

EVIDENCE-BASED EVOLUTIONARY RESEARCH AND DEVELOPMENT OF THE PRACTICAL PHYLOGENETICS: VERIFICATION OF THE GRAVITY-CORRESPONDING EVOLUTIONARY LAW BY MEANS OF BIOMATERIALS

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ABSTRACT

From studies of developing artificial bone marrow chambers with hematopoiesis, the author evidenced that the evolution of the hemopoiesis in endoskeletons is dependent upon correspondence of organisms against the increased gravitational force during terrestrialization¹⁻³. After that the author proposes the Gravity-corresponding Evolutionary Law in vertebral phylogeny⁴⁻⁸. Metamorphosis of the endoskeleton by biomechanical stimuli is known as functional adaptation named Wolff's Law⁹. The Wolff's Law is the system of metamorphosis in accordance with Use and Disuse Theory of Lamarck precisely restricted in the morphology of skeletal organs, which occurs during within one generation. The evolutionary research can be carried out not only biomechanically as Use and Disuse Theory of Lamarck, but morphologically as Biogenetic Law of Haeckel, i.e., close morphological correlation between the ontogeny and phylogeny⁷. In this paper comparative anatomy concerning skeletons, i. e., cartilage and bones of the amphibian, reptiles, and the mammals is carried out. Skeletal morphology of the vertebrates depends not only upon modality of repeated movements of skeletal organs (inner factors by Lamarck) but biomechanical stimuli influencing outer side upon organisms (outer factors by Lamarck, i.e., environmental factors). Therefore, if metamorphosis can be observed among same animal kind of the same phylogenetic stage, there should be differences of inner or outer factors during evolution of these animals. Differences in the skeletal and muscle system of amphibian as well as reptiles and mammals can be seen as follows: 1) The viscerocranium system ① The tooth and jawbone system ② The branchial system ③ The ossicles of the auditory system ④ Morphology of nostrils ⑤ The masticatory muscle system ⑥ The diaphragm, the heart and lung system ⑦ The collarbones.

INTRODUCTION

Experimental evolutionary studies are carried out using chondrichthyes *Triakis*, *Heterodontus*, and neoteny-type Mexican salamanders (*Ambystoma*) through artificial terrestrialization. The comparative morphology of these animals of the viscerocranium among ontogeny and phylogeny in five organs are carried out. The formation of the diaphragm and the lungs correlation with pericardial sack as well as collarbone are investigated and divergence of the mammals and the amphibian, reptiles is evidenced. As conclusion through comparative anatomy it is evidenced that origins of mammals and reptiles are different at the stage of archetype vertebrates before terrestrialization, i.e., chondrichthyes. Not only skeletons in phylogeny but also the evolutionary processes of the lungs as well as the tongue are quite different of the amphibian as well as reptiles and the mammals.

The definition of the vertebrates is a chordate having a bony backbone with the various degrees of ossification. Characteristic organs of the vertebrates are the first the vertebrae and the second the gut respiratory system, i.e., the gills and lungs¹⁰⁻¹¹. Phylogenetic tree of the vertebrates can be studied exactly by investigating not only the skeletal system but the respiratory system. Conventionally evolutionary researches as well as phylogenetic studies have been carried out vigorously by investigation the skeletal systems, i.e., the bone and the tooth.

In this paper the author studies evolutionary metamorphosis during the second revolution, i.e., terrestrialization experimentally using dog sharks (*Heterodontus japonicus*) and ahlotols (Mexican salamanders) with experiment applying biomechanical stimuli, and comparing these phylogenetic metamorphosis with ontogenetic processes of mammals (rats). The author calls these experimental studies as practical phylogenetics. Before the experiments the author investigated developmental studies on artificial bone marrow chambers to solve the riddle of the bone marrow hemopoiesis.

From these developmental researches it is known that phylogenetics can be investigated practically by studies on the skeletal system, and biomechanical stimuli can be the cause of the evolution of the vertebrates. Therefore, applying biomechanical as well as physicochemical stimuli (energies) in broad sense including substance with mass (oxygen, nutrition and minerals), evolutionary stage can be changed in present animals experimentally, the author develops the experimental evolutionary researches using these methods.

