under discussion do not markedly differ because the second finger and the toe of Siberian salamander and the fourth toe of frog coincide by both criteria (spatial and temporal); only in the front limb of *Rana* the earliest digit lies preaxial to the central artery (Fig. 5d).

A middle position of the earliest digital anlage may be explained quite simply. Cells forming this anlage are in fact the cells of a distal portion of the primary mesenchymal core, i.e., they are the oldest cells of the digital plate; accordingly, they are the first to differentiate. The second to become cartilaginous is the digit which lies on the opposite side of the central artery, in the other (lesser) part of the primary core. In urodeles, the two earliest digits are *II* and *I*, in the hind limb of the frog *IV* and *III* (Fig. 2c), in its front limb *III* and *IV*. Subsequent digits are being added owing to the accumulation of skeletogenous tissue preaxial and/or postaxial to the first two or, in other words, owing to broadening of the primary mesenchymal core. Both in Caudata and Anura, the digital plate broadens *from the midline* (!) but there is an essential difference in the character of its growth. In urodeles, the skeletogenous mesenchyme and then digits are being added on the postaxial side, whereas in anurans mainly on the preaxial one. In frog, such kind of asymmetry of the digital plate is particularly noticeable in the front limb where already the primary core extends farther in its preaxial part (Fig. 5d) and not in the postaxial as it is in the hind limb (Fig. 1c).

Thus, modern amphibians demonstrate the two opposite, as it were, modes of extension of the digital

complex in their ontogeny — the “preaxial” (Fig. 6c) and the “postaxial” ones (Fig. 6a). And what will happen if both potentialities are simultaneously realized in the same limb? One can see the result in Fig. 6b where an artificial digital complex is created by the mutual imposition of two natural complexes (Fig. 6a, c) whose zero digits are combined. Hence, it can be suggested that the polydactylose limbs of *Tulerpeton* (Lebedev, 1984), *Ichthyostega*, and *Acanthostega* (Coates and Clack, 1990) formed just owing to a simultaneous activity of the “preaxial” and “postaxial” potentialities. Of course, the other way of multiplication of digits was also possible, at the expense of a very intensive increase in only one half of the digital plate.

“From the midline” broadening of the digital plate characterizes the digital complex as a kind of the biserial aggregate. Biseriality shows itself more clearly in the hypothetical set (Fig. 6b), whereas digital complexes of modern amphibians are more or less asymmetrical (Fig. 6a, c). Notably, even such classical biserial structures as the fins of *Neoceratodus* (Dipnoi) are not strictly symmetrical since their radialia are disposed thicker and are more numerous on one side than on the other (Druzinin, 1933).

The notion of a “zero digit” can be used for composing a digital formula of a new type. I propose to make up such a formula for the right paw viewed from above. The formula of the pentadactylose complex of urodeles (Fig. 6a), for example, looks like 10123, where zero (0) means a zero digit, the numbers of figures on both sides from it mean the numbers of digits within the preaxial (to the left) and postaxial (to the right) parts of the paw; in addition, the extreme figures (1 and 3) indicate the number of the pre- and postaxial digits in themselves. The digital formula of the front four-dactylose paw of frog looks like 2101, of its hind pentadactylose limb (Fig. 6b) 32101. The hypothetical set (Fig. 6b) may be described by the formula 3210123. Other methods of registration were also discussed. For example, such variants as 103, 1-0-3, 10111 could be written instead of the formula 10123 but all of them seemed less convenient for understanding.

Introduction of a new numeration system does not mean the rejection of the traditional mode of marking digits: it is possible to use both schemes in parallel. In order to distinguish them more clearly, digits in the new formula are marked by Arabic, and

not Roman, numerals; true, Arabic numerals are also more convenient graphically.