MATERIALS AND METHODS

For experimental evolutionary researches 50 cm length chondrichthyes *Triakis* and *Heterodontus* and 20 cm length neoteny-type ahlotols are used. Comparative anatomy between them is carried out. Morphological changes in ontogeny of the rats are studied by the atlas *The House Mouse*¹². *Triakis* and *Heterodontus* are artificially landed for one hour a day during 10 days. After that they are dissected and observed the pericardial sac around the heart. Neoteny-type ahlotols are artificially landed for 3 months with moisture without water pool. After that they are dissected. Specimens are prepared and morphological studies as well as histological observations are carried out.

The tooth system, bronchial arch system, and the collarbone are observed and compared between them. The development of diaphragm and morphology of nostrils are studied and compared.

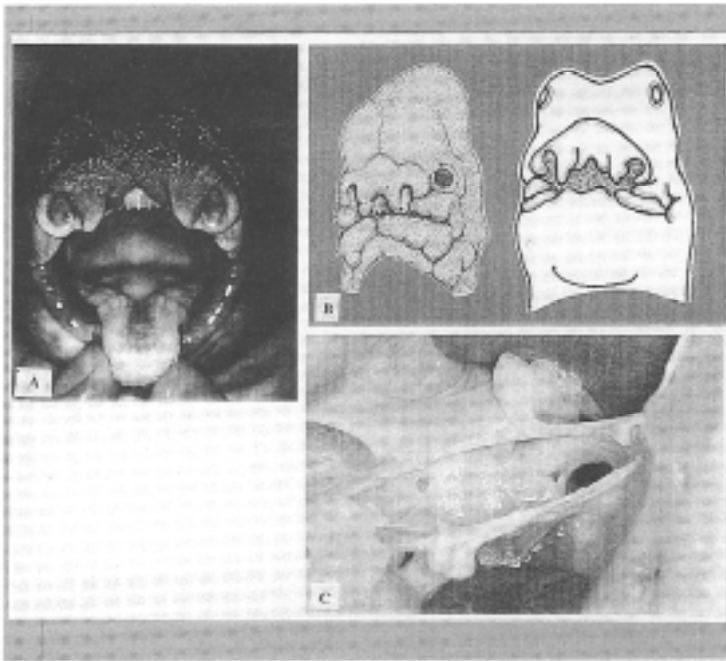


Figure 1
 A- *Heterodantus japonicus* (H.J.)
 B- Human embryo and H.j.
 C- Air cells in pericardiac membrane

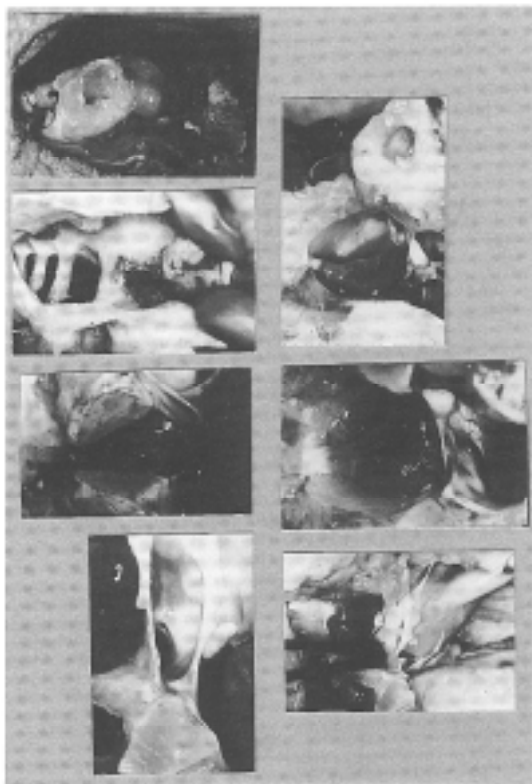


Figure 2 Dissection of H.j. after landing

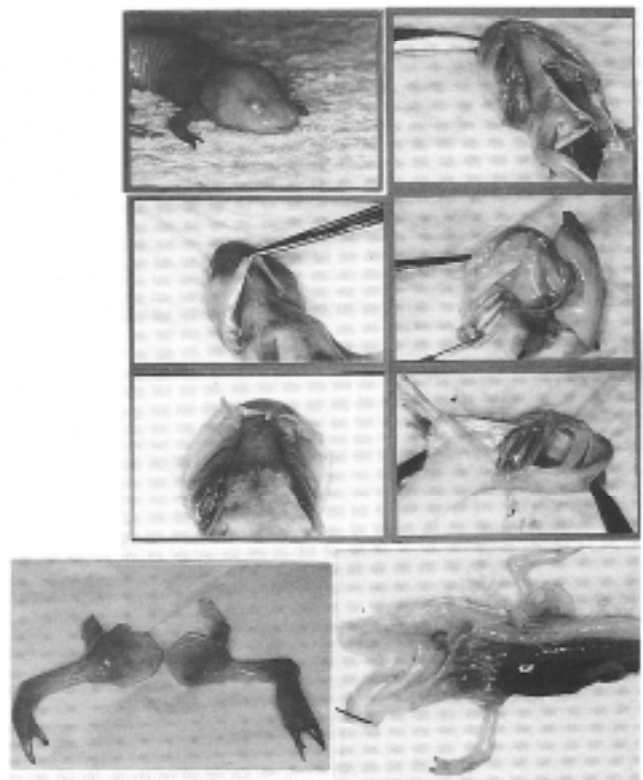


Figure 3
 A- Dissection of aholotol after landing

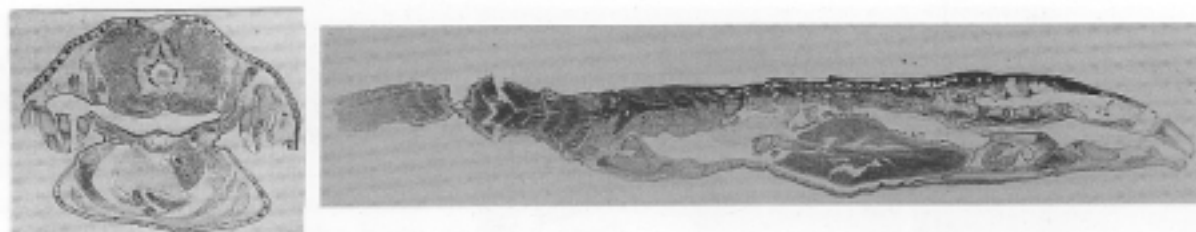


Figure 3
B- Cross and longitudinal section of aholotol after landing

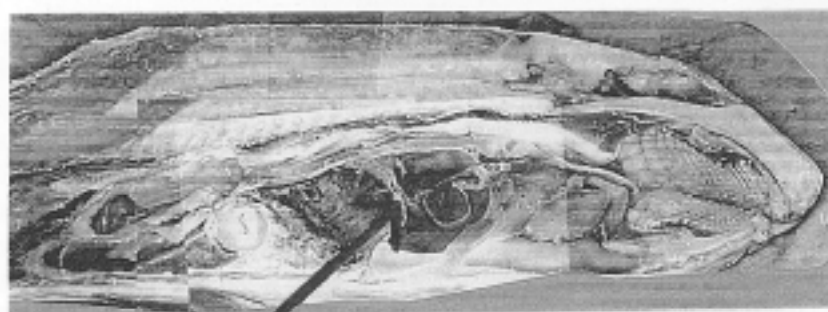


Figure 4 Longitudinal section of *Heterodontia japonicus*

RESULTS

1) The viscerocranium system

① The tooth system of *Triakis* and *Heterodontus* are compared. *Heterodontus* has three kinds of tooth system quite resembling to the mammalian system (Figure 1-A). *Triakis* has the homodontia system as the amphibians and reptiles system. ② The Branchial arch system of *Heterodontus* and tongue development are quite similar to these of the mammals. A part of muscles of the branchial system function as the masticatory system in *Heterodontus*. These of *Triakis* are quite similar to these of the amphibians and reptiles. Branchial arches and muscles gathered together to make a movable tongue.

③ It is well known that a part of jawbone turned into auditory skeletons in phylogenic, which were disclosed by Gaubb. The auditory ossicles of reptiles can not evolve into mammalian ossicles biomechanically.

④The nostril and ⑤ Masticatory system

Morphology of viscerocranium of *Heterodontus* quite resembles that of mammals in the teeth and the nose. The caput of human embryo 32 days after fertilization is almost the same as adult dog shark (*Heterodontus*) just like the recapitulation theory of Haeckel (Figure 1-B). On the contrary, the gill system of *Triakis* is quite similar to amphibian *aholotol*, which fuses to close fenestration of waterway of the branchial system to form the tongue in landing (Figure 3-A).