Mesopodial Complex

Among modern tetrapods urodeles have the largest number of mesopodial elements, in Palaeozoic temnospondyls (*Eryops*, *Trematops*, *Greererpeton*) this number reached 12, and in theoretical schemes it could be still more (e.g., Schmalhausen, 1915). The lesser number of carpals and tarsals in anurans and amniotes is usually considered to be the result of reduction or fusing, and many authors tried to find the lost (“latent”) elements in larvae and embryos. These elements were actually found but, as a rule, only at the mesenchymal stages. Using the method of autoradiography, Hinchliffe and Griffiths (1983) demonstrated that in axolotl (Caudata), clawed frog (Anura), chick (Aves), and mouse (Mammalia) the number of mesenchymal anlagen and of definitive elements was approximately the same. It was concluded that “...the limb prechondrogenic pattern in these species is already very specialized, almost as specialized in fact as are their adult limb skeleton...” (p. 118). I readily accept this conclusion proceeding from my own observations on limb development in Siberian salamander and common frog.

A desire of scientists to find the entire set of canonical elements in the developing paw of all animals was a source of much controversy in the interpretation of the morphogenetic process and definitive structure of the mesopodial skeleton. The other cause of controversy is that the mesopodial elements are not clearly defined in principle. When Gegenbaur (1864) introduced the universal system of designations he took the newt limb as a model and “tied” the skeletal complexes of other tetrapods to it. In their turn, the proximal and distal carpalia (tarsalia) were “tied” to the stable limb segments – zeugopodial bones and digits. Accordingly, such names appeared as ulnare, radiale, fibulare, tibiale, carpalia (tarsalia), distalia 1 – 5. We saw, however, that digits of *Salamandrella* and *Rana* which have the identical numbers in the traditional numeration system occupy a different position with regard to the central artery. The same refers to the mesopodial elements. In Siberian salamander only two elements, namely radiale (tibiale) and radiale 1 (tibiale 1), lie preaxial to the central artery. All others, seven in number, lay down

postaxial to this vessel (Borkhvardt, 1994a). In frog the picture is opposite as a whole.

So, the autopodial skeletons in Caudata and Anura have the different structural plans or, more exact, the different developmental plans. One indisputable distinction is that in urodele paw the skeletal elements, and before them the skeletogenous mesenchyme, are added mainly on the postaxial side, whereas in anuran paw on the preaxial one. Such disproportion reveals itself in the temporal parameters as well. In urodeles, digits III – V (in *Salamandrella* III – IV) and small extreme postaxial cartilages, e.g., postminimus, appear in ontogeny noticeably later than the others. On the contrary, cartilages of frog paw are late at the preaxial edge. If a hypothesis about the morphogenetic role of blood vessels (see above) is correct, a different organization of arterial flows in urodele and anuran limb buds — two stem arteries in the former and only one in the latter (rather, two and one pair) — will also appear as an important distinction between the plans of limb development in Caudata and Anura.

On the basis of what was said above, I refuse to compare the individual mesopodial elements of urodeles and anurans. Specifically, there is no sense to discuss the scheme of Holmgren (1933) who also sharply opposed the mesopodial complexes of Caudata and Anura but proceeded from quite different general conceptions and operated with the traditional terms which, especially centralia, had in fact no clear definition. True, some classical terms were used in the present article as well but this was done only for the simplification of the text, without any special meaning. In principle, one could try to elaborate a new list of symbols for the mesopodial skeleton on the basis of a stable marker, namely, the central artery, as it was done for digits. Unfortunately, now it is not clear what way to choose. Possibly, it would be expedient to work out a special terminology for every developmental plan but now it is not possible to say how many such plans exist in tetrapods.

CONCLUSION

After establishing the fundamental, as he thought, distinction between mesopodial complexes of Caudata and Anura, Holmgren (1933) considered it rightful to derive the limbs of these animals from the fins of different sarcopterygians. Should one do the same on the basis of a new demarcation? I think,

not. It is hardly possible to decide at what historical stage the limbs of modern amphibians diverged if only the extant animals are being studied. Hence, the question arises: what is the evolutionary significance of polydactylose limbs of Devonian *Tulerpeton*, *Ichthyostega*, and *Acanthostega*? It would seem that the recent discoveries confirmed the old idea that the pentadactylose limb originated from the limb with more digits, e.g., seven. Of course, the probability of such course of events is higher now but again it is not worth to accept it completely since the limbs of Devonian amphibians could be dead-lock variants. At the same time, the information stated above does not contradict but rather supports a hypothesis that the tetrapod limb was derived from the biserial or at least centro-axial fin that at the larval stage was "split" by the stem arteries (Borkhvardt, 1994b).

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