2) The diaphragm, heart and lung system

Artificially landed *Triakis* and *Heterodontus* are dissected. In *Triakis* connective tissues with numerous air cells are observed between chest fins and pericardial sac. If numerous air cells join with gill apparatus the lungs resembling amphibian appear. In *Heterodontus* two air cells are observed between inner and outer membrane of pericardial sac, of which latter becomes to be diaphragm after the lungs developed. If these two cells join with gill apparatus, the lungs resembling mammalian appear.

In the rat atlas in ontogeny in early developmental stage the lung had developed into center of the heart, so diaphragm develops from pericardial sac of caudal side. At the caudal side of pericardial sac of two artificially terrestrialized *Heterodontus japonicus*, two air sacs resembling air bladder are observed between inside and outside of the serous pericardial membrane (Figure 2), of which the one on the right side is larger than the one on the left. (Figure 1-C). On the contrary, control *Heterodontus japonicus* without landing has no air sac between either inside or outside of pericardial sac (Figure 4, arrow). Triakis after landing has intact pericardial sac, but marked formation of air sacs are observed between fin muscles and pericardial sac of the heart. 3) The origin of collarbone is found in cyclostomata as pericardial cartilage, which is a part of peri-branchial cartilage covering hemopoietic organs of gill grand. The pericardial cartilage of cyclostomata is succeeded to the chondrichthyes as fused cartilage in ventral side of pericardial cavity, which are joined with fins and of which movement can promote function of the heart (Figure 4). This cartilage is succeeded to amphibians and reptiles as 2 plate-type collarbones, which are in ventral side of pericardial sac and are join to each foreleg (Figure 3, 5). To mammalian the collarbones are succeeded from chondrichthyes as stick like bones, which are at the apex of the lungs. This deference between amphibian, reptiles as well as aves and mammals are dependent upon difference of development of the lungs and diaphragm.

DISCUSSION

Phylogenetic researches can be carried out effectively by skeletal development. Prior to the development of the artificial bone marrow chambers, the author developed in vivo artificial root of the gomphotic type, which induces the cementum around it and represents the mammalian tooth system using sintered hydroxyapatite. These are hybrid-type artificial organs, which induce highly differentiated cells hetero-topically. On artificial roots surface cementoblasts are induced hybridly from mesenchymal cells of periostium by biomechanical stimuli. In artificial bone marrow chambers highly differentiated hemopoietic cells are induced hybridly by biomechanical stimuli as well. In phylogeny the bone marrow hemopoiesis had developed during the second revolution of the vertebrates i.e., terrestrialization and the gomphotic tooth system had developed during the third revolution of the vertebrates i.e., the mammalian birth. In terrestrialization 1/6 G in water turned into 1G on land. At the same time 1% content of oxygen in water turned into 21 % in air. It is considered that during gill respiration in air content of oxygen in blood became 5 to 10 times higher in landing. Around the heart surplus oxygen was discharged to form air bladder. When air bladders break through to the sixth gill apparatus, the gill become the airway and the bladders become the lungs.

Conventional phylogenetics tells us that the reptiles evolve into the mammals. However, the vertebrates evolve by biomechanical stimuli, which influence the organisms throughout phylogenic time span. Evolving modality by biomechanics is in accordance with the Wolff's Law. Therefore, amphibian lungs, which have no muscles around, never induce diaphragm. In this research through comparative studies on ontogeny of mammals (rats) and amphibian (salamanders), the development of the lung system between them is observed completely different. The morphological changes in second revolution occurred through billion years can be observed by neoteny-type ahotols through 5 months, which are artificially landed by reducing water. Drastic changes of the skeletons, heart, skins, and gill system can be seen to form the tongue.

The formation of the lungs from branchial glands is quite different between reptiles and mammals.

From developmental studies on mammalian embryo, it is known that the lung buds extend into pericardial sac of the embryo. On the contrary, those of reptiles extend across the esophagus dorsally to the iliac bone. The differences of the lung development between reptiles and mammals are known by comparative anatomy of landed aholotols with developing embryo of rats. From these research it is known that, divergence between reptiles and mammals had started in the stage of terrestrialization. By newly developed experimental research methods, the authors can evidence Heterodontus to be archetype of the mammals.

ACKNOWLEDGMENT

This research has been supported by a Grant-in-Aid for Scientific Research (A)(1)09309003 from the Ministry of Education, Science and Culture, Japan.

